Form 49
[Rule 13.19]

Clerk's Stamp

COURT FILE NUMBER	
COURT	COURT OF QUEEN'S BENCH OF ALBERTA
JUDICIAL CENTRE	WETASKIWIN
PLAINTIFFS	DR. BLAINE ACHEN, DR. GERT GROBLER DR. NADR JOMHA AND DR. TYLER MAY
DEFENDANT	ALBERTA HEALTH SERVICES
DOCUMENT	**AFFIDAVIT OF DR. NADR JOMHA**

ADDRESS FOR SERVICE AND CONTACT INFORMATION OF PARTY FILING THIS DOCUMENT	Ackroyd LLP Barristers and Solicitors 1500, 10665 Jasper Avenue Edmonton, Alberta T5J 3S9 Attention: Richard C. Secord [redacted]	Justice Centre for Constitutional Freedoms #253, 7620 Elbow Drive SW Calgary, Alberta T2V 1K2 Attention: Eva Chipiuk

AFFIDAVIT OF DR. NADR JOMHA
Sworn on December 7, 2021

I, Dr. Nadr Jomha, of the City of Edmonton, in the Province of Alberta, SWEAR AND SAY THAT:

1. I am one of the Plaintiffs herein, and as such have a personal knowledge of the matters hereinafter deposed to, except where they are based on information and belief, in which case I verily believe them to be true.

Background Personal Information

2. I graduated with my Degree as a Medical Doctor in 1990 at the University of Alberta (the "University"). I completed my residency in Orthopaedic Surgery at the University in 1995 and received a Degree in Orthopaedic Surgery as a Fellow of The Royal College of Surgeons from the Royal College in Canada in 1995, followed by a Master of Science at the University in 1996, and a PhD in Experimental Surgery at the University in 2003. I began my career at the University as a Clinical Associate in September 1998 after

completing Knee (1997-1998) and Foot and Ankle Surgery (January 1998 - August 1998) Fellowships at the North Sydney Sports Medicine Clinic in Sydney, Australia.

3. I currently hold the position of Professor in the Department of Surgery Division of Orthopaedic Surgery at the University. I was granted a Professorship position in 2015. I have been the Director of Orthopaedic Research for the Division of Orthopaedic Surgery at the University since 2003. My clinical work consists of lower extremity joint reconstruction in the elective and traumatic realms. The University is one of the busiest Orthopaedic Trauma hospitals in Canada. I perform general orthopaedic trauma surgery, but I also receive complex lower extremity trauma referred specifically to me from other trauma surgeons at the University as well as the northern half of Alberta.

4. I am the only Orthopaedic Surgeon at the University (and one of a few in Edmonton) that performs complex knee ligament reconstruction after trauma. I was enrolled in University for 16 years to achieve my level of education and expertise including obtaining a BMSc, then MD, followed by surgical training to obtain my FRCS(C), and then I completed an MSC in Experimental Surgery and a PhD in Experimental Surgery. This was augmented by another full year of subspecialty training in knee joint reconstruction and then 8 months in foot and ankle reconstruction.

5. I have approximately 18 years of post-secondary training to get where I am. I have held my current clinical position for over 23 years. I have operated on over 10,000 patients and seen many more for non-operative treatment. I operate on approximately 500 patients per year and see a similar number for non-operative management of musculoskeletal injuries per year.

6. Since March of 2020 to present (the "Pandemic"), I regularly operated on Covid-19 positive and Covid-19 suspected patients understanding the risk to my personal safety during opaque times. I performed all my trauma care and surgical duties even when personal protective equipment ("PPE") was scarce, and the threat of Covid-19 was at its maximum due to a lack of knowledge of the virus at the start of the Pandemic. Throughout the Pandemic, I continued to work every day without fail. During the past 20 months, I operated on approximately 401 trauma patients and 154 elective patients. During that time, I have also had 1856 urgent care patient clinic visits and 151 new elective patient care visits.

7. I will continue to operate on Covid-19 positive patients and Covid-19 suspected patients until I am told by my supervisors or AHS that I cannot work just like I operate and care for HIV positive patients, Hepatitis B and C positive patients, and anyone else who needs my help. It has been my goal in life to help people irrespective of their situation in life.

8. It was my experience that the volume of trauma at the Hospital was initially quite low in the first few months of the Pandemic, likely due to decreased human activity in the initial phases of Covid-19, but subsequently, the trauma volume picked up. I find that the level of activity at the Hospital is quite similar to pre-pandemic levels in respect of the intensity and the volume of trauma cases while elective cases have decreased significantly even though surgeons are available. Accordingly, it is my observation that the Hospital is not overwhelmed as a result of unvaccinated patients.

Irreparable Harm to me Personally and Professionally

9. I am known in my local community of Muslims and Arabs as a role model as I am one of the first Muslim Arabs to obtain a position such as mine in Edmonton. I have worked extremely hard to establish my clinical practice and the research program for the University. I was the first fully trained foot and ankle surgeon in Edmonton and unofficially mentored subsequent recruits in my subspecialty who have become leaders in the field. Orthopaedic lower extremity reconstruction surgery and research is who I am. Losing everything I built will be devastating after the past 37 years of hard, tireless work often working 100-hour weeks during my training and 80 hours weeks for many years thereafter. A stoppage for any significant period will destroy much of what has been accomplished over those many years.

10. From a personal perspective, I cannot even imagine the extent of the loss that I will face both personally and professionally. I have 2 children aged 12 and 8, one of which has a formal diagnosis of autism. He requires special attention including social and scholastic support. I am committed to caring for them financially until their first post-secondary degree is completed so that is up to another 14 years for my 8-year-old. This could be longer for my son who has autism as we don't know how his independence will grow as he ages. I have also committed spousal support for their mother. I am newly remarried with 3 stepchildren. Although they are older (aged 16, 18, and 20), they still attend school and are partially dependent on me. I am committed to my dependents for years to come and providing for them will be extremely difficult without my work as a surgeon. I also have the

usual expenses such as a mortgage and car payments that need to be made. I have no idea how I will manage my financial obligations if I am terminated or placed on an unpaid leave of absence.

11. Neither my supervisors or Alberta Health Services ("AHS") have told me how this will impact me long term. I do not know if this will affect my ability to practice medicine in the future or if I will be able to continue my research. Given that AHS controls all the hospitals where trauma is performed, I will effectively be denied the opportunity to treat trauma patients and any patients that require joint reconstruction. It is also my understanding that AHS has instructed all private surgical centers to enforce their policies and rules, which means that I will effectively be denied the opportunity to surgically treat patients with even lesser elective disorders.

12. AHS has not provided any reasonable accommodation for my situation and position; they have only continued to threaten me and try to coerce me into taking the Covid-19 injection.

Policy is not in the Public Interest

13. My elective work consists of deformity correction and arthritic joint reconstruction. During my career, I have been referred many very complex and difficult cases from foot and ankle surgical colleagues, including a 17-year-old male who was essentially incapacitated with great difficulty walking due to a fracture of his talus (ankle) bone with subsequent collapse of the bone. I designed and produced a custom talar bone replacement prosthesis and implanted it successfully in that patient in 2011. Since then, I have evolved the prosthetic design and implanted another 10 prostheses, I continue to evolve the design of the prosthesis with my research team. I currently have 2 patients waiting for the production of this prosthesis and am expecting another patient from the Calgary region. I developed these implants together with engineers at the University and we have published papers on the development of this implant and its clinical results. I am the only surgeon in Alberta (one of few in the world) that performs talar bone prosthesis implantation that enables these patients to get back to walking and productive lives.

14. I created a collaboration with the Comprehensive Tissue Center ("CTC") (Edmonton's tissue bank) and have developed a talar bone and cartilage transplant program which will save the province thousands of dollars each year. This program provides a joint resurfacing option to patients that would otherwise be prohibitively expensive or

unavailable due to lack of donor tissue. I continue to collaborate with the CTC to expand this program to other bones and joints such as the knee joint where the demand is high. This collaboration will stop if I am denied my clinical work.

15. I have been involved in and conducted clinical trials that have impacted how Orthopaedic Surgery is delivered in various areas such as Achilles tendon repairs, tibial shaft fractures, distal radial fractures, and open fractures.

16. As a fellowship-trained foot and ankle surgeon (1 of only 5 in Edmonton), my clinical practice is extremely busy with very long waitlists to see me and to get an operation. I have over 130 patients very early in the assessment process waiting to see me with some referrals I accepted dating back to February of 2020. In addition, the Central intake foot and ankle patient screening clinic has approximately a 2-3 year wait time just to get onto my accepted consultation list behind the 130 patients noted.

17. I have 70 patients booked and waiting for surgical repair. That is approximately 6 months of elective operating in normal times. I have dozens of patients in various stages of injury treatment and surgical repair that require continued care, preferably by me, their operating surgeon.

18. My elective patients have suffered significantly due to the Pandemic and the closures instituted by AHS. Patients with the great difficulty walking due to lower extremity deformity could not get into the operating room due to the closures in the Spring and Summer of 2020. Patients that require follow-up surgery for complications also have been delayed potentially compromising their outcomes. Waitlists have been poor for many years with waitlists from 2 to 3 years to see a Foot and Ankle Surgeon due to a chronic lack of resources including an insufficient number of Foot and Ankle Surgeons to service Northern Alberta. My removal will significantly exacerbate that.

19. In my role as Director of Orthopaedic Research, I am intimately involved in all the research produced by the Division of Orthopaedic Surgery (including a summary booklet of the research conducted each year since 2004). Attached hereto and marked as **Exhibit "A"** to this my Affidavit is the Orthopaedic Research Review for 2019. I am a key member of the Edmonton Orthopaedic Research Committee and have been a member of the Department of Surgery Research Committee for almost 20 years. I have trained all

Orthopaedic Residents in the University Orthopaedic Surgical training program in research for the past 18 years.

20. I have helped develop a combined Masters Fellowship with the School of Public Health at the University to train the next generation of surgeon scientists. I have been a Principal Investigator as a researcher since 2003 and have developed a vast network of collaborators in medicine and other disciplines such as engineering locally and nationally. My cryobiology research (investigating methods to repair cartilage and meniscus at low temperatures) is currently in a translational stage, we are performing animal surgeries, and we expect to have products available in Alberta within the next 2 to 5 years to aid in cartilage repair, meniscal repair, and joint reconstruction with bone and cartilage transplants. The University is recognized as a world leader in part due to the work of our cryopreservation group over the past 27 years. Cryobiology has been recognized as one of the most promising avenues to increase tissue availability to treat severe joint injury and disease and well as other tissues and organs. To my knowledge I am the only Orthopaedic Surgeon in Canada with this training and experience (including a PhD in Experimental Surgery related to Cryobiology) to continue this research.

21. I have nearly 100 (97 with some pending) peer-reviewed publications related to my research and have been awarded the best Orthopaedic Surgery Canadian Researcher by the Canadian Orthopaedic Research Society. I am recognized locally and nationally as a research leader and have been asked to review research for national agencies such as the Canadian Institutes for Health Research as well as many international journals.

22. I am the only trauma surgeon at the University of Alberta that reconstructs complex knee ligament injuries. I am the only surgeon in Alberta that has performed talar prosthetic implantation. I have established and am further developing a bone and cartilage transplant program for which I am uniquely qualified. Loss of my ability to work will truncate all these activities putting patients at increased delays for treatment and I believe some patients will get their treatment denied altogether. My bone and cartilage tissue transplantation program has already saved Alberta thousands of dollars by sourcing our tissue locally instead of importing it from the US. This program will not be further developed with my departure. The talar prosthesis program I developed for people with severe talar collapse after trauma or of spontaneous onset which decreases pain and restores ankle range of motion and ability to function will stop. Further development of the prosthesis to improve

outcomes in the current model will stop as I am the originator of the current prosthesis and its improvements.

23. I am the most senior Orthopaedic researcher in Northern Alberta and have run the Division of Orthopaedic Surgery research program for almost 20 years. I have been the Orthopaedic representative on the University Department of Surgery Research Committee for approximately the same number of years. I have designed the training program (with one other person) for all Orthopaedic residents and have overseen the research for the Division of Orthopaedic Surgery for the resident trainees and surgical staff. Our division works to improve patient care via appropriate research. My leadership and experience in this area will be lost.

The AHS Policy and My Request for Accommodation

24. On September 14, 2021, AHS Policy 1189 was put in place and was to become effective October 31, 2021 (the "Policy"). Attached hereto and marked as **Exhibit "B"** to this my Affidavit is a true copy of the **Policy.**

25. On or about October 12, 2021, I submitted my blood to Kinexus Bioinformatics to have an antibody test completed. On November 10, 2021, I received an email from Steven Pelech of Kinexus Bioinformatics stating that my blood did come back positive with several antibodies for Covid-19. Attached hereto and marked as **Exhibit "C"** to this my Affidavit is a true copy of that email with the results. My case of Covid-19 was evidently asymptomatic as I do not have any recollection of being sick with Covid-19 during this period. This could be possible because I was adhering to daily practices obtained from reading peer-reviewed articles on the use of honey, vitamin D, and zinc in preventing and treating viral illnesses.

26. To my knowledge, I did not transmit Covid-19 to AHS staff or patients or anyone else. I was never identified as a possible transmitter or notified as a close contact of a Covid-19 case. This is because I take exceptional health and safety precautions, including PPE and handwashing.

27. When the Policy first came out, I had a personal meeting with my Chair, Dr. David Williams, and I advised him that for personal reasons I was not going to get vaccinated. He stated he was unclear about what would happen but that he would likely put me on an unpaid leave of absence ("LOA") with AHS. As a fee-for-service physician that works exclusively

at AHS facilities, an LOA with AHS means no patient contact and no pay. He suggested there was no other option available to his knowledge at that time. In early September, I met with my Site Chief of Orthopaedic Surgery, Dr. Angela Scharfenberger, and advised her the same. We did not discuss many options as she was not aware of any paths forward.

28. I subsequently wrote an email to my Orthopaedic group at the University, my academic lead (Dr. Ed Masson), and a researcher I collaborate with (Dr. Lauren Beaupre) to inform them of what was happening and to gauge who might be able to help with care of my patients. One colleague symphaized with my situation and questioned the legality AHS is enforcing the Policy. Attached hereto and marked as **Exhibit "D"** to this my Affidavit are true copies of those emails.Other foot and ankle surgeons that agreed to help my current patients but they are already overloaded with a multi-year waiting lists to be seen, let alone operated on. Since then, I have not had much contact with administrators with regards to my AHS position except for the occasional brief hallway chat without much guidance.

29. On October 16, 2021, which was AHS' deadline for workers to declare their vaccination status, I submitted a document stating that I did not want to disclose my vaccination status as it is protected health information. Attached hereto and marked as **Exhibit "E"** to this my Affidavit is a copy of my non-disclosure letter.

30. On November 8, 2021, I sent an email to Dr. Zygun's assistant advising that I am not happy with how AHS is enforcing the Policy and that I need more time to consider options. Attached hereto and marked as **Exhibit "F"** to this my Affidavit is a copy of my email.

31. On November 8, 2021, I also submitted a religious exemption request to AHS. Attached hereto and marked as **Exhibit "G"** to this my Affidavit is a copy of my request for a religious exemption. On November 18, 2021, I received a denial of my religious exemption from AHS. Attached hereto and marked as **Exhibit "H"** to this my Affidavit is a copy of the denial of my religious exemption.

32. I again sent a similar message on November 23, 2021, to Dr. Zygun and Dr. Manns. Dr. Manns sent a reply to set up a zoom meeting to discuss options. In the zoom meeting Dr. Manns acknowledged that given that my position and the work, I can only work in hospitals that are exclusively run by AHS in Alberta, the options provided by AHS do not apply to me. Notwithstanding that Dr. Manns acknowledged that none of the options provide any accommodation that applies to me, Mr. Manns sent me an email pressing me to make a

decision. Attached hereto and marked as **Exhibit "I"** to this my Affidavit is a true copy of those emails.

My Professional Judgement

33. After an extensive review of the scientific research and medical data, it is my professional, medical opinion that someone with natural immunity is the safest person in the Hospital with regards to Covid-19 based on scientific literature and medical data.

34. In May of 2021, a study published in the prestigious Nature journal stated: "Overall, our data provide strong evidence that SARS-CoV-2 infection in humans robustly establishes the two arms of humoral immune memory: long-lived BMPCs and memory B cells." And they concluded: "Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans." Attached hereto and marked as **Exhibit "J"** to this my Affidavit is a true copy of the article in Nature entitled *SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans*.

35. In a very large study done in Austria with 8,900,000 participants regarding re-infection after infection with SARS-CoV-2, it was noted: "...we observed a relatively low tentative re-infection rate of SARS-CoV-2 in Austria that suggests a similar protection against SARS-CoV-2 infection compared to vaccine efficacies." Attached hereto and marked as **Exhibit "K"** to this my Affidavit is a true copy of the article in Wiley entitled *SARS-CoV-2 reinfection risk in Austria*.

36. In a pre-print study done in the United States investigating the rate of re-infection in health care workers found 74,557 re-infection-free days "...adding to the evidence base for the robustness of naturally acquired immunity." Attached hereto and marked as **Exhibit "L"** to this my Affidavit is a true copy of the health care study entitled Continued Effectiveness of COVID-19 Vaccination among Urban Healthcare Workers during Delta Variant Predominance.

37. As of November 25, 2021, the Brownstone Institute shows 135 scientific studies that demonstrate natural immunity is equal or better than vaccine-induced immunity. This number continues to increase almost daily. The Brownstone Institute is made of scientists and medical doctors around the world who are collecting scientific evidence in support of various aspects of Covid-19 including natural immunity for Covid-19. The mission of the Brownstone Institute is "... to come to terms with what happened, understand why discover

and explain alternative paths, and prevent such events from happening again" with regards to Covid-19. Attached hereto and marked as **Exhibit "M"** to this my Affidavit is a list of the 135 studies on the Brownstone Institute.

38. Increasingly, research shows vaccinated people can contract and transmit Covid-19. A study of healthcare workers in Vietnam (Chau 2021) and the United States (Riemersma 2021) show that not only are vaccinated healthcare workers contracting Covid-19 post-vaccination, but they are also asymptomatic Covid-19 spreaders. Another published paper from Israel, being a highly vaccinated population, shows nosocomial outbreak caused by the SARS-CoV-2 Delta variant. An outbreak caused by the SARS-CoV-2 Delta variant in a secondary care hospital in Finland, shows that vaccinated people carry high viral loads of the SARS-CoV-2 virus. Attached hereto and marked as **Exhibit "N", "O", "P" and "Q"** respectively, to this my Affidavit are copies of those studies.

39. As of August 6, 2021, the Center for Disease and Control Prevention's ("CDC") Morbidity and Mortality Weekly Report ("MMWR") indicated that vaccinated people were able to transmit the disease. Furthermore, the CDC also confirmed that they were not collecting data in the US demonstrating transmission of Covid-19 from Covid-19 recovered people. Attached hereto and marked as **Exhibit "R" and "S",** respectively, to this my Affidavit is a copy of the CDC's MMWR, and a copy of the CDC letter.

40. Finally, the CDC Covid Response Team just put out a pre-print study and concluded "As this field continues to develop, clinicians and public health practitioners should consider vaccinated persons who become infected with SARS-CoV-2 to be no less infectious than unvaccinated persons." Thus, this paper confirms that differentiation between vaccinated and unvaccinated in terms of potential for spread should not be made. A follow-on from that could be that recovered covid patients are less of a threat than either of these groups due to robust natural immunity gained after the infection. Attached hereto and marked as **Exhibit "T"** to this my Affidavit is a copy of the CDC study.

41. The Covid-19 vaccines carry significant known lethal risks such as myocarditis, Guillain Barre Syndrome, and blood clots along with other suspected risks including, increased risk of an acute coronary syndrome, death, anaphylaxis, stroke, myocardial infarctions. These adverse effects are reported in multiple databases such as the Vaccine Adverse Events Reporting System (VAERS) organized by the CDC and Food and Drug Administration ("FDA") in the US where 18,854 reports of deaths and over 890,000 adverse events have

been reported in regards to the Covid-19 vaccine as of November 29, 2021. Attached hereto and marked as **Exhibit "U"** to this my Affidavit is a copy of the VAERS page from November 29, 2021.

42. The VAERS numbers are consistent with those reported in the European database including the European Medicines Agency which reported 749,979 adverse events as of Oct 28, 2021, and the UK database - Yellow Card Reporting - 395,049 reports of 1,294,956 potential events as of November 24, 2021. Attached hereto and marked as **Exhibit "V"** to this my Affidavit is a copy of the European and UK pages as of December 6, 2021.

43. Given that an overwhelming majority of studies prove that individuals with naturally acquired immunity:

    a. have been shown to have equal or better immunity than a vaccine-induced immunity;

    b. are very rarely re-infected with Covid-19; and

    c. are unlikely to transmit Covid-19.

    Given the above, there is no medical or scientific benefit to myself or anyone with naturally acquired immunity to take the Covid-19 vaccines.

44. Furthermore, and more concerning as a medical doctor, there are known and significant medical risks associated with the vaccines, particularly for individuals with natural immunity. It has been recently shown that prior Covid-19 infection increases the risk of adverse events post-vaccination (Mathioudakis; Self-Reported Real-World Safety and Reactogenicity of COVID-19 Vaccines: A Vaccine Recipient Survey). Other research has found vaccines can generally be linked to chronic inflammation and autoimmune disorders (Kostoff. Vaccine- and natural infection-induced mechanisms that could modulate vaccine safety. Toxicology Reports). Attached hereto and marked as **Exhibit "W" and "X"**, respectively, to this my Affidavit are copies of those studies.

45. Based on a thorough review of the scientific and medical data, there are more long-term risks that prevent me from taking the current Covid-19 vaccines than I can accept. I have a very strong family history of Alzheimer's dementia which can be precipitated or

exacerbated by chronic inflammation. Attached hereto and marked as **Exhibit "Y"** to this my Affidavit is a copy of the Alzheimer's study.

46. My grandfather was diagnosed with Alzheimer's at 71 years of age, my mother at 61 years of age, and one of my brothers at approximately 56 years of age. I fear daily that I will get Alzheimer's. I live my life doing the best I can to limit anything that can be considered a risk for Alzheimer's such as cutting out sugar and processed foods, keeping my mind active, keeping physically active, abstaining from all intoxicants including alcohol. I supplement my diet with krill oil and coconut oil-based on my readings of the literature all in an effort to eliminate any possible external factors that could induce this disease. There is no long-term data on these vaccines, and they should still be considered experimental. We just do not know how they are going to affect people in the long term, especially people with predispositions to other diseases. I cannot put myself at risk, even if theoretical when there is no demonstrable benefit of the vaccine for me or those around me.

47. As a qualified medical professional, based on the current scientific and medical data, and given the known risks of the Covid-19 vaccinees versus the known benefits of the Covid-19 vaccine, I cannot consent to take the Covid-19 vaccine at this time.

48. The pressure, coercion, and threats from AHS are in conflict with everything we have learned and swore an oath to as medical professionals including informed consent, duty to disclose, and patient autonomy. It goes against the explicitly written consent instructions from AHS and the College of Physicians and Surgeons of Alberta. Attached hereto and marked as **Exhibit "Z"** to this my Affidavit is a copy of AHS' Informed Consent Policy and the College of Physicians and Surgeons information on Informed Consent. In my 31 years in the medical field during my training and practice, I have never attempted to provide a medical treatment against that patient's personal consent. Bodily autonomy is sacred from a medical and religious point of view.

49. In my religion and belief system, Islam, coercion, and compulsion are never allowed. I cannot allow myself to be coerced into a medical procedure that has no benefit for me or those around me.

50. The Policy is going against science, my own medical understanding, and against my natural immunity. In addition, the Policy is infringing on my Charter rights, human rights, and the Nuremberg Code to take an experimental medical intervention.

51. It is unreasonable and unethical for AHS to put me on an involuntary, unpaid leave of absence or terminate me when reasonable, safe, and efficient alternatives to preserve workforce capacity and support the healthcare system are available, AHS is causing irreparable harm to me personally and the public health care system in Alberta generally.

52. It is unreasonable and unethical for AHS to dismiss my legitimate medical concerns and religious believes without any accommodation. The ultimate impacts and harm that will be caused to me personally and professionally are yet unknown. I do not know if I will be able to perform surgery in Alberta again. I am being coerced with my livelihood about something I don't believe in personally or professionally. My record and reputation are excellent. I am known to be a leader in my field respected in my role. I have never faced disciplinary action or competency concerns about my patient care and practice.

53. I undertake to indemnify the Defendant in the event of a loss of this application.

54. I swear this affidavit *bona fide*, in support of the within action and injunction application and for no improper purpose.

Exhibit "A"

UNIVERSITY OF ALBERTA

Orthopaedic Research Review for 2019

This is **Exhibit "A"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at Edmonton Alberta, this

[redacted]

Eva Chipiuk
Barrister & Solicitor

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Research Director:
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Research Director:

Nadr Jomha
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I write this as the world reacts to the novel coronavirus infections sweeping the world, and wonder what the new research landscape will look like. Currently all our research is essentially shut down. There are no patients to enroll in clinical studies and the basic science labs have been ordered to close. We all anxiously await the opportunity to generate new knowledge as we did before the advent of the pandemic.

This newsletter reviews the accomplishments of the Division of Orthopaedic Surgery in 2019. Inside you can read about advances made both clinically and in the basic science realms. We are pleased to provide interesting insights from eight different areas of research (Trauma, Knee, Arthroplasty, Foot and Ankle, Upper Extremity, Paediatrics, Spine and Basic Sciences). Each area continues to expand and develop new knowledge, and this has resulted in well over one hundred publications and presentations directly influencing the practice of orthopaedic surgery throughout the world. We are very excited about the future of research in our Division and hope that you enjoy reading about what we accomplished in 2019. If you have any comments or questions, please contact us.

Spine Research

Faculty: Doug Hill, Dr Eric Huang, Dr Edmond Lou, Dr Jim Mahood, Dr Marc Moreau, James Raso, Sarah Southon, Dr Kyle Stampe

Affiliates: Dr Samer Adeeb, Dr Kajsa Duke, Dr Lawrence Le, Dr Eric Parent, Dr Francois Roy, Dr Aleksandra King, Dr Lindsey Westover, Natalie Wittmeier

Technical Staff: Kenwick Ng, Duc Nguyen, Dichong Song

Research Coordinator: Kathleen Shearer

Administrative Support: Ann Papps

Schroth Therapist: Graham Murray

Using Non-ionizing Radiation Approaches to Assess and Assist Treatment of Spinal Deformity

Surface topography and ultrasound scanning methods are the two most promising approaches that may be able to assess and predict the severity of scoliosis without requiring ionizing radiation. The ultrasound method is also able to measure the Cobb angle on the plane of maximum deformity.

Customized k-nearest neighbourhood analysis in the management of adolescent idiopathic scoliosis using 3D markerless asymmetry analysis

Drs Adeeb, Westover, Parent

Markerless surface topography (ST) analysis (Fig. 1) has been proposed for monitoring AIS to reduce the X-ray radiation exposure. This study aimed to define a custom neighbourhood classifier algorithm for AIS classification to improve the accuracy, sensitivity, and specificity of predicting curve severity and curve progression in AIS. Curve severity was predicted with 80% accuracy (sensitivity = 81%; specificity = 79%) for thoracic-thoracolumbar curves and 72% (sensitivity = 93%; specificity = 53%) for lumbar curves. This represents an improvement over the previous method's curve severity accuracies of 77% and 63% for thoracic-thoracolumbar and lumbar curves, respectively. Additionally, curve progression was predicted with 93% accuracy (sensitivity = 83%; specificity = 95%) representing a substantial improvement over the previous method's accuracy of 59%. The current method has shown the potential to further reduce radiation exposure for AIS patients by avoiding X-rays for mild and non-progressive curves identified using ST analysis.

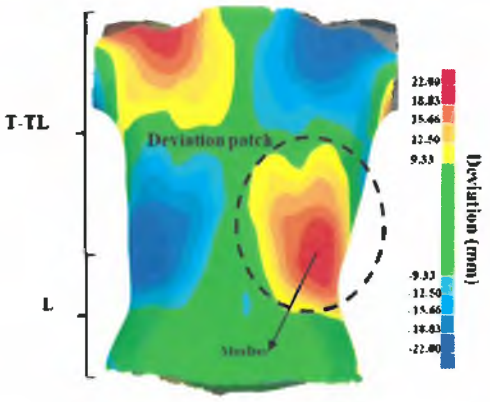


Figure 1. Asymmetry analysis map.

Reliability of Cobb angle measurements on the plane of maximum curvature using ultrasonic imaging method

Dr Lou – principal investigator

Study Design:
Retrospective reliability study.

Objectives:
To investigate the intra- and inter-rater reliability of Cobb angle measurements on the plane of maximum curvature (PMC) using ultrasound (US) images on children with adolescent idiopathic scoliosis (AIS).

Background:
Cobb angle measurement on posteroanterior (PA) radiographs is the gold standard to assess curve severity. However, the PA Cobb angle does not reflect the true three-dimensional deformity.

Methods:
One hundred and one children with AIS (87 F and 14 M; age: 13.7 ± 1.7 years old) were recruited and 157 curves were recorded by clinicians on radiographs. Three raters, R1, R2, and R3, with 0, 4, and 20+ years of experience, respectively, measured the PA Cobb, vertebral axial rotation (VAR), PMC Cobb, and PMC orientations on US images (Fig. 2). The true PMC orientations were determined using 3D reconstructions of the PA and lateral EOS radiographs. The reliability of R1's measurements on PMC orientations were validated using inter-method (ultrasound vs EOS) measurements with the intra-class correlation co-efficients (ICC). Inter- and intra-rater reliabilities, standard error measurements (SEM), and Bland–Altman bias were used to report the PMC Cobb and VAR measurements.

Results:
Inter-method, inter- and intra-rater ICC [2,1] values for all reliability assessments were greater than 0.9. The mean absolute differences and the standard error measurements for both PMC Cobb and VAR were less than 4° and 0.5°, respectively. The PMC orientation was strongly correlated ($r^2 = 0.88$) between both measurement modalities. There appeared to be a positive association between the difference of PMC and PA Cobb when the PA Cobb and the maximum VAR were greater than 30° and 14°, respectively.

Conclusion:
The PMC Cobb and VAR can be measured reliably on US images. Future studies should validate the PMC Cobb angle and include participants with a wider Cobb angle range.

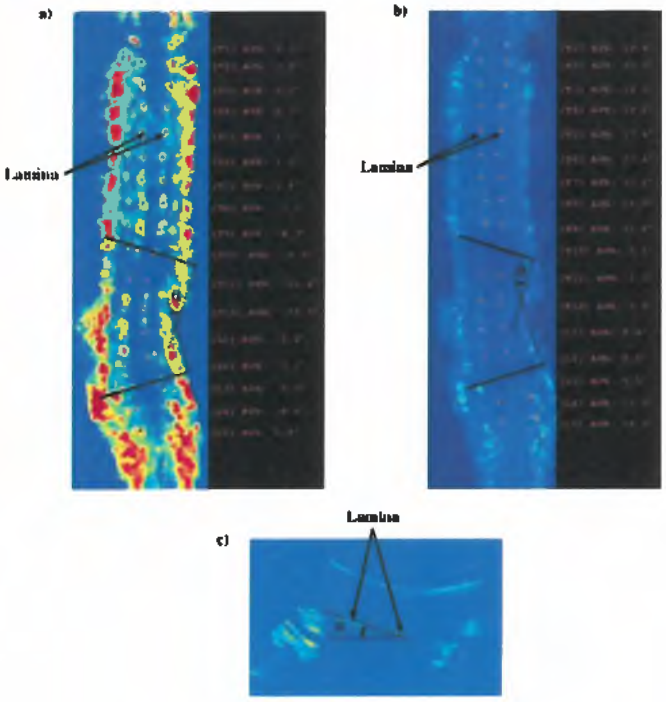


Figure 2. Ultrasound Cobb angle measurements (θ) on a) the coronal plane, b) the plane of maximum deformity, including values for the VAR (α) of each vertebra, and c) the transverse plane.

Lumbosacral compensations during Schroth physiotherapeutic scoliosis-specific exercise corrections in adolescent idiopathic scoliosis

Dr Parent – principal investigator

Patients with double curves can correct the vertebral rotation, interapical distances, and curve angles during Schroth exercises. However, Schroth exercises may trigger lumbosacral compensations. This study quantified the immediate effect of Schroth postural corrections on the L3–L4 and L4–L5 vertebral angles. A total 36 participants with AIS, double curves, and 3+ months of Schroth training were imaged using 3DUS in 16 Schroth-supervised positions including four relaxed positions and their passively corrected, corrected without leg activation, and fully corrected Schroth positions. L3–L4 and L4–L5 coronal angles were measured. Counter-tilt was defined as a vertebral angle opening opposite to the lumbar convexity. L3–L4 and L4–L5 angles showed counter-tilting in all positions except for the tilt towards the convexity in relaxed side-lying (1 ± 2°). The only significant difference among relaxed positions was more counter-tilt of L3–L4 in relaxed standing (1 ± 3°) than in side-lying ($p < 0.05$). Prone comparisons found no differences among L3–L4 angles. Active correction in prone created more counter-tilt at L4–L5 (3 ± 3°) compared to passive corrections (1 ± 2°), but this disappeared with full correction using the hip.

L3–L4 was counter-tilted in all other side-lying positions: passively corrected (1 ± 2°), actively corrected (2 ± 3°), and actively corrected with leg lift (2 ± 4°), compared to tilting towards the convexity in relaxed side-lying position (1 ± 2°). No changes occurred at L4–L5 among side-lying positions.

The L3–L4 counter-tilt in sitting, both actively corrected (3 ± 3°) and actively corrected with leg lift (2 ± 3°), were increased compared to relaxed sitting (0 ± 3°). The L4–L5 angle in actively corrected sitting (3 ± 5°) was also increased compared to relaxed sitting (1 ± 2°).

There were no differences in L3–L4 or L4–L5 angles across the four maximally corrected positions.

Conclusion:
Counter-tilting was observed in most relaxed and all Schroth-corrected positions. The clinical importance and long-term effects of this small increase in counter-tilt are unknown. Continued research is underway.

Immediate effect of daily living (ADL) postures on spinal curvatures and rotation from 3D ultrasound images in adolescent idiopathic scoliosis

Dr Parent – principal investigator

Spinal alignment was quantified using non-invasive 3D ultrasound (3DUS) imaging, and the immediate effects of 18 ADL positions on curve measurements in AIS were compared in order to propose posture recommendations designed to prevent curve progression.

Participants ($n = 25$) with double curves were scanned in 18 positions: standing, model (left [L] & right [R]), sitting

(natural, cross-legged [L&R], lotus [L&R], leaning forward, and sideways [L&R] on a desk), lunge [L&R], wearing a backpack and shoulder bag [L&R], and side-sitting [L&R]. We measured axial vertebral rotation differences (AVR twist), and thoracic and lumbar curve angles.

Lunges: LungeL significantly lowered lumbar angles, by 8.9°, but slightly worsened thoracic and twist angles, by 3.6° and 3.3°, respectively.

Bags: Backpack and BagL reduced AVR twist by 3.4–4.8° compared to BagR, but BagR produced lower thoracic and lumbar angles than BagL by 3.3–3.9°.

Conclusion:
ModelL and Sit-LeanL were the best standing and sitting positions, respectively, improving all angles. Side-SitR was among the best positions for lumbar angles but among the worst for thoracic and twist angles.

Non-surgical Treatment for Scoliosis

Effects of Schroth exercises added to standard care in adolescents with idiopathic scoliosis (AIS) on full-torso surface topography parameters: A randomized controlled trial (RCT)

Dr Parent – principal investigator

This RCT in AIS aimed to determine the effect of Schroth exercises added to standard care (SCHROTH) as compared to standard care alone (CTRL) on the external deformity. This is a preliminary analysis of the first seventy-two consecutive participants with AIS in the ongoing Schroth Exercise Trial for Scoliosis. Participants were aged 10–18 years, with curves of 10–45° and Risser 0–5. They were recruited from a scoliosis clinic and randomized to either the SCHROTH (n = 39) or CTRL (n = 33) group. A Schroth home program adapted to each curve type was taught weekly. All participants received standard care. Postural measurements were recorded at baseline and 6 months using full-torso surface topography. Measurements were extracted using custom software following digitization of landmarks. There was a significantly larger improvement in the frontal alignment of the centroid of the torso cross-sections (deviation of 12.5 ± 0.9 reduced to 9.8 ± 0.8) with Schroth, while no change occurred in controls (10.2 ± 0.8 vs ± 10.3 ± 0.7). While differences appeared to favour the Schroth group for the three other variables investigated, differences did not reach statistical significance.

Conclusion:
While the effects of 6 months of Schroth exercises appear beneficial, especially in the frontal plane, the results were variable. Future analyses will include curve type specific subgroup analyses, additional parameters, and markerless asymmetry analysis.

A randomized clinical trial of 3D printed brace for the treatment of adolescent idiopathic scoliosis (AIS)

Dr Lou – principal investigator

Introduction:
Bracing is the only proven non-surgical treatment for AIS. Using 3D ultrasound (3DUS) during brace design has demonstrated considerable benefits. Recently, 3D printing technology in orthotic applications has become more mature, but the effectiveness of using 3D printed braces for the treatment of AIS is still unknown. An ongoing longitudinal randomized clinical trial (RCT) has been conducted, comparing the effectiveness of 3D printed braces to that of plaster casting techniques.

Aim:
Comparison of the effectiveness of 3DUS plus 3D printed brace to plaster casting techniques for the treatment of AIS.

Materials and Methods:
Recruited participants were 1) aged 10–16 years, 2) diagnosed with AIS, and 3) prescribed full-time braces. Additionally, they had 4) a maximum Cobb angle between 20° and 40°, and 5) Risser sign greater than 3. All participants were randomly assigned (50% probability) to either the standard brace (control) or 3D printed brace (intervention) group. During the brace design clinic, standing and bending 3DUS images were acquired and measured to determine the spinal flexibility and to set the target for in-brace correction. Orthotists used a custom brace design frame which simulates wearing a brace by applying corrective forces via directional arms at proposed brace pad locations. 3DUS was used to determine the correction, and the force locations were altered to determine the optimum location and direction of pressure pads. The control group's braces were designed and manufactured with the plaster wrap method, while the intervention group's were designed using a Vorum Spectra scanner to acquire the 3D trunk shape, and Canfit software to tune and personalize the file for 3D printing. Nylon12 material with ~3 mm thickness was used for the 3D printed braces for the intervention group (Fig. 3). All subjects used their braces for 3–8 months.

Results:
Thirteen participants, 8 (6 F, 2 M) in the control and 5 (4 F, 1 M) in the intervention group, with a mean age 13 ± 1 years and a total of 19 curves were recruited. All participants had flexible spine with flexibility ranging from 0.8° to 2.0°. For the control and intervention groups, the average pre-brace Cobb angles were 26° ± 7° and 26° ± 6°, respectively. The average in-brace Cobb angle correction for all treated curves was 47 ± 29% (control) and 44 ± 17% (intervention), respectively. There were no statistically significant differences ($p > 0.05$) between both groups for pre-brace and in-brace correction. Two participants had opportunities to try both types of braces for a few days. They both reported that the 3D printed braces were lighter and more comfortable.

Figure 3. A subject wears a 3D printed Nylon12 brace.

Conclusion:
All subjects had good in-brace correction of the treated curves. The 3DUS plus the 3D printing technology provided a good brace design with effectiveness similar to the traditional brace. The 3D printed brace was lighter, thinner, and lower-cost compared to the traditional brace design and manufacturing process.

Surgical Treatment for Scoliosis

Reconstruction and positional accuracy of 3D ultrasound on vertebral phantoms for adolescent idiopathic scoliosis spinal surgery

Dr Lou – principal investigator

Purpose:
Determine the positional, rotational, and reconstruction accuracy of a 3D ultrasound system to be used for image registration in navigation surgery.

Methods:
A custom 3D ultrasound for spinal surgery image registration was developed using OptiTrack Prime 13W motion capture cameras and a SonixTablet ultrasound system (Fig. 4). Temporal and spatial calibration were completed to account for time latencies between the two systems and to ensure accurate motion tracking of the ultrasound transducer. A mock operating room capture volume with a pegboard grid was set up to allow phantoms to be placed at a variety of pre-determined positions to validate accuracy measurements. Five custom-designed ultrasound phantoms were 3D printed to allow for a range of linear and angular dimensions to be measured when placed on the pegboard.

Results:
Temporal and spatial calibrations were completed with measurement repeatabilities of 0.2 mm and 0.5° after calibration. The mean positional accuracy was within 0.4 mm, with all values within 0.5 mm of the critical surgical regions and 96% of values within 1 mm inside the full capture volume. All orientation values were within 1.5°. Reconstruction accuracy was within 0.6 mm and 0.9° for geometrically shaped phantoms and 0.5 and 1.9° for vertebrae-mimicking phantoms.

Conclusions:
The accuracy of the developed 3D ultrasound system meets the 1 mm and 5° requirements of spinal surgery from this study. Further repeatability studies and evaluation on vertebrae are needed to validate the system for surgical use.

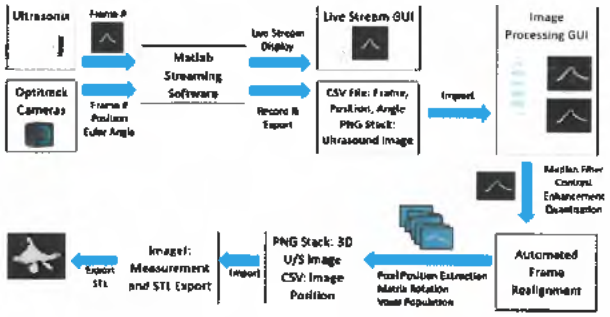


Figure 4. Schematic of 2D ultrasound to 3D ultrasound conversion

Refereed Journal Publications

1. Schreiber S, Parent EC, Hill DL, Hedden DM, Moreau MJ, Southon SC (2019). Patients with adolescent idiopathic scoliosis perceive positive improvements regardless of change in the Cobb angle – Results from a randomized controlled trial comparing a 6-month Schroth intervention added to standard care and standard care alone. SOSORT 2018 Award winner. *BMC Musculoskeletal Disorders*. 20(1): 319. DOI: 10.1186/s12891-019-2695-9.
2. Hadizadeh M, Kawchuk GN, Parent E (2019). Reliability of a new loaded rolling wheel system for measuring spinal stiffness in asymptomatic participants. *BMC* Musculoskeletal Disorders, 20(1): 176. DOI: 10.1186/s12891-019-2543-y.
3. Ghaneei M, Ekyalimpa R, Westover L, Parent E, Adeeb S (2019). Customized k-nearest neighbourhood analysis in

the management of adolescent idiopathic scoliosis using 3D markerless asymmetry analysis. *Computer Methods in Biomechanics and Biomedical Engineering*, 22(7): 696–705. DOI: 10.1080/10255842.2019.1584795.

4. Wong AYL, Parent EC, Dhillon SS, Prasad N, Samartzis D, Kawchuk GN (2019). Differential patient responses to spinal manipulative therapy and their relation to spinal degeneration and post-treatment changes in disc diffusion. *European Spine Journal*, 28(2): 259–269. DOI: 10.1007/s00586-018-5851-2.
5. Abdollah V, Parent E, Battié MC (2019). Reliability and validity of lumbar disc height quantification methods using magnetic resonance images. *Biomedical Engineering/Biomedizinische Technik*, 64: 111–117.
6. Chan A, Parent E, Wong J, Narvacan K, San C, Lou E (2019). Does image guidance decrease pedicle screw–related complications in surgical treatment of adolescent idiopathic scoliosis: A systematic review update and meta-analysis. *European Spine*, 29: 694–716. DOI: 10.1007/s00586-019-06219-3 [Epub ahead of print].
7. Ng K, Duke K, Lou E (2019). Investigation of future 3D printed brace design parameters: Evaluation of mechanical properties and prototype outcome. *Journal of 3D Printing in Medicine*, 3(4): 171–184. DOI: 10.2217/3dp-2019-0012 [Epub ahead of print].
8. Vo Q, Le LH, Lou E (2019). A semi-automatic 3D ultrasound reconstruction method to assess the true severity of adolescent idiopathic scoliosis. *Journal of Medical & Biological Engineering & Computing*, 57: 2115–2118. DOI: 10.1007/s11517-019-02015-9.
9. Tran T, Sacchi MD, Ta D, Nguyen VH, Lou E, Le LH (2019). Nonlinear inversion of ultrasonic dispersion curves for cortical bone thickness and elastic velocities. *Annals of Biomedical Engineering*, 47(11): 2178–2187. DOI: 10.1007/s10439-019-02310-4.
10. Trac S, Zheng R, Hill D, Lou E (2019). Intra- and inter-rater reliability of Cobb angle measurements on the plane of maximum curvature using ultrasonic imaging method. *Spine Deformity*, 7(1): 18–26. DOI: 10.1016/j.jspd.2018.06.015.
11. Chan A, E Parent, Lou E (2019). Reconstruction and positional accuracy of 3D ultrasound on vertebral phantoms for adolescent idiopathic scoliosis spinal surgery. *International Journal of Computer Assisted Radiology and Surgery*, 14(3): 427–439. DOI: 10.1007/s11548-018-1894-4.

Abstract Publications and Conference Presentations

1. Eberhardt J, Parent E, Shaker M, Su A, Shearer K, Lou E. Spinal curves and rotation in daily living posture measured using 3D ultrasound imaging in adolescent idiopathic scoliosis. SOSORT 2019, San Francisco, CA, USA (paper 24, p. 73).
2. Su A, Parent E, Goonasekera M, Lou E. The intra- and inter-evaluator reliability of frontal and rotational spinal measurements from 3D ultrasound imaging for adolescents with idiopathic scoliosis while performing exercises. SOSORT 2019, San Francisco, CA, USA (paper 64, p. 115).
3. Su A, Parent E, Fong E, Schreiber S, Moreau M, Lou E. Immediate frontal and transverse deformity reductions during Schroth physiotherapeutic scoliosis-specific exercise corrections in patients with adolescent idiopathic scoliosis. SOSORT 2019, San Francisco, CA, USA (paper 65, p. 116).
4. Gergelé, É, Parent E, Gross D. Accuracy of the Örebro musculoskeletal pain questionnaire for selecting successful interventions for workers with spinal conditions. Proceedings of the Rehabilitation Medicine Research Day, June 2019, Corbett Hall, University of Alberta, Edmonton, AB, Canada (poster).
5. Zeng H, Chen H, Lou E, Ta D, Zheng R. Segmentation of spinous process on ultrasound images based on gradient vector flow snake model – A preliminary study. 2019 International Congress on Ultrasonics, 3–6 October 2019, Bruges, Belgium.
6. Chen H, Zeng H, Lou E, Ta D, Zheng R. Improvement of the 3D ultrasound spine imaging technique using multiple plane interpolation and fast dot-projection methods. 2019 International Congress on Ultrasonics, 3–6 October 2019, Bruges, Belgium (pp. 1440–1443).
7. Chen H, Zheng R, Lou E, Ta D. Imaging spinal curvatures of AIS patients using 3DUS free-hand fast reconstruction method. 2019 IEEE Ultrasonics Symposium, 6–9 October 2019, Glasgow, Scotland.
8. Ng K, Lou E, Duke K, Hill D, Donaeur A, Tilburn M. A randomized clinical trial of 3D printed brace for the treatment of adolescent idiopathic scoliosis (AIS). Proceedings of SOSORT Annual Meeting, 24–26 April 2019, San Francisco, CA, USA.
9. Chan A, Parent E, Lou E. Accuracy of screw placement in 3D ultrasound-based spinal navigation in adolescent

idiopathic scoliosis phantom vertebrae. WCHRI Research Day 2019, 6 November 2019, Edmonton, AB, Canada (oral).

10. Khodaei M, Le LH, Parent E, Lou E. Prognosis factors of curve progression in adolescent with idiopathic scoliosis: A systematic review. WCHRI Research Day 2019, 6 November 2019, Edmonton, AB, Canada (oral).
11. Wong J, Reformat M, Lou E. Feasibility of using machine learning to aid clinicians to predict curve progression past moderate severity for children with adolescent idiopathic scoliosis. WCHRI Research Day 2019, 6 November 2019, Edmonton, AB, Canada (oral).
12. Pham TT, Le LH, Lou E. Correlating ultrasonic soft tissue-bone reflection coefficients with curve severity in adolescent idiopathic scoliosis: A preliminary study. WCHRI Research Day 2019, 6 November 2019, Edmonton, AB, Canada (poster).
13. Sayed T, Khodaei M, Lou E. Intra- and inter-rater reliabilities and accuracy of kyphotic angle measurements on ultrasound images for children with adolescent idiopathic scoliosis – A pilot study. WCHRI Research Day 2019, 6 November 2019, Edmonton, AB, Canada (poster).
14. Chan A, Parent E, Lou E. 3D-ultrasound navigation for pedicle screw insertion for posterior spine fusion surgery. Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff, AB, Canada (oral).
15. Chan A, Parent E, Lou E. Image registration of 3D ultrasound (3DUS) onto CT vertebral surfaces for pedicle screw navigation in adolescent idiopathic scoliosis (AIS) surgery. The 20th IMAST, 16–20 July 2019, Amsterdam, Netherlands (oral).
16. Chan A, Parent E, Mahood J, Lou E. Image registration of 3D ultrasound vertebral surfaces on CT vertebrae for pedicle screw navigation in idiopathic scoliosis surgery. Faculty of Engineering Graduate Symposium 2019, 3–5 July 2019, Edmonton, AB, Canada (poster).
17. Khodaei M, Le LH, Lou E. Association of ultrasound reflection signals with curve progression on children with AIS. Radiology Audit and Research Day 2019, 15 May 2019, University of Alberta, Edmonton, AB, Canada (oral).
18. Nguyen KCT, Kaipatur NR, Marques F, Lou E, Major P, Le LH. Inter-rater and intra-rater reliabilities of ultrasound imaging to measure the alveolar bone level in adolescents. Radiology Audit and Research Day 2019, 15 May 2019, University of Alberta, Edmonton, AB, Canada (oral).
19. Pham TT, Le LH, Lou E. Can the ultrasound echoes from spinal scans provide bone quality information? Radiology Audit and Research Day 2019, 15 May 2019, University of Alberta, Edmonton, AB, Canada (oral).
20. Ng K, Lou E, Duke K, Hill D, Donaeur A, Tilburn M. A randomized clinical trial of 3D printed brace for the treatment of adolescent idiopathic scoliosis (AIS). SOSORT Annual Meeting, 24–26 April 2019, San Francisco, CA, USA (oral).

Invited Presentations

1. Lou E. An artificial intelligent smart brace system for the treatment of adolescent idiopathic scoliosis. Advanced Biomedical Engineering and Instrumentation Summit 2019, 3–5 June 2019, San Francisco, CA, USA.
2. Parent E. Evidence based multidisciplinary approach to adolescent idiopathic scoliosis instructional course. American College of Rehabilitation Medicine, 4 November 2019, Chicago, IL, USA.
3. Parent E. Patient reported outcome measurements (as part of symposia): Toward a standardized minimum dataset for clinical practice and research. Proceedings of the 14th International SOSORT meeting 2019, 25–27 April 2019, San Francisco, CA, USA.
4. Parent E. Clinical assessment of patients with scoliosis. 1st International Conference on Scoliosis Management, 12–13 April 2019, Akıngüç Auditorium and Art Center, Istanbul Kültur University, Istanbul, Turkey.

Awards and Honors

1. The 2nd best oral presentation in the Women and Children's Health Research Institute Research Day, 6 November 2019
2. The best poster presentation in the Women and Children's Health Research Institute Research Day, 6 November 2019
3. The 2nd best oral presentation in the Alberta Biomedical Engineering Conference, 25–27 October 2019
4. Parent E – President Elect of the Society on Scoliosis Orthopaedic and Rehabilitation Treatment 2018–2019.

Research Funding

Western Economic Diversification Canada
AI-supercomputing Hub for Industry and Academic Collaboration (2018–2021).

Glenrose Rehabilitation Hospital Foundation
A new ultrasound imaging method to monitor hip dislocation for CP (2018–2020).

Development of 3D circuit board prototyping, wearable devices, and artificial intelligence system for children with scoliosis (2019–2022).

Federal Government of Canada (NSERC, CIHR, SSHRC) NCE award
A Centre of Excellence – Canadian Arrhythmia Network (2015–2020).

Alberta Cancer Foundation
Portable swallowing therapy unit: interfacing technology and rehabilitation medicine to provide accessible care for patients with chronic swallowing difficulties (2014–2019).

Scoliosis Research Society
Using ultrasound and health records to non-invasively identify cases with curve progression or not in children with AIS (2018–2020).

Prediction of curve progression in idiopathic scoliosis without treatment (2018–2020).

NSERC – Discovery Grant
3D ultrasound imaging and spatial pressure measurement system to investigate spinal curve response inside a brace (2015–2020).

Women and Children's Health Research Institute
Customized 3D printed spinal braces for the treatment of adolescent idiopathic scoliosis. Innovation grant (2016–2019).

The Northern Alberta Benefits Society for Scoliosis
Research into scoliosis (2018).

Physiotherapy Foundation of Canada (Ortho Canada)
The Adult Degenerative Scoliosis Exercise Trial (ADSET Pilot) (2019–2021).

The Edmonton Orthopaedic Research Committee
Advanced assessment and treatment for scoliosis (2018–2019).

Alberta Spine Foundation
Development of 3D ultrasound reconstruction program to assist orthopaedic surgeons to insert pedicle screws during spine surgery (2015–2020).

Sick Kids Foundation (SKF) CIHR IHDCYH
New investigator research grant – Multicentre Schroth exercise trial for scoliosis (Multicentre SETS study) (September 2013–December 2020).

Edmonton Civic Employees Clinical Research Grant
Long-term effects of Schroth scoliosis-specific exercises: A matched controlled study (2019–2020).

Alberta Ministry of Economic Development, Trade, and Tourism (Edmonton RCP-19-001-MIF)
Centre for Autonomous Systems in Initiative (2018–2023).

Faculty of Rehabilitation Medicine, University of Alberta
The adult idiopathic scoliosis exercise trial pilot. Mid-career stimulus grant (2020–2021).

International Mechanical Diagnosis and Therapy Research Foundation (IMDTRF)
Combined mechanical diagnosis and treatment and transforaminal epidural steroid injections versus usual care for chronic lumbar radiculopathy: An RCT. Research grant (January 2017–2019).

Training Highly-qualified Personnel in 2019

Ph.D. Graduate Students
- Andrew Chan (NSERC, AITF, TD Interdisciplinary, QE II, 75th Faculty of Medicine)
- Ahmed Badr (Egypt PhD Scholarship)
- Mahdieh Khodaie (Medical Sci. Graduate Scholarship, WCHRI)
- Tho Tran (AITF, WCHRI)
- Kim-Cuong Thi Nguyen (AITF)
- Thanh-Tu Pham
- Jason Wong (QE II, WCHRI, NSERC)
- Malik Alanazi (Saudi Bureau)

M.Sc Graduate Students
- Steffen Adria
- Daniel Crumback

Visiting Student (Masters)
- Éloi Gergerlé

MScPT Students
- J Eberhardt
- A Su

Orthopaedic Fellow
- Bigyan Bhandari

Orthopaedic Resident

- Teresa Li

Undergraduate Students

- Binh Le (Mitacs International)
- Brett Baker
- Brendan Coutts
- Solvin Sigurdson (NSERC USRA)
- Cameran Leung
- Tehzeeb Sayed
- Kennedy Tralnberg
- Jade Rosenberger
- Nicholas Yee
- Alexander Yee

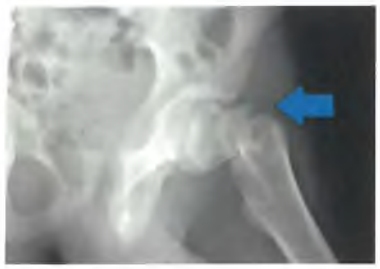
TRAUMA RESEARCH

Faculty: Dr L Beaupre, Dr M Menon, Dr R Stiegelmar, Dr D Weber

Affiliates: B Garib, Dr M Ferguson-Pell, Dr M Jacka, Dr A Jones, S Kang, Dr A Juby, Dr J Powell, Dr A Ramadi, Bone and Joint Health Strategic Clinical Network, Alberta Bone and Joint Health Institute, Canadian Collaborative for Hip Fracture

Hip Fracture Studies

Cost-effectiveness of hip fracture liaison services (FLS) in Alberta



The Alberta Bone and Joint Health Institute and the STOP-Fracture Research Team undertook a cost-effectiveness analysis of more than 1200 patients who were included in the initial implementation of the Hip-FLS (H-FLS) at two pilot sites in Alberta between June 2015 and March 2018. Osteoporosis medication following fracture was initiated in 60% of H-FLS patients relative to 21% receiving usual care (from our published work). Every 1000 H-FLS patients would experience 12 fewer hip fractures and 37 fewer total fragility fractures than patients receiving usual care. Despite the H-FLS costing > $1300/patient, over the study horizon, the H-FLS led to only a $54 incremental cost/patient with a modest gain of 8 QALYs/1000 patients. The incremental cost-effectiveness ratio (ICER) of $6,750/QALY gained was less than the $27,000 cost-effectiveness threshold. Eliminating the 9-month follow-up resulted in incremental savings of $218/patient while also reducing 6-month follow-ups increased cost-savings to $378/patient. A H-FLS implemented into standard practice significantly improved osteoporosis medication use to prevent future fractures and was cost-effective with potential to become cost-saving.

Impact of time to surgery on mortality after hip fracture: The HIP ATTACK randomized clinical trial

The HIP ATTACK RCT compared accelerated surgery to usual care to determine if this could reduce mortality and major complications. HIP ATTACK was performed at 69 hospitals in 17 countries, including the University of Alberta Hospital. Patients with a hip fracture, aged 45 years or older, were randomly assigned to either accelerated surgery (goal to perform surgery within 6 hours of diagnosis at hospital admission) or standard care. Co-primary outcomes of mortality and major complications within 90 days of randomization were assessed by evaluators blinded to group allocation. Over 27,000 patients were screened, of whom 7780 were eligible. 2970 of these were subsequently enrolled and randomized to receive accelerated surgery (n = 1487) or standard care (n = 1483). The median time from hip fracture diagnosis to surgery was 6 h (IQR 4–9) in the accelerated-surgery group and 24 h (10–42) in the standard-care group (p < 0.0001). Death occurred in 140 (9%) patients assigned to accelerated surgery, and 154 (10%) assigned to standard care died (p = 0.40). Major complications occurred in 321 (22%) accelerated-surgery and 331 (22%) standard-care participants (p = 0.71). Accelerated surgery for patients with hip fracture

did not significantly lower the risk of mortality or a composite of major complications compared with standard care. The University of Alberta contributed 30 participants to this trial, which was recently published in *The Lancet*.

An outreach rehabilitation program for nursing home residents after hip fracture may be cost-saving

We compared the cost-effectiveness of 10 weeks of outreach rehabilitation (intervention) versus usual care (control) for ambulatory nursing home residents after hip fracture. 77 participants were allocated in a 2:1 ratio to receive a 10-week rehabilitation program (intervention) or usual care (control) (46 intervention; 31 control). Using a payer perspective, we estimated incremental cost and incremental effectiveness. Groups were similar at study entry; the mean age was 87.9 ± 6.6 years, 54 (71%) were female, and 58 (75%) had severe cognitive impairment. Inpatient re-admissions were two times higher among controls, with an overall cost savings of $3350/patient for intervention participants over the first post-fracture year, offsetting the $2300/intervention participant for the outreach rehabilitation. The incremental cost/patient was –$621 for intervention participants. A 10-week outreach rehabilitation intervention for nursing home residents who sustain a hip fracture may therefore be cost-saving, through reduced post-fracture hospital re-admissions. These results, which support further work to evaluate post-fracture rehabilitation for nursing home residents were recently published in the *Journal of Gerontology*.

Other Hip Fracture Research

Regional versus general anesthesia for promoting independence after hip fracture surgery (REGAIN).

Analyses were carried out to determine impact probability of mortality of delays to surgery, admission and discharge settings, and hospital type on outcomes after hip fracture using Canadian Institute for Health Information (CIHI) data. Three manuscripts have been published, with several more under review or in preparation.

Trauma Studies

STOP-Fracture: A multi-pronged secondary fracture prevention initiative

As an extension of our 'Implementation Science' study, STOP-Fracture, we obtained further funding in 2019 through CIHR to undertake knowledge translation activities. In April, we held a provincial workshop with 40 in-person attendees and approximately 30 remote attendees to discuss the current Alberta programs and future work needed. As a result of that workshop, we created 2 arms of work.

In the first arm, we created an osteoporosis expert working group to develop 'Bone Health' modules on fall prevention, medication, nutrition and exercise. Using an expert in adult learning, we created 15 educational modules that can be utilized by Alberta healthcare providers in seniors' residential institutions and/or communities. The modules include PowerPoint presentations and patient educational tools and activities as well as evaluation materials. These modules will be housed at the Alberta Bone and Joint Health Institute for use on request by Alberta healthcare providers.

The second arm of work was to create a Bone Health 'app' prototype for patients. Following development of the prototype, a focus group was held with members of the Canadian Osteoporosis Patient Network to get their insights on how the prototype could be improved. We are currently working towards final development and commercialization of the app.

Infrapatellar versus suprapatellar reamed intramedullary nailing for fractures of the tibia (INSURT) RCT

The University of Alberta joined this multicentre, randomized, prospective trial in November 2018 and has randomized 34 patients to date into the trial, making it one of the leading enrollment sites. The trial is comparing two different approaches for IM nailing: infrapatellar and suprapatellar. Although both are currently used, few studies have directly compared their impact on knee pain while kneeling. Participants will be followed up to two years postoperatively.

Other Trauma Research

PRIHS Stop Fracture study. 3-arm study examining osteoporosis management after fragility fracture. This work is aligned with three strategic clinical networks: Bone and Joint Health, Seniors' Health, and Primary Care. Quantitative and qualitative evaluations are underway.

The effects of resistance training characteristics on patient outcomes after hip fracture. A systematic review and meta-analysis. This systematic review is examining which components of resistance training programs have the greatest impact on patient recovery of function after hip fracture.

Publications

1. Sheehan KJ, Fitzgerald L, Hatherley S, Potter C, Ayis S, Martin FC, Gregson C, Cameron ID, Beaupre L, Wyatt D, Lipczynska S, Sackley C (2019). Equity in rehabilitation interventions after hip fracture surgery: A systematic review age and ageing. *Age and Aging*, 48(4): 489–497 (IF 4.01) [published early online, 10 April 19]. DOI: 10.1093/ageing/afz031.
2. Beaupre LA, Soboley B, Guy P, Kuramoto L, Kim JD, Sheehan KJ, Bohm E, Morin SN, Sutherland JM, Dunbar M, Harvey E, Jaglal S, for The Canadian Collaborative Study of Hip Fractures (2019). Discharge destination following hip fracture in Canada among previously community-dwelling older adults, 2004–2012: Database study. *Osteoporosis International*, 30(7): 1383–1394 [published early online, 3 April 2019]. DOI: 10.1007/s00198-019-04943-6. PMID: 30937483.
3. Beaupre LA, Khong H, Smith C, Kang S, Evens L, Jaiswal P, Powell J (2019). The impact of time to surgery after hip fracture on mortality at 30- and 90-days: does a single benchmark apply to all? *Injury*, 50(4): 950–955 (IF 2.199) [published early view, 28 March 2019]. DOI: 10.1016/j.injury.2019.03.031. PMID: 30948037.
4. Beaupre LA, Magaziner JS, Jones CA, Jhangri G, Johnston DWC, Wilson D, Majumdar SR (2019). Rehabilitation after hip fracture for nursing home residents: A controlled feasibility trial. *The Journals of Gerontology: Series A*, 74(9): 1518–1525 (IF 4.902) [published online, 9 February 2019]. DOI: 10.1093/gerona/glz031. PMID: 30753303.
5. Sheehan KJ, Smith TO, Martin FC, Johansen A, Drummond A, Beaupre L, Magaziner J, Whitney J, Cameron ID, Price I, Hommel A, Sackley C (2019). Conceptual framework for an episode of rehabilitative care after surgical repair of hip fracture. *Physical Therapy*, 99(3): 276–285 (IF 2.799) [published online 23 January 2019]. DOI: 10.1093/ptj/pzy145. PMID: 30690532.
6. Neuman MD, Gaskins LJ., Montgomery B, Menio D, Long J, Fleisher LA., Beaupre L, Ahn J (2019). Feasibility and acceptability of a peer mentoring program for older adults following hospitalization for hip fracture. *Journal of the American Medical Directors Association*, 20(2): 218–220.e2 (IF 5.325) [published online 13 November 2018]. DOI: 10.1016/j.jamda.2018.09.038. PMID: 30446475.

Presentations

1. Menon MR, Beaupre L, Almaazmi K, Tsui B. Preoperative nerve blocks for hip fracture patients: A pilot randomized trial. Orthopaedic Trauma Association (OTA) Annual Meeting 2019, 25–28 September 2019, Denver, CO, USA. Selected as a top paper.
2. Almaazmi K, Menon MR, Tsui B, Beaupre L. Preoperative nerve blocks for hip fracture patients: A pilot randomized trial. Canadian Orthopaedic Resident's Association (CORA) Annual Meeting, June 2019, Montreal QC, Canada.

Research Funding

CIHR MPD Grant Institute of Aging
STOP-Fracture: A multi-pronged KT initiative.

Alberta Innovates
Health solutions strategies targeting osteoporosis to prevent recurrent fractures (STOP-Fracture Study).

Simon Fraser Research Fund (sub-contract)
Infrapatellar versus suprapatellar teamed intramedullary nailing for fractures of the tibia (INSURT) RCT.

KNEE RESEARCH

Faculty: Dr C Hui, Dr D Otto, Dr M Sommerfeldt

Affiliates: Dr J Jaremko, Dr S Adeeb, Dr M Chan, Dr L Westover, Dr H Rouhani, Dr V Abdollah, S Nathanail, J Sheehy, Glen Sather Sports Medicine Clinic

Residents & trainees: Dr J Bowes, Dr J Karpyshyn, Dr K Hickie, Dr M Clarke, Dr M McIntosh, AJ Baptiste Jr, L Graf-Alexiou, N Mohamed, Y Qui, R Fathian

NOVOCART® 3D

NOVOCART® 3D autologous chondrocyte implant system phase 3 prospective randomized clinical trial

We recently opened enrollment in this large, international, multicentre randomized clinical trial investigating a novel autologous chondrocyte implantation (ACI) product made by Aesculap Biologics, LLC. This system is a biologic-device combination product composed of ex vivo expanded autologous chondrocytes seeded on a bioresorbable biphasic collagen scaffold. This phase 3 clinical trial compares NOVOCART® 3D to microfracture in the treatment of articular cartilage defects of the knee. Dr Sommerfeldt is currently the only NOVOCART® 3D investigator in Alberta and one of few Canadian investigators.

A biomechanical comparison of graft preparation techniques for all-inside ACL reconstruction

The graft for the all-inside technique is typically constructed using the semitendinosus tendon due to its favourable biomechanical properties and the similar elasticity and force-elasticity curve to the native ACL. No studies have explored the added strength of a graft with secondary fixation sutures. We compared the biomechanical properties of four-stranded grafts prepared in five different configurations. The study results suggest the all-inside ACL graft with free ends secured to the adjustable tibial loop (secured end-to-end fixation) is biomechanically superior to the simple interrupted sutures technique. Adding secondary fixation to the tibial button did not significantly change the biomechanical properties. This study has been accepted for publication in a peer-reviewed journal and was scheduled for presentation at the Canadian Orthopaedic Association Meeting 2020.

Are pre- and postoperative activity levels as measured by a wearable sensor associated with return to play outcomes following anterior cruciate ligament reconstruction?

Measurement of activity levels can provide valuable information to the rehabilitation team about patient recovery and may improve the understanding of timing return to activity. Self-report measures of physical activity are heavily relied on in both clinical and research settings, but self-report data often differ from objective measurements of activity.

Wearable activity tracking technology has emerged as a tool to address these self-report concerns. Recent advances in wearable technologies provide novel opportunities to monitor progression through a participant's rehabilitation journey and can assist health care professionals in tailoring rehabilitation and activity prescriptions for each client. Currently, there is a paucity of research investigating participant physical activity levels in the time periods surrounding ACL reconstruction. Additionally, associations between preoperative and early postoperative activity levels and return to play outcomes are unknown. We plan to investigate the relationship between level of activity and participant return to play outcomes. We have secured funding for this study and are beginning patient recruitment.

Do novel individualized group-based neuromuscular training programs after anterior cruciate ligament reconstruction improve return to activity compared to usual care pathways in the Edmonton Zone?

Various rehabilitation delivery methods are available to patients following their ACL reconstruction (ACLR), but there is no available evidence supporting the most suitable post-ACLR rehabilitation program pathway for these patients. Rehabilitation sessions can be delivered via one-on-one clinician–patient treatments, in individualized group-based

(IGB) sessions, or in a home-based format. Our research question is: Does return-to-activity status at 12 months post-ACLR differ between individuals participating in IGB NMT return-to-activity programs versus those completing individual-based single-provider usual care (IUC) post-ACLR? We will also compare whether patient-reported and objective outcomes are improved in IGB programs versus IUC. This project is currently under review for grant funding.

Quantifying asymmetry and lower limb muscle function with force plate technology

Functional neuromuscular deficits may persist up to two years following ACL surgery, even after return to full activity/play has occurred. Current return-to-play criteria/assessments may not sufficiently evaluate the dynamic functional movement demands facing active individuals. Force plate systems have emerged as novel tools which accurately and objectively assess the neuromuscular system during dynamic functional tasks. In our previous work, engineering students created a mathematical tool to instantaneously analyse countermovement and non-countermovement jumps performed by participants on dual force plates. This preliminary work quantified lower limb asymmetries and aided dynamic assessment of lower limb muscle function in various healthy athletic populations. We are seeking funding to expand this work in the ACLR population; we hope to demonstrate the application of force plate technology as a tool to assist clinicians and patients in return-to-play/activity decisions.

Does topical antibiotic solution on anterior cruciate ligament autografts decrease rates of infection? A systematic review and meta-analysis

Septic arthritis is a rare but devastating complication following anterior cruciate ligament (ACL) reconstruction (ACLR). It often necessitates further surgery and IV antibiotic use, and may require removal of the ACL graft. The major source of bacterial contamination is from skin contact during graft harvesting. A novel strategy to potentially decrease the risk of infection in ACLR involves pre-soaking the autograft in an antibiotic-loaded solution prior to implantation. This systematic review determined that pre-soaking the ACL autograft in topical antibiotic solution appears to decrease infection rates, but further study is needed. This project was presented at the Canadian Orthopaedic Association Meeting 2019.

Other Knee Research

A biomechanical comparison of knee braces with application for posterolateral corner reconstructions. Multiple published studies support the operative repair or reconstruction of posterolateral corner injuries. Consensus data promote the use of knee orthoses postoperatively to protect the reconstruction or repair during rehabilitation, but as yet no specific type of brace has been established as superior. The purpose of this study is to determine the ability of various knee brace types to withstand varus compressive forces in a mechanical model. We have mechanically tested 6 different brace types and are analysing the results. This project was accepted for poster presentation at the Canadian Academy of Sport and Exercise Medicine Meeting 2020.

Epidemiology of knee injury and ACL reconstruction in Alberta, Canada. The current study aims to investigate knee injury epidemiology across the age continuum in both sexes. Data on distribution and risk factors of knee injuries is important to sports medicine physicians and orthopaedic surgeons, and in addition to improving clinical knowledge, may help to prevent, diagnose, and treat sports-related knee joint injuries. We are collaborating with the Injury Prevention Centre, part of the School of Public Health at the University of Alberta.

Tibial slope–adjusting osteotomies in revision anterior cruciate ligament reconstructions. Previous cadaveric and modelling studies have explored the relationship between the degree of posterior tibial slope and the function of the ACL. Performing a high tibial osteotomy to reduce the posterior tibial slope may reduce strain on the ACL graft. This procedure may be particularly important for those undergoing multiple revision ACL reconstructions; however, clinical evidence is sparse. This review aims to explore the implications of slope-adjusting osteotomies for patients with recurrent ACL tears.

Publications

1. Cavendish P, Everhart J, Peters N, Sommerfeldt M, Flanigan D (2019). Osteochondral allograft transplantation for knee cartilage and osteochondral defects: A review of indications, technique, rehabilitation, and outcomes. *Journal of Bone and Joint Surgery Reviews*, 7(6): e7. DOI: 10.2106/JBJS.RVW.18.00123.

Presentations

1. Zhao B, Tiessen J, Khan N, Kung J, Hui C, Sommerfeldt M. Does topical antibiotic solution on anterior cruciate ligament autografts decrease rates of infection? A systematic review and meta-analysis. Canadian Orthopaedic Association (COA) Annual Meeting, 19–22 June 2019, Montreal, QC, Canada (poster).

Invited Presentations

1. Sommerfeldt, M. Exercise as a primary and secondary prevention strategy for ACL injury and re-injury. Glen Sather Sports Medicine Clinic's Annual David Reid Day, September 2019 (oral).

Unpublished Presentations

1. Karpyshyn, J. Effect of diameter on failure rate of four-stranded anterior cruciate ligament grafts: Literature review. 2019 Orthopaedic Research Day, University of Alberta, Edmonton, AB, Canada. 1st prize literature review.

Posters

1. Qui Y, Westover L, Adeeb S, Nathanail S, Sepehri M, Hickie K, Sommerfeldt M. Investigating stiffness of knee braces in varus loading. Faculty of Engineering Undergraduate Research Day, December 2019, University of Alberta, Edmonton, AB, Canada.

Research Funding

Edmonton Civic Employees Charitable Research Award

A biomechanical comparison of graft preparation techniques for all-inside ACL reconstruction.

A biomechanical comparison of knee braces with application for posterolateral corner reconstructions.

Canadian Academy of Sports and Exercise Medicine Research Award

Are pre- and postoperative activity levels as measured by a wearable sensor associated with return-to-play outcomes following anterior cruciate ligament reconstruction?

ARTHROPLASTY RESEARCH

Faculty: Dr L Beaupre, Dr D Durand, Dr B Herman, Dr C Hui, Dr G Lavoie, Dr E Masson, Dr C Weeks

Affiliates: Dr A Jones, Dr S Adeeb, Dr M El-Rich, L Jasper, Dr J Spence, Dr A Vette, Dr M Funabashi, Dr A Ramadi

Residents & trainees: Dr T Boettcher, Dr B Congdon, Dr M Goplen, Dr A Negm, C Wayne

The effect of obesity and sarcopenic obesity on knee biomechanics and gait parameter in people with severe osteoarthritis

Individuals living with obesity are at increased risk for developing knee osteoarthritis (OA) compared to those of normal weight. Quantitative gait analyses via three-dimensional (3D) motion capture have shown that individuals living with obesity or OA have altered knee kinematics and kinetics compared to those of normal weight or those without OA. In addition, obesity with low muscle mass and high fat mass, termed sarcopenic obesity (SO), may create greater risk for poor postoperative outcomes than obesity alone. Sarcopenia is the age-related, progressive loss of skeletal muscle mass and strength, negatively affecting patients' function. Despite potential significant clinical implications, knee biomechanics and postoperative outcomes have not been evaluated among TKA candidates with SO. This pilot work will compare knee biomechanics in patients with Class II obesity only or with Class II SO to that in sex- and age-matched adults (within 5 years) who are normal/overweight (BMI 18.5–29 kg/m^2) and undergoing TKA. Walking and functional tasks (e.g. sit-stand-sit) will be assessed at 1 year post-TKA using the Computer-Assisted Rehabilitation ENvironment (CAREN) at the Glenrose Hospital. This work is supported by an Alberta Innovated Clinical Postdoctoral Fellowship.

Pre-surgical rehabilitation to improve outcomes after total joint arthroplasty in patients with depression or anxiety: A randomized feasibility trial

In 2017–2018, approximately 129,000 total joint arthroplasties (TJA) were performed in Canada, with more than 10,000

occurring annually in Alberta. Not all patients report postoperative improvements in pain and function, though, and pre-existing depression and anxiety in particular can negatively impact post-TJA outcomes. A local cohort study of more than 700 participants found an association with poorer patient-reported outcomes at 1, 3, and 6 months after TJA in patients reporting preoperative depressive/anxiety symptoms even after adjusting for age, sex, comorbidities, preoperative pain and function, and social support. Depression and anxiety are common in people with osteoarthritis, with 23% and 28% of patients suffering from lower limb osteoarthritis reporting depression and anxiety, respectively. This pilot randomized trial will determine the feasibility and outcomes of a pre-surgical rehabilitation program (PRP) for people awaiting TJA who have pre-existing depression/anxiety, with the goal of better preparing them for surgery. The program includes a postoperative follow-up to address concerns after discharge from hospital and encourage improved physical activity. We anticipate that TJA patients' recovery and outcomes will be improved by enhancing readiness for surgery through cognitive and behavioural pain- and self-management skills, and by introducing postoperative exercises and mobility approaches. This pilot trial is currently under review for funding.

Using Fitbits to improve physical activity after TKA

Although there is increased use of Fitbits by both researchers and patients, there is little research on the validity of Fitbits in older adults with mobility challenges, and none in the population of older adults following total knee arthroplasty (TKA). This study determined the concurrent validity of the Fitbit as compared to a validated research grade accelerometer, the SenseWear Armband (SWA), for measuring physical activity in older adults following TKA. At 3 months post-operatively, 47 patients wore both SWA and Fitbits for 5 days. Good correlation was observed between the measurement of steps (ICC = 0.79), average daily energy expenditures (EE) (ICC = 0.78), and average daily EE < 3 metabolic equivalents (METS) (ICC = 0.79). Fitbits tended to overestimate time spent in < 3 METS (ICC = 0.52) and underestimate time spent in the higher intensities (ICC = 0.56 for 3–6 METS and 0.42 for ≥ 6 METS). This study demonstrates that the Fitbit may be appropriate to measure steps and energy expenditure in older adults following TKA, but caution should be used when measuring time spent in specific activity intensities.

Other Arthroplasty Research

Using press-fit TKA in younger, high-demand patients. A retrospective review of patients who have undergone press-fit TKA to determine clinical outcomes after TKA such as survivorship, aseptic loosening, pain, revision surgery (if applicable), and postoperative complications within 1 year of surgery.

Gait in patients with a medial pivot TKA vs those with a fixed-bearing TKA. A randomized, comparative trial to demonstrate that total knee arthroplasty (TKA) performed using medial pivot TKA is superior to standard TKA prostheses in terms of knee biomechanics (knee kinematics and kinetics) and spatio-temporal gait parameters at 1 year post-TKA.

Reducing OR door–opening through staff education. Pre/post evaluation of reducing OR door–opening during TJA using a multi-modal educational intervention.

Impact of steroid injection in the hip on development of avascular necrosis. We are investigating the incidence of AVN following hip cortisone injections using population-based administrative health data.

Vanguard TKA RCT. Comparison of fixed-bearing and mobile-bearing TKA. Long-term follow-up (to 10 years) is continuing.

Coaching for older adults (with OA) for community health (COACH Study). Impact of a telephone intervention on improving physical activity after TKA.

Survivorship of THA in younger patients: A systematic review. This review will consolidate current evidence on outcomes, including survivorship of younger patients undergoing THA.

Publications

1. Goplen CM, Randall J, Kang SH, Vakilian F, Jones CA, Voaklander DC, Beaupre LA (2019). The influence of refill gaps on the prevalence of long-term opioid therapy among patients with knee arthritis using administrative data. *Journal of Managed Care & Specialty Pharmacy*, 25(10): 1064–1072. DOI: 10.18553/jmcp.2019.25.10.1064.
2. Chen SK, Voaklander D, Perry D, Jones CA (2019). Falls and fear of falling in older adults with total joint arthroplasty: A scoping review. *BMC Musculoskeletal Disorders*, 20(1): 599. DOI: 10.1186/s12891-019-2954-9.
3. Goplen CM, Verbeek W, Kang SH, Jones CA, Voaklander DC, Churchill TA, Beaupre LA (2019).

Preoperative opioid use impacts patient outcomes after total joint arthroplasty: A systematic review and meta-analysis. *BMC Musculoskeletal Disorders*, 20(1): 234. DOI: 10.1186/s12891-019-2619-8, PMID: 31103029.

4. Beaupre LA, Hammal F, DeSutter C, Stiegelmar R, Masson E, Finegan BA. (2019). Impact of a standardized referral to a community pharmacist-led smoking cessation program before elective joint replacement surgery. *Tobacco Induced Diseases*, 17 (February): 14. DOI: 10.18332/tid/101600.
5. Kolaczek S, Hewison C, Caterine S, Berardelli R, Beveridge T, Herman B, Hurtig M, Gordon K, Getgood A (2019). 3D strain in native medial meniscus is comparable to medial meniscus allograft transplant. *Knee Surgery, Sports Traumatology, Arthroscopy*, 27: 349. DOI: 10.1007/s00167-018-5075-3.
6. King LK, Marshall DA, Farris P, Woodhouse L, Jones CA, Noseworthy T, Bohm E, Dunbar M, Hawker GA (2019). Use of recommended non-surgical knee osteoarthritis management in patients prior to total knee arthroplasty: A cross-sectional study. *Journal of Rheumatology*, jrheum.190467. DOI: 10.3899/jrheum.190467.
7. Bahari H, Vette AH, Hebert JS, Rouhani H (2019). Predicted threshold against forward and backward loss of balance for perturbed walking. *Journal of Biomechanics*, 95: 109315. DOI: 10.1016/j.jbiomech.2019.109315.
8. Roberts BWR, Hall JC, Williams AD, Rouhani H, Vette AH (2019). A method to estimate inertial properties and force plate inertial components for instrumented platforms. *Medical Engineering & Physics*, 66: 96–101. DOI: 10.1016/j.medengphy.2019.02.012.

Presentations

1. Goplen CM, Jones CA, Voaklander D, Churchill T, Beaupre LA. Preoperative opioid use negatively impacts one-year outcomes after total knee arthroplasty. Canadian Orthopaedic Association (COA) Annual Meeting, 19–22 June 2019, Montreal, QC; Canada. Canadian Orthopaedic Resident's Association (CORA) Annual Meeting, June 2019, Montreal, QC, Canada.
2. Boettcher T, Jones CA, Macleod R, Kang SK, Beaupre LA. Effect of preoperative depression on patient-reported function and pain post–total joint arthroplasty (total hip/knee arthroplasty). Canadian Orthopaedic Association (COA) Annual Meeting, 19–22 June 2019, Montreal, QC, Canada. Canadian Orthopaedic Resident's Association (CORA) Annual Meeting, June 2019, Montreal QC, Canada.
3. Park E, Jones A, Forhan M. Hearing patients' voices: Including patient perspectives for meaningful interactions. 10th International Shared Decision Making Conference, 8–10 July 2019, Quebec City, QC, Canada. Abstract: https://fourwaves-sots.s3.amazonaws.com/static/media/uploads/2019/06/28/isdm2019-oralsessionsbooklet-2019-06-28.pdf (no. 275, p. 107).
4. Park E, Jones A, Forhan M. Living with osteoarthritis and obesity: Patients' healthcare experiences. Canadian Association of Occupational Therapists Conference, 29 May–1 June 2019, Niagara Falls, ON, Canada.
5. Park E, Jones A, Forhan M. Hearing patients' voices: Including patient perspectives through digital storytelling. Alberta Strategies for Patient-Oriented Research (AbSPOR), 13–15 May 2019, Summer Institute, Edmonton, AB, Canada.
6. Bahari H, Forero J, Hebert JS, Vette AH, Rouhani H. Assessment of gait stability during perturbed walking. 27th Congress of the International Society of Biomechanics (ISB 2019), 31 July–4 August 2019, Calgary, AB, Canada.
7. Noamani A, Riske S-B, Vette AH, Rouhani H. Balance evaluation of elderly fallers using wearable inertial sensors: A clinical study. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff, AB, Canada.
8. Forero J, Hall J, Vette AH, Hebert JS. A performance assessment tool quantifies high-level balance abilities in normative and impaired participants with the CAREN. Forum of the Canadian Institute for Military and Veteran Health Research (CIMVHR 2019), 21–23 October 2019, Ottawa-Gatineau, ON, Canada.
9. Noamani A, Nazarahari A, Vette AH, Rouhani H. Evaluation of wearable inertial sensors for quantification of standing balance. 27th Congress of the International Society of Biomechanics (ISB 2019), 31 July–4 August 2019, Calgary, AB, Canada.
10. Vette AH, Hall JC, Forero J, Hebert JS. Application and evaluation of the extrapolated centre of mass as a clinical gait stability measure. World Congress of the International Society of Posture and Gait Research (ISPGR), 30 June–4 July 2019, Edinburgh, Scotland.
11. Hall JC, Roberts BWR, Williams AD, Rouhani H, Vette AH. Quantifying inertial properties and force plate inertial components in instrumented platforms. 27th Congress of the International Society of Biomechanics (ISB 2019), 31 July–4 August 2019, Calgary, AB, Canada.

Research Funding

Diabetes, Obesity and Nutrition SCN
The effect of obesity and sarcopenic obesity on knee biomechanics and gait parameter in people with severe osteoarthritis.

Covenant Health Foundation
Impact of an educational intervention to reduce OR door–openings during surgery.

Division of Surgical Research Clinical Research Grant
The impact of preoperative opioids on patient-reported outcomes following primary elective total knee arthroplasty.

Biomet Canada
Clinical evaluation of the Vanguard deep dish rotating platform knee.

NESH-W Innovation Grant
Coaching for older adults (with OA) for community health (COACH).

FOOT & ANKLE RESEARCH

Faculty: Dr L Beaupre, Dr G Goplen, Dr N Jomha, Dr P Leung, Dr E Pedersen, Dr A Scharfenberger

Affiliates: Dr S Adeeb, Dr S Dhillon, Dr M El-Rich, Dr M Funabashi, Dr A Ramadi

Residents & trainees: Dr J Bowes, AJ Baptiste Jr, Dr M Goplen, Dr L Heinrich, Dr T Li, Dr A Perreault, J Sun, R Sun, D Stone, K Vats

Universal talus development

We continue to develop a universal talar bone replacement and published a landmark study this past year. Talar avascular necrosis can be a devastating complication mainly following trauma or steroid use, or it can occur idiopathically (Fig. 5).

Standard treatment is a tibiotalar–calcaneal fusion, which provides reasonable pain relief but creates a stiff ankle and hindfoot that may limit function. A newer alternative is a custom talar bone replacement. This has provided reasonably good long-term results in a small number of patients but can be technically difficult, time-consuming and costly to manufacture. We have been developing a universal talar prosthesis. Our research has shown that tali are very similar in shape, even among people of the opposite sex, with the main variation being scale. With this knowledge, we began developing a talus bone replacement with a range of sizes of the same shape. These could be manufactured in bulk to both improve access to this treatment and decrease costs. After implanting 6 universal talar prostheses with good results (Fig. 6), we published a paper in 2019 on the first universal, non-custom talus bone replacement in the world. In fact, the paper reports on two implants because the patient required replacement of both talus bones. This was a very exciting event for our team and has spurred us on to now refine the shape of the talar implant to decrease the impact load exerted by the metal implant on the normal cartilage and bone. We will also investigate materials to reduce contact loads against normal cartilage and bone. We are very excited about translating this research into the next generation of talar bone implants.

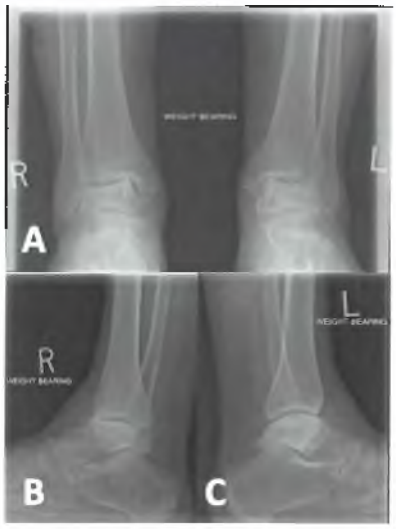


Figure 5. Preoperative bilateral AP and lateral radiographs of ankles showing significant talar collapse due to talar avascular necrosis.

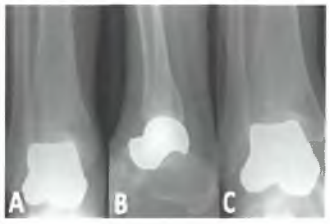


Figure 6. Three radiographic views after implantation of the first universal talar replacement.

Pain management for complex elective hind-foot and ankle surgery

Elective complex foot and ankle surgery (e.g. ankle and hind-foot fusion or correction of hind-foot deformities) are common orthopaedic procedures performed at the University of Alberta Hospital. Persistent postoperative pain (PPP) is common after

these procedures, and opioid medications form part of the multimodal regime for postoperative pain management. In 2019 we continued this work, completing the retrospective analysis and pursing development of a consensus statement.

Retrospective data review:
We performed a retrospective chart review and administrative data review using the Pharmacy Information Network to examine pre-, peri-, and post-surgical opioid consumption in 88 patients. Of these patients, over half (n = 45) had used opioid medication preoperatively, with 19 (22%) being long-term opioid users before their surgery; general practitioners prescribed 78% of these opioids. Despite undergoing similar surgical procedures (p > 0.36), the group exposed to opioids preoperatively and the long-term users both consumed significantly more opioids during their hospital stay (p = 0.004) and were more likely to be discharged to rehabilitation facilities (p = 0.02). Further, no patients who were opioid-naïve preoperatively became long-term opioid users postoperatively, while 13 (68%) preoperative long-term opioid users remained long-term opioid users postoperatively. These results support the need for patient and provider education regarding preoperative opioid use. This work is being prepared for publication.

North American survey and Delphi survey

We also surveyed North American foot and ankle surgeons with (n = 116 respondents across North America) to determine current practices in pain management.

We are currently in the process of developing consensus guidelines for pre-, peri- and postoperative management of patients undergoing complex foot and ankle surgery. A multidisciplinary steering group (n = 10) and an expert panel (n = 35) were created to undertake a Delphi consensus process. 1405 preliminary statements regarding important principles of perioperative pain management (from preoperative surgeon encounter to 6 months postoperatively) are being synthesized with the consensus process, and guideline development will be completed by Summer/Fall 2020.

Ankle stiffness after malleolar fracture

We completed a longitudinal cohort study on 155 patients who underwent open reduction and internal fixation to examine return to activity postoperatively. Our work examined a) how 'stiffness' could best be defined clinically and b) factors associated with return to activity within 1 year, including the impact of 'stiffness'. Overall, only 52% of participants indicated that they had returned to activity at their normal level within 12 months of ankle fracture.

Our first work demonstrated that responses to a simple question put to patients about whether they were stiff or not correlated well with at least a 2-centimetre side-to-side discrepancy on the weight-bearing lunge test (κ = 0.39), and that stiffness measured by this side-to-side discrepancy was common over time, with 98%, 80%, and 72% of patients reporting this level of stiffness at 6 weeks, 6 months and 1 year postoperatively, respectively.

However, in our longitudinal analysis, using simply the ratio lunge (affected lunge/unaffected lunge) proved to be the most effective means clinically to determine who would return to activity within 1 year of hip fracture (p = 0.03). These works are in preparation for publication in a peer-reviewed journal.

Other Foot and Ankle Research

Foot and ankle injuries in the WCB patient population. Administrative review of the prevalence of foot and ankle injuries in the working population and factors associated with return to work using WCB data.

Publications

1. Bowes J, Adeeb S, Grosvenor A, Beaupre L, Jomha NM (2019). Development and implantation of a universal talar prosthesis. *Frontiers in Surgery*, 6: 63. DOI: 10.3389/fsurg.2019.00063.
2. Stiegelmar C, Li Y, Beaupre L, Pedersen ME, Dillane D, Funabashi M (2019). Perioperative pain management and chronic postsurgical pain after elective foot and ankle surgery: A scoping review. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie*, 66: 953–965. DOI: 10.1007/s12630-019-01370-3 [Epub ahead of print].

Presentations

1. Li T, Stiegelmar C, Funabashi M, Dillane D, Beaupre L, Pedersen ME. Chronic postoperative pain after elective foot and ankle surgery: A scoping review. Canadian Orthopaedic Association (COA) Annual Meeting.

19–22 June 2019, Montreal, QC, Canada. Canadian Orthopaedic Resident's Association (CORA) Annual Meeting, June 2019, Montreal, QC, Canada.

2. Liu T, Jomha N, Westover L, Adeeb S. The Investigation of the average shape and variations of the human talus bone. Proceedings of the XXVII Congress of the International Society of Biomechanics (ISB) and the 43rd Annual Meeting of the American Society of Biomechanics (ASB), 31 July–4 August 2019. Calgary, AB, Canada.
3. Adeeb S, Palizi M, Algohary Y, Trovato A, Islam K, Kim M, Jomha N. Geometry and mechanics of diarthrodial joints with emphasis on the talus joint. Proceedings of the 16th International Symposium on Computer Methods in Biomechanics and Biomedical Engineering, 14–16 August 2019, New York, NY, USA.
4. Sun R, Baptiste AJ Jr, Westover L, Adeeb S, Jomha N. Wear on articulating cartilage by titanium alloy and cartilage. 60th Annual National Student Research Forum, 18–19 April 2019, Galveston, TX, USA.

Invited Presentations

1. Jomha NM. Foot and ankle biomechanics. The Tom Smallman Canadian Orthopaedic Association Basic Science Course, 21 October 2019, Ottawa, ON, Canada.

Research Funding

WCB Alberta/ECE Foundation
Ankle stiffness after malleolar fracture: A study of incidence and predictive factors.

American Foot and Ankle Society
Development of best practices: Guidelines for pain management after complex foot and ankle surgery.

Surgery SCN Seed Grant
Opioid usage after complex foot and ankle surgery: Current prescriber and patient practice patterns.

UPPER EXTREMITY RESEARCH

Faculty: Dr A Badre, Dr R Balyk, Dr L Beaupre, Dr J Bergman, Dr M Bouliane, Dr J Bury, Dr R Chan, Dr M Furey, Dr R Glasgow, Dr A Lalani, Dr M Lapner, Dr D Sheps

Affiliates: Dr S Adeeb, Dr J Chepeha, Dr T deFreitas, Dr S Dhillon, Dr J Jaremko, C Luciak-Corea, Dr H Rouhani, A Silveira, F Styles-Tripp

Residents & trainees: Dr A AlEidan, Dr T Bornes, Dr. C Bouchard, Dr J Bowes, Dr D Durham, Dr G Harding, J Luk, Dr S Westberg, H Zielinski

Early functional return to work following distal biceps repair

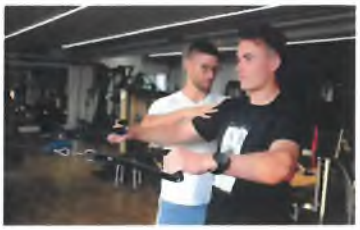
This randomized trial compared early mobilization to standard 6-week postoperative immobilization post-distal biceps tendon rupture (DBTR). 102 male participants with DBTR using endobutton technique were randomized to early mobilization (EM) (n = 50; self-weaned from sling and performed pain-free active ROM during the first 6 weeks) or to standard immobilization (SI) (n = 52; wore a splint for 6 weeks with no active ROM). EM participants had significantly more passive forearm supination, passive forearm pronation, and passive forearm extension than SI participants at early assessments. EM participants had significantly better quick-DASH scores over time compared to SI. No group differences occurred in return to work, pain, ROM, or strength. Two (3%) participants (1 EM and 1 SI) had a full-thickness tear at ultrasound testing 12 months postoperatively. Early motion after distal biceps tendon repair is safe, but does not appear to have clinically important benefits. This work has been submitted for publication.

Reoperation rates in surgically managed olecranon fractures

Recent heterogeneous data suggest that the hardware removal rate related to implant prominence following plate fixation of

the olecranon is between 17% and 54%. This study determined the reoperation rate following plate fixation of the olecranon with contoured anatomic plates in the Edmonton Zone. 600 surgically treated olecranon patients were identified, with 325 patients undergoing plate fixation. Chart review determined 90 (28%) patients had reoperations. Reoperation due to hardware prominence was 16% while hardware failure (5.3%), infection (2.8%), and contracture (2.8%) were less common. Patients undergoing reoperation had a higher incidence of type III olecranon fractures (17% vs 8%) and Monteggia pattern injuries (14% vs 5%). Compared to previous reports, we found lower rates of reoperation with contoured anatomic plating. This work is currently being prepared for submission to a peer-reviewed journal.

Appropriate and efficient management of shoulder injuries: Who needs surgery?

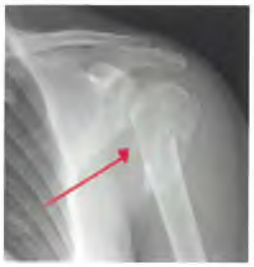


We determined if a decision rule, as part of a standardized rehabilitation program, could detect patients who require early surgical consult and which patient characteristics were associated with needing surgery. Of 143 participants, 32 (22%) required surgical consult with 16 (11%) going on to have surgery. Rehabilitation was effective for the remaining participants (78%). The need for surgery was associated with full-thickness rotator cuff tears, increased medication use, older age, short-term disability, decreased range of motion (ROM), and decreased external rotation strength. Initial rehabilitation using a decision rule detected those who required surgical consult for generalized shoulder pathology; most patients recovered with rehabilitation. This work is being prepared for submission to a peer-reviewed journal.

Impact of a standardized rehabilitation program for patients with shoulder pathology

This study examined the impact of a standardized rehabilitation program on pain, ROM, strength, and health related quality of life (HRQL). The rehabilitation program improved pain, ROM, strength, WORC, and quick-DASH scores over 6 months. Most participants (89%) improved with rehabilitation. Appropriate shoulder rehabilitation can improve pain, ROM, strength, and HRQL within 6 months for the large majority of patients presenting with generalized shoulder pathology. This work will also be submitted for publication.

Radiographic parameters that could predict outcome of proximal humerus ORIF



We evaluated mechanical features of proximal humerus fracture fixation that enhance stability in patients treated with ORIF using a locking plate. Loss of reduction was seen in 79 of 355 (22%) patients. Significant loss of reduction was associated with increasing age, increasing fracture severity (4-part compared to 2-part fracture), and poor head–shaft angle (HSA) alignment. Patients achieving shaft impaction (SI), shaft medialization (SM), and calcar reduction (CR) were significantly less likely to experience loss of reduction, even after controlling for age, fracture severity, and HSA alignment. SI was the most important factor to achieve. Loss of reduction increased reoperation rate (34% vs 7%). This work is under review at a peer-reviewed journal.

Other Upper Extremity Research

Marijuana use patterns in Canadian upper-extremity patients at the time of legalization. This study, conducted by the Wrist Evaluator Canada (WECAN) research group, aims to better understand marijuana consumption practices among upper-extremity patients presenting to an orthopaedic clinic. The Western Upper Limb Facility (WULF) contributed 192 surveys to Phase 1 (2019). We plan to repeat the survey in September 2020 to determine if marijuana use increases with legalization and increased access.

Radiographic assessment of ulnar shortening osteotomy: The Canadian experience. This Canada-wide collaboration through the Wrist Evaluators Canada (WECAN) research group will retrospectively evaluate the heterogeneity of surgical techniques (e.g. types and location of osteotomy, free-hand vs jig-based osteotomy, etc.) and their impact on radiographic outcomes.

The use of intra-articular corticosteroid injection to treat osteoarthritis of the carpometacarpal joint: A randomized control trial. This randomized clinical trial (RCT) will compare 6-month outcomes following fluoroscopic-guided intra-articular corticosteroid or saline injections in adult patients with primary carpometacarpal osteoarthritis (OA).

Management of post-traumatic elbow contractures (PERK II). Led by Dr K Hildebrand from Calgary, Prevention of post-traumatic contractures with ketotifen II (PERK II) is a phase 3 randomized multicentre trial examining post-traumatic elbow contracture using 3 parallel groups (ketotifen 2 mg or 5 mg, or lactose placebo twice daily orally for 6 weeks). The Edmonton groups aims to recruit 23 patients over 2 years.

Four-corner vs three-corner vs capitolunate arthrodesis for post-traumatic wrist arthroplasty: A systematic review of outcomes. This systematic review provides a comprehensive background for a multicentre randomized controlled trial to address the current evidence gap regarding the appropriate partial fusion technique (four-corner, three-corner, or capitolunate) for patients with debilitating post-traumatic wrist arthritis.

Randomized comparison of partial wrist fusion with or without triquetral excision. This national multicentre double-blind RCT will compare clinical and radiographic outcomes of four-corner arthrodesis (no triquetral excision) to three-corner and capitolunate arthrodesis with triquetral excision.

Biomechanical evaluation of different partial wrist fusion techniques on radiolunate load and contact. Triquetral excision has been recently advocated to improve motion and grip strength in partial wrist fusion to manage patients with debilitating post-traumatic wrist arthritis. This biomechanical study will examine the load and contact across the radiolunate articulation with triquetral excision.

US/MRI evaluation of interosseous membrane in anterior Monteggia fracture–dislocations. Recent biomechanical studies found that the interosseous membrane plays a significant role in radial head instability in anterior Monteggia injuries, but its role in acute and chronic injuries is unclear. We will examine how interosseous membrane integrity in acute and chronic Monteggia injuries affects clinical outcomes.

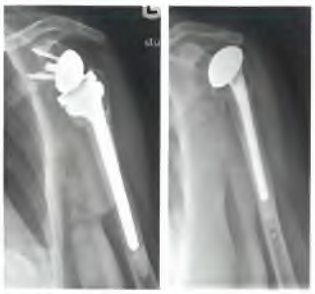
Systematic scoping review of rehabilitation following arthroscopic Bankart repair. We investigated evidence for postoperative rehabilitation following Bankart repair. There was a paucity of evidence investigating the impact of rehabilitation approaches after arthroscopic Bankart repair strength and return to activity/work. This work has been prepared for submission to a peer-reviewed journal.

The impact of using a shoulder-specific clinical pathway among WCB patients with rotator cuff–related pathology (RCRP). This project will assess the impact of the Alberta Workers' Compensation Board's shoulder pathway in order to better manage patients with RCRP as compared to usual care. We believe that using a shoulder-specific clinical pathway will expedite recovery and decrease health care use.

Exercise therapy (ET) for chronic rotator cuff (RC)–related pathology: A systematic review and network analysis. This network meta-analysis aims to answer the questions: 1) Is ET targeting scapula and rotator muscles more effective compared with ET targeting only rotator cuff muscles?; 2) How long should ET continue?; 3) Do adjunct therapies provide additional benefits?; and 4) Which patients benefit from ET?

Patient-specific bone grafting in reverse total shoulder arthroplasty in patients with large glenoid defects: A pilot study. This study will determine if a novel three-dimensional (3D) patient-specific cutting guide for glenoid bone grafts can establish accurate glenoid baseplate positioning without increasing postoperative complications.

Glenoid reconstruction study. This study will evaluate the clinical and anatomic outcomes utilizing a novel double suture button fixation system that eliminates and simplifies arthroscopic distal tibial bone grafting of the anterior glenoid. The trial is led by Ian Lo (University of Calgary); SURGE will contribute 15 participants.



Total shoulder arthroplasty vs reverse shoulder arthroplasty. This study will compare disease-specific quality of life in patients with glenohumeral osteoarthritis (OA) who a) are 65 years of age or older and undergo anatomic total shoulder arthroplasty (TSA) or a reverse shoulder arthroplasty (RSA), or b) have more severe deformities of the glenoid and undergo a TSA with either a posterior augmented glenoid component, or an RSA with bone graft. The primary outcome measure will be the Western Ontario Osteoarthritis of the Shoulder Index (WOOS) score at 24 months postoperatively.

Publications

1. Chepeha J, Silveira A, Sheps D, Beaupre LA (2020). Evaluating the uptake and acceptability of standardized postoperative rehabilitation guidelines using an online approach. *Physical Therapy*, 100(2): 225–237. Available online 21 November 2019, DOI: 10.1093/ptj/pzz161.

2. Sheps D, Silveira A, Beaupre L, Styles-Tripp F, Balyk R, Lalani A, Glasgow R, Bergman J, Bouliane M (2019). Early active motion versus sling immobilization after arthroscopic rotator cuff repair: a randomized controlled trial. *Arthroscopy*, 35(3): 749–760. DOI: 10.1016/j.arthro.2018.10.139.
3. Edwards A, Chepeha J, Jones CA, Sheps DM, Beaupre L (2019). Can clinical assessment differentiate partial thickness rotator cuff tears from full thickness rotator cuff tears? A secondary analysis. *Disability and Rehabilitation*. Published online 9 February 2019, DOI: 10.1080/09638288.2018.1563637.
4. Dehghan N, Furey MJ, Schemitsch L, Ristevski B, Goetz T, Schemitsch EH, Canadian Orthopaedic Trauma Society (COTS), McKee MD (2019). Long-term outcomes of total elbow arthroplasty for distal humerus fracture: Results from a prior randomized clinical trial. *Journal of Shoulder and Elbow Surgery*, 28(11): 2198–2204. DOI: 10.1016/j.jse.2019.06.004.
5. Badre A, Axford DT, Banayan S, Johnson JA, King GJW (2019). The effect of torsional moments on the posterolateral rotatory stability of a lateral ligament deficient elbow: An in vitro biomechanical investigation. *Clinical Biomechanics*, 67: 85–89. DOI: 10.1016/j.clinbiomech.2019.05.002.
6. Badre A, Axford DT, Banayan S, Johnson JA, King GJW (2019). Role of the anconeus in the stability of a lateral ligament and common extensor origin-deficient elbow: An in vitro biomechanical study. *Journal of Shoulder and Elbow Surgery*, 28(5): 974–981. DOI: 10.1016/j.jse.2018.11.040.

Presentations

1. Chan R, Silveira A, Bowes J, Westberg S, Beaupre L, Lapner M. Distal radius fractures in the late middle-aged: Surgical or conservative treatment – Feasibility of a randomized control trial. 2019 American Society for Surgery of the Hand (ASSH), 5–7 September 2019, Las Vegas, NV, USA.
2. Silveira A, Luk J, Tan M, Kang SH, Sheps D, Bouliane M, Beaupre L. Early active mobilization following rotator cuff repair: A meta-analysis of the current evidence. International Congress of Shoulder and Elbow Surgery (ICSES) 2019, 17–20 September, Buenos Aires, Argentina.
3. Badre A, Banayan S, Axford DT, Johnson JA, King GJW. Role of an adjustable hinged elbow orthosis in the rehabilitation of a lateral collateral ligament deficient elbow: An in vitro biomechanical study. 14th IFSSH (International Federation of Societies for Surgery of the Hand) Triennial Congress, 17–21 June 2019, Berlin, Germany.
4. Badre A, Axford DT, Banayan S, Johnson JA, King GJW. The effect of torsional moment of forearm weight on the posterolateral rotatory instability of a lateral collateral ligament deficient elbow: A novel biomechanical modeling and in vitro investigation. 14th IFSSH (International Federation of Societies for Surgery of the Hand) Triennial Congress, 17–21 June 2019, Berlin, Germany.
5. Badre A, Axford DT, Banayan S, Johnson JA, King GJW. Role of anconeus in the stability of a lateral ligament deficient elbow: An in vitro biomechanical study. 14th IFSSH (International Federation of Societies for Surgery of the Hand) Triennial Congress, 17–21 June 2019, Berlin, Germany.
6. Badre A, Perrin M, Albakri K, Suh N, Lalone E. Gender differences in distal radius morphology and its effect on the anatomic fit of standard volar locking plates. Canadian Orthopaedic Association (COA) Annual Meeting, 19–22 June 2019, Montreal, QC, Canada.
7. Badre A, Axford DT, Banayan S, Johnson JA, King GJW. The effect of torsional moments to the forearm on the posterolateral rotatory instability of a lateral collateral ligament–deficient elbow. A novel biomechanical modeling and in vitro investigation. Canadian Orthopaedic Association (COA) Annual Meeting, 19–22 June 2019, Montreal, QC, Canada.
8. Perrin M, Lalone E, Suh N, Badre A. Analysis of three-dimensional anatomical variance and fit of the distal radius to current volar locking plate designs. 2nd Meeting of the International Combined Orthopaedic Research Societies (ICORS), 19–22 June 2019, Montreal, QC, Canada.
9. Badre A, Banayan S, Axford DT, Johnson JA, King GJW. Role of an adjustable hinged elbow orthosis in the rehabilitation of a lateral collateral ligament–deficient elbow: An in vitro biomechanical study. 2nd Meeting of the International Combined Orthopaedic Research Societies (ICORS), 19–22 June 2019, Montreal, QC, Canada.
10. Badre A, Axford DT, Banayan S, Johnson JA, King GJW. Role of anconeus in the stability of a lateral ligament–deficient elbow: An in vitro biomechanical study. 2nd Meeting of the International Combined Orthopaedic Research Societies (ICORS), 19–22 June 2019, Montreal, QC, Canada.

Posters

1. Silveira A, Luk J, Tan M, Kang SH, Sheps D, Bouliane M, Beaupre L. Early active mobilization following rotator cuff repair: A meta-analysis of the current evidence. This is Public Health Week, 4–8 November 2019, University of Alberta, Edmonton, AB, Canada.
2. Badre A, Axford DT, Banayan S, Johnson JA, King GJW. Role of anconeus in the stability of a lateral ligament–deficient elbow: An in vitro biomechanical study. International Congress of Shoulder and Elbow Surgery (ICSES) 2019, 17–20 September, Buenos Aires, Argentina.
3. Badre A, Banayan S, Axford DT, Johnson JA, King GJW. Role of an adjustable hinged elbow orthosis in the rehabilitation of a lateral collateral ligament–deficient elbow: An in vitro biomechanical study. International Congress of Shoulder and Elbow Surgery (ICSES) 2019, 17–20 September, Buenos Aires, Argentina.
4. Axford DT, Banayan S, Johnson JA, Badre A, King GJW. The effect of brace hinge angle on the rehabilitation of a lateral collateral ligament–deficient elbow: An in vitro biomechanical study. Ontario Biomechanics Conference 2019, 8–10 March 2019, Alliston, ON, Canada.

Unpublished Presentations

1. Hickie K, Beaupre L, Sheps D, Chan R, Silveira A, Bergman J, Lalani A, Lapner M. Early functional return to work. 2019 Orthopaedic Research Day, University of Alberta, Edmonton, AB, Canada. 1st prize clinical paper.
2. Bouchard C, Chan R, Borne T, Beaupre L, Silveira A, Hemstock R. Hardware complications following plate fixation of the olecranon. 2019 Orthopaedic Research Day, University of Alberta, Edmonton, AB, Canada.
3. AlEidan A, Beaupre L, Silveira A, Bouliane M, Heinrichs L. Radiographic parameters of proximal humerus Fractures Managed by open reduction and internal fixation that could predict patient outcome. 2019 Orthopaedic Research Day, University of Alberta, Edmonton, AB, Canada.

Research Funding

WCB Alberta

Early mobilization following arthroscopic rotator cuff repair – A systematic review.

Appropriate and efficient management of shoulder injuries: Who needs surgery?

WCB Alberta/Edmonton Civic Employees Research Competition

Early functional return to work following distal biceps repair: A randomized control trial.

Edmonton Civic Employees Research Competition/ Covenant Health Research Foundation

Early mobilization following rotator cuff repair – Postoperative ultrasound imaging.

Northern Alberta Shoulder Research Centre (Covenant Health)

Supports upper extremity research personnel at the Grey Nuns Community Hospital.

Sturgeon Community Hospital Foundation

Supports upper extremity research personnel at the Sturgeon Community Hospital.

PAEDIATRIC RESEARCH

Faculty: Dr Lauren Beaupre, Dr Sukhdeep Dulai

Affiliates: Dr John Andersen, Dr Martha Funabashi, Dr Jacob Jaremko, Justin Lewicke, Siyavash Nia, Dr Matthew Prowse, Dr Abhilash Rakkunedeth, Dr Ailar Ramadi, Dr Richard Thompson, Dr Albert Vette, Beth Watkins, Dr Joe Watt, Dr Dornoosh Zonoobi

Trainees & students: Dr Matt Clarke

Multicentre Collaborations

Physician wellness in orthopaedic surgery: A multinational survey study

The consequences of physician burnout have been well-reported and include, most notably, adverse effects on patient care and increased physician suicide rates. In this multicentre collaboration, we sought to understand the rates of well-being and self-reported risk factors for burnout amongst orthopaedic surgeons and trainees in Canada, the United States, England, Scotland, Australia and New Zealand. While all of these countries boast first-world living and working conditions, it is hypothesized that wellness rates will vary significantly and may be related not only to personal factors but also to institutional and health system differences.

In collaboration with the 2019 American–British–Canadian Travelling Fellowship, a cross-sectional survey was conducted and the results have been analysed. The manuscript is currently in progress.

Multicentre pin site infection study in paediatric patients

External fixator devices (EFD), which are widely used to treat various musculoskeletal conditions, use percutaneous transosseous pins or wires to obtain stabilization of the fractures or osteotomies. Pin site infections have been the most commonly reported complication of EFDs, with incidence reported between 11% and 100%. Pin site infections can be superficial, but when deep, they can result in, for example, fasciitis or toxic shock syndrome. With the increasing use of EFDs and potentially high pin site infection rates, documenting the rate of pin site infections, comparing rates across sites, and investigating the factors affecting the rate of pin site infections will provide clinically useful information.

We are therefore collaborating in this multicentre study to measure and document pin site infection rate and pin site care across Canada, the US, and the UK and create a pin site infection database. The study will determine the rate of pin site infections document the factors affecting the rate of pin site infections, and compare methods of pin site care across participating surgical centres.

Development of a quality of life instrument for children with lower limb deformities – Qualitative interviews

Lower limb deformities describe a range of conditions that may result from congenital defects in fetal development or are acquired during growth. Children with disabilities have been found to experience limitations to mobilization, difficulties in normal development, and the need for assistance with daily activities. In addition, they appear to have increased risk of behavioral and emotional problems, as well as psychological and social adjustment problems. These issues, along with the treatment procedures involved for various lower limb deformities, can significantly affect the children's quality of life (QOL).

It has been well recognized that any medical treatment should focus not only on physical health, but also on mental health and social well-being; thus, traditional clinical- and laboratory-based outcomes need to be complemented by patient-based measures focusing on the specific patient's concerns in order to comprehensively evaluate interventions. To our knowledge, there is currently no validated patient-reported outcome (PRO) measure to assess the QOL of children with lower limb deformities.

We are participating in a multicentre study that proposes to fill this gap by developing a new patient-reported QOL instrument. Semi-structured qualitative interviews will be conducted with patients and families to gain an understanding of their experiences with lower limb deformity and their perceptions of QOL. Using this information, a quality-of-life instrument for children with lower limb deformities will be developed.

Paediatric Orthopaedic and Rehabilitation Medicine Research Team of Alberta (PORRTAL)

Characterization of plantar pressure patterns in able-bodied children using dynamic pedobarography

In this study, we used dynamic pedobarography to collect foot pressure data during walking to develop a normative, age-standardized data set of impulse patterns in children with normal feet (Fig. 7). In future studies, this information will be used to enhance the evaluation of foot deformities and their response to surgical intervention. We have completed data collection and analysis at this time, and a manuscript is currently in development.

Figure 7. A typical example of a foot's maximum pressure map for a single gait cycle (stance phase). Using the visual information on pressure data and foot geometry, a rater identifies and segments four regions of the foot in the following order: (1) the hallux (M01); (2) the heel (M02); (3) the medial forefoot (M03); and (4) the lateral forefoot (M04).

The utility of flexible carbon fibre ankle–foot orthoses in idiopathic toe walkers

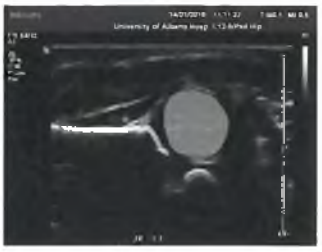
This study is examining the efficacy of a flexible carbon fiber ankle–foot orthosis in achieving a normal heel strike and ankle rockers in children with idiopathic toe walking who have adequate dorsiflexion range to allow for a foot-flat position in stance.

University of Alberta Developmental Dysplasia of the Hip (DDH) Study Group

AI-augmented 2D cine ultrasound reliability and accuracy of hip dysplasia diagnosis

Conventional image-aided diagnosis of developmental dysplasia of the hip (DDH) uses two-dimensional ultrasound (2DUS), which relies heavily on user training to accurately capture the 'Graf plane' that best represents the acetabular bone. An obvious limitation of 2DUS is that it does not capture the complex three-dimensional shape of the acetabular bone (and femoral head). 2D cine sweeps offer a more complete and potentially more reliable visualization of the infant's hip, but the manual interpretation of sweeps is tedious and time-consuming. Artificial intelligence (AI) augmented interpretation of 2D cine sweeps would radically simplify its usage in DDH detection. We propose a technique for automated detection of DDH and compare it to expert clinical diagnosis. We tested the technique on a set of 600 hip scans and the classifier gave an area under the receiver-operating-characteristic (ROC) curve (AUC) of 0.96. The AI-based approach accurately categorized 100% of dysplastic and normal hips from cine sweeps. The approach gave the correct diagnosis directly from the initial 2D cine scan in 89% (90/101) of the cases which were categorized as borderline on initial 2DUS, thereby reducing the need for follow-up scans. This AI-based approach shows promise for a larger-scale clinical study and could potentially result in more widespread use of cine ultrasound and AI in DDH detection.

Assessment of developmental hip dysplasia by 3D ultrasound



A prospective cohort study to correlate findings on 3D ultrasound with clinical and imaging findings; to examine the feasibility and accuracy of 3D ultrasound measurement of hip dysplasia via development of 3D hip dysplasia models through mathematical analysis of ultrasound images from routine clinical care; and to explore the feasibility and accuracy of ultrasound deep learning for the development of an automated process of evaluation for developmental hip dysplasia (DDH).

A prospective, international hip dysplasia registry with follow-up to skeletal maturity: An analysis of risk factors, screening practices, and treatment outcomes

This international multisite study has developed a comprehensive, prospective, international registry for all patients with DDH. Data collected will be analysed to improve the understanding of DDH, its consequences, and its treatment outcomes. To date, we have enrolled more than 20 patients in the registry from our site.

Publications

1. Vette AH, Funabashi M, Lewicke J, Watkins B, Prowse M, Harding G, Silveira A, Saraswat M, Dulai S (2019). Functional, impulse-based quantification of plantar pressure patterns in typical adult gait. *Gait & Posture*, 67: 122–127. DOI: 10.1016/j.gaitpost.2018.09.029.
2. Vafaeian B, Adeeb S, El-Rich M, Dulai SK, Jaremko JL (2019). Prediction of mechanical behavior of cartilaginous infant hips in Pavlik harness: A subject-specific simulation study on normal and dysplastic hips. *Journal of Orthopaedic Research*, 37(3): 655–664. DOI: 10.1002/jor.24213.
3. Mostofi E, Chahal B, Zonoobi D, Hareendranathan A, Roshandeh KP, Dulai SK, Jaremko JL (2019). Reliability of 2D and 3D ultrasound for infant hip dysplasia in the hands of novice users. *European Radiology*, 29(3): 1489–1495. DOI: 10.1007/s00330-018-5699-1.

Invited Presentations

1. Dulai S. Building networks to improve patient care and surgeon well-being. New Zealand Orthopaedic Association Continuing Orthopaedic Education Meeting, May 2019, Christchurch, New Zealand.
2. Dulai S. Building networks to improve patient care and surgeon well-being. Royal Australasian College of Surgeons Orthopaedic Meeting, May 2019, Melbourne, Australia.
3. Dulai S. 3D ultrasound in DDH. Queensland University of Technology, May 2019, Brisbane, Australia.
4. Dulai S. The effect of time to treatment on the rate of deep infection in open fractures. Gold Coast University Hospital Orthopaedic Conference, May 2019, Gold Coast, Australia.
5. Dulai S. Optimizing acute pain management in paediatric orthopaedic outpatients. Ipswich Hospital Orthopaedic Conference, May 2019 Brisbane, Australia.
6. Dulai S. The good, the bad and the ugly of RCTs in paediatric orthopaedics. Queen's Medical Centre in Nottingham/Nottingham College of Medicine/University of Nottingham Orthopaedic Conference, May 2019, Nottingham, England.
7. Dulai S. The good, the bad and the ugly of RCTs in paediatric orthopaedics. University of Edinburgh Orthopaedic Conference, May 2019, Edinburgh, Scotland.
8. Dulai S. Building networks to improve patient care and surgeon well-being. Wrightington Hospital Orthopaedic Conference, April 2019, Wrightington, England.
9. Dulai S. Is 3D Ultrasound in DDH prodigal or a panacea? Royal London Hospital/St Bartholomew's University Orthopaedic Conference, April 2019, London, England.
10. Dulai S. Is 3D ultrasound in DDH prodigal or a panacea? Stanmore Orthopaedic Conference, April 2019, Royal National Orthopaedic Hospital, London, England.

Unpublished Presentations

1. Clarke M, Prowse M, Lewicke J, Watkins B, Vette A, Dulai S. What is the gold standard to measure femoral and tibial rotational alignment in children? 2019 Orthopaedic Research Day, University of Alberta, Edmonton, AB, Canada.

Research Funding

Women and Children's Health Research Institute Innovation Grant

Can babies be screened for hip dysplasia by a quick 3D ultrasound scan read by a computer just as well as by visiting a specialist hospital clinic?

Glenrose Rehabilitation Hospital Clinical Research Fund

Characterization of plantar pressure patterns in able-bodied children using dynamic pedobarography.

Glenrose Rehabilitation Hospital Clinical Research Fund

The utility of flexible carbon fibre ankle–foot orthotics in idiopathic toe walkers.

Canadian Orthopaedic Foundation Operational Research Grant

Paediatric orthopaedic research.

Awards

1. S Dulai was awarded an international ABC Travelling Fellowship in 2019.

BASIC SCIENCE

Joint Tissue Regeneration Laboratories

Faculty: Dr Adetola Adesida, Dr Nadr Jomha, Dr Andrei Manolescu

Research associates: Dr Leila Laouar

Technical staff: Aillette Mulet-Sierra, Melanie Kunze, Kenneth Wong

Affiliates: Dr Khalid Ansari, Dr Fred Berry, Dr Janet AW Elliott, Dr Daniel Graf, Dr Greg Korbutt, Dr Joanne Lemieux, Dr Locksley McGann, Dr Martin Osswald, Dr Vinay Prasad, Dr Jana Rieger, Dr Hasan Uludag, Dr Samer Adeeb, Dr Yaman Boluk, Dr Mark Sommerfeldt, Dr Gail Thornton, Dr Fred West, Dr Lindsey Westover, Dr Frank Wuest

Joint tissue engineering

Dr Adesida – principal investigator

The biomechanically functional component of articular cartilage, its extracellular matrix (ECM), has been reported to sequester biological stimuli for the formation of cartilage-forming cells (chondrocytes) from adult-derived mesenchymal stem cells (MSC). Therefore, we first explored the possibility that meniscus-derived ECM may sequester bioactive molecules that can convert synovial fluid-derived MSCs to meniscus fibrochondrocytes (Liang *et al., Acta Biomaterialia* 2018, 80: 131–143; IF 6.638). More recently, we explored whether meniscus-derived ECM could restore the ability of cell culture-expanded human meniscus fibrochondrocytes to make the meniscus's native ECM without externally added biological signals in the form of growth factors (Fig. 8). Our findings revealed that without the addition of external growth factors (i.e. TGF-β3), human meniscus-derived ECM alone could restore the capacity of cultured human meniscus fibrochondrocytes to synthesize meniscus-like ECM (Liang *et al., Annals of Biomedical Engineering* 2020, 48(3): 968–979 [Epub 30 May 2019]; IF 3.47).

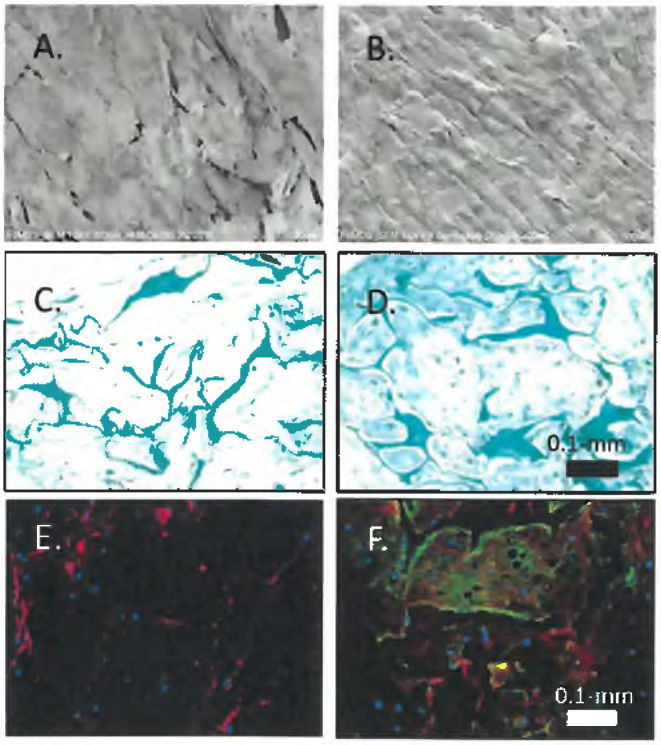


Figure 8. (A & B) Scanning electron microscopy of meniscus fibrochondrocyte (MFC)-based tissues after 21 days' culture in a meniscus-derived decellularized matrix (DCM) without (A) and with (B) supplementation of 10 ng/mL TGF-β3. (C & D) Safranin O staining of meniscus extracellular matrix (ECM) molecules of MFC-based tissues after 21 days' culture in DCM without (C) and with (D) supplementation of 10 ng/mL TGF-β3 DAPI staining for cell nuclei is overlaid with immunofluorescence images in blue. (E & F) Type I collagen and type II collagen immunofluorescence of meniscus ECM molecules of MFC-based tissues after 21 days' culture in DCM without (E) and with (F) supplementation of 10 ng/mL TGF-β3 DAPI staining for cell nuclei.

Vitrification of joint tissues

Dr Jomha – principal investigator

It has been a number of years since our group successfully cryopreserved/vitrified intact human articular cartilage. We are steadily working to optimize the protocol and make it clinically relevant to enable transition to a clinical tissue transplantation bank. This includes making a delicate procedure robust to variances that can occur in a busy tissue lab, which means devising techniques to prevent cryoprotectant agent toxicity and developing packaging

methods that enable easy and reproducible exposure to incredible extremes in conditions including cooling in liquid nitrogen at a temperature of –196 °C.

Over the past years we have decreased the time it takes to achieve successful vitrification from 9.5 hours to 7 hours. We are now focused on two different aspects. The first is to scale up the process. Most of our work is done with 10-mm diameter bone and cartilage dowels (osteochondral dowels), which have some utility, but it is most practical if we can do this with larger tissue fragments such as whole femoral condyles. This will also increase the scope of use for the tissue because it can then be used to treat large joint defects. As we increase the tissue size, the volume of surrounding vitrification solution also has to increase to keep it immersed, but this brings with it heat transfer issues which can lead to ice formation (which kills the cells and damages the internal matrix architecture) and cracking (which can physically disrupt the articular cartilage). Experimenting mainly with cartilage from pigs slaughtered for use at a deli, we developed a novel method to store the treated femoral condyles in a vacuum sealed bag with no surrounding vitrification solution. We documented that this method greatly increases our cell viability when compared to use of a surrounding solution and that it eliminates cracking. We will work to confirm this with human femoral condyles as the tissue becomes available through the Comprehensive Tissue Centre.

Our second approach is to move towards clinical implementation of this technology. We recently documented that we are able to successfully vitrify 1-mm cubes of articular cartilage from both pigs and humans with greater than 80% cell recovery and good metabolic activity, even after storing for 6 months in liquid nitrogen at –196 °C. With this data, we successfully obtained funding from the University (of Alberta) Hospital Foundation to do a comparison trial of transplanting 1-mm cubes of fresh vs vitrified cartilage in full-thickness femoral condylar defects in pigs. This procedure mimics the one currently in clinical practice. It will be the first animal model investigation using vitrified articular cartilage, and if successful will justify moving to a human clinical trial for the use of vitrified cartilage.

Building on our success with articular cartilage, we have begun looking further into the vitrification of menisci for the purpose of transplantation. Our initial studies are characterizing the biomechanical effects of freezing and vitrification. It is early in this research but, from the perspective of maintaining the mechanical properties of fresh tissue, vitrification appears to have advantages over freezing. We will continue to explore this avenue to clarify whether an improved transplantation product can be achieved using vitrification.

SLCA2 proteins – New molecular reports of chondrocyte differentiation and degeneration

Dr Manolescu – principal investigator

Last year, our preliminary results, obtained through generous funding from the Edmonton Orthopaedic Foundation and the Edmonton Civic Employee foundation, came to fruition. Our data pertaining to the characterization of chondrocyte degeneration enabled us to gather enough evidence to initiate an application for funding to the Alberta Spine Foundation. The resulting grant application, entitled 'In vivo anatomical and functional imaging of intervertebral disc degeneration using hexose analogues, tagged with near infrared (NIR) fluorophores and positron emission tomography (PET)', was successfully reviewed and funded.

Our previous joint experiments proved the critical role of GLUT proteins in hypoxia-driven cellular functions. Using a library of hexose-based PET tracers, our group successfully developed innovative molecular imaging techniques for fructose and glucose metabolism in hypoxia-controlled cellular models. Based on our results, we were invited to apply to last year's competition for multidisciplinary grants held by The Canadian Glycomics Network (GlycoNet). GlycoNet is a Network of Centres of Excellence that focuses on the role of glycans (carbohydrates) in health in the areas of therapeutic proteins and vaccines, antimicrobials, and chronic disease, in particular diabetes and obesity, genetic diseases involving errors in glycan metabolism, and cancer. This granting agency functions under the auspices of 22 universities across Canada with the goal of identifying high-impact translational research. Last year we applied for a second multidisciplinary grant entitled 'Novel molecular probes targeting GLUT5 – Diagnostic and medical imaging applications and discovery of fructose inhibitors as potential therapeutic drugs'. Once again, our team was successful and the research was funded.

Publications

1. Zhalmuratova D, La TG, Yu KT, Szojka ARA, Andrews SHJ, Adesida AB, Kim CI, Nobes DS, Freed DH, Chung HJ (2019). Mimicking 'J-shaped' and anisotropic stress-strain behavior of human and porcine aorta by fabric-reinforced elastomer composites. *ACS Applied Materials & Interfaces*, 11(36): 33,323–33,335 (IF 8.456). DOI: 10.1021/acsami.9b10524.
2. Dzobo K, Motaung K, Adesida A (2019). Recent trends in decellularized extracellular matrix bioinks for 3D printing: An updated review. *International Journal of Molecular Sciences*, 20(18): 4268 (IF 4.183). DOI: 10.3390/ijms20184628.
3. Szojka ARA, Lyons BD, Moore CN, Liang Y, Kunze M, Idrees E, Mulet-Sierra A, Jomha NM, Adesida AB (2019). Hypoxia and TGF-β3 synergistically mediate inner meniscus–like matrix formation by fibrochondrocytes. *Tissue Engineering Part A*, 25(5–6): 446–456 (IF 3.616). DOI: 10.1089/ten.tea.2018.0211.
4. Liang Y, Szojka ARA, Idrees E, Kunze M, Mulet-Sierra A, Adesida AB (2020). Re-differentiation of human meniscus fibrochondrocytes differs in three-dimensional cell aggregates and decellularized human meniscus matrix scaffolds. *Annals of Biomedical Engineering*, 48(3): 968–979 (IF 3.47) [Epub 30 May 2019]. DOI: 10.1007/s10439-019-02272-7.
5. Barin FR, de Sousa Neto IV, Vieira Ramos G, Szojka A, Ruivo AL, Anflor CTM, Agualimpia JDH, Domingues AC, Franco OL, Adesida AB, et al. (2019). Calcaneal tendon plasticity following gastrocnemius muscle injury in rat. *Frontiers in Physiology*, 10: 1098 (IF 3.201). DOI: 10.3389/fphys.2019.01098.
6. Wiafe B, Adesida AB, Churchill T, Kadam R, Carleton J, Metcalfe PD (2019). Mesenchymal stem cell therapy inhibited inflammatory, and profibrotic pathways induced by partial bladder outlet obstruction and prevented high-pressure urine storage. *Journal of Pediatric Urology*, 15(3): 254.e1–254.e10 (IF 1.726). DOI: 10.1016/j.jpurol.2019.03.003.
7. Wu K, Laouar L, Dong R, Elliott JAW, Jomha NM (2019). Evaluation of five additives to mitigate toxicity of cryoprotective agents on porcine chondrocytes. *Cryobiology*, 88: 98–105 (IF 2.1). DOI: 10.1016/j.cryobiol.2019.02.004.

Research Funding

Operating

Edmonton Civic Employees Charitable Assistance Fund (ECE)
Revisiting meniscus fibrochondrocytes: A new paradigm in meniscus tissue engineering.

Alberta Cancer Foundation (ACF; Mickleborough Research Program)
Engineering high-quality autologous cartilage grafts for nasal reconstruction.

Natural Sciences and Engineering Research Council (NSERC) Discovery Grant
Meniscus fibrochondrocytes mechano- and hypoxia-transduction.

Canadian Institute of Health Research (CIHR) Project Grant
Precision engineering of cartilage grafts for nasal reconstruction.

Canadian Institutes of Health Research (CIHR)
Knee meniscus reconstruction using stem cells.

Edmonton Orthopaedic Research Committee (EORC)
Regeneration of joint tissues.

University (of Alberta) Hospital Foundation (UHF)
Transplantation of vitrified particulate articular cartilage in a pig model.

Equine Guelph Research Committee
Vitrified equine MSC cartilage for cartilage repair.

Alberta Spine Foundation
In vivo anatomical and functional imaging of intervertebral disc degeneration using hexose analogues, tagged with near-infrared (NIR) fluorophores and positron emission tomography (PET).

The Canadian Glycomics Network (GlycoNet) is a Network of Centres of Excellence
Novel molecular probes targeting GLUT5—Diagnostic and medical imaging applications and discovery of fructose inhibitors as potential therapeutic drugs.

Equipment

Natural Sciences and Engineering Research Council (NSERC) Research Tools and Instruments (RTI)
Gravity unloading and mechanobiology suite for characterization of engineered connective microtissues.

Training of High-quality Personnel

Visiting Scholars

- Dr Rita Marqueti-Durigan (Universidade de Brasília, Brazil)
- Dr Gabriel Ochube (Ahmadu Bello University, Nigeria)

Postdoctoral Fellows

- Dr Matthew Anderson-Baron
- Dr Kar Way Yong

Graduate Students

- Guoju Hong (doctoral student)
- Enaam Idrees (doctoral student)
- Xiaoyi (Michelle) Lan (master's student)
- Dr Yan Liang (doctoral student)
- Alexander Szojka (doctoral student)
- Bridget Wiafe (doctoral student)
- Dr Kezhou Wu (doctoral student)

Orthopaedic Residents

- Dr Tyson Boettcher
- Dr Charles Bouchard
- Dr Luke Heinrichs

Summer Undergraduate Students

- Dr Ryan Chee
- Mary Crisol
- Rachel Dong
- Esra Erkut
- Victoria Goncalves
- Jenny He (medical student)
- Clayton Molter
- Samia Rahman
- Austyn Roelofs (University of Victoria)
- John Sevik (medical student)
- Meredith Stadnick (medical student)
- Itai Wine

Invited Talks

1. Jomha NM. Orthopaedic tissue transplantation: What we do and where we are going. Alberta Transplantation Institute, 30 October 2019, Edmonton, AB, Canada.
2. Jomha NM. Functional anatomy: Cartilage and meniscus. The Tom Smallman Canadian Orthopaedic Association Basic Science Course, 21 October 2019, Ottawa, ON, Canada.
3. Adesida AB (guest lecturer). Cartilage tissue engineering. University of Ibadan Biomedical Engineering Conference, 6 February 2019, Ibadan, Nigeria (online delivery).
4. Adesida AB (plenary session speaker). Molecular lessons: Bioengineered meniscus under simulated microgravity. Ascension 2019 Annual Space Conference of Students for the Exploration and Development of Space (SEDS), 1–3 March 2019, University of Alberta, Edmonton, AB, Canada.
5. Adesida AB (keynote lecturer). Engineering functional cartilages. 35th Annual Meeting of Canadian Biomaterials Society, 21–24 May 2019, Quebec City, QC, Canada.
6. Adesida AB (guest lecturer). Cartilage tissue engineering in health and space. 2019 Health International Conference, 8 August 2019, Federal University of Technology, Owerri, Nigeria.
7. Adesida AB (invited lecturer). Engineering cartilage for nasal reconstruction. Alberta Cancer Foundation Board of Trustees, 22 August 2019, Marriott Courtyard, Edmonton, AB, Canada.
8. Adesida AB (invited lecturer). Engineering cartilage for nasal reconstruction. Cross Cancer Institute Grand Rounds, 3 September 2019, Edmonton, AB, Canada.

Student Presentations

Podium

1. Szojka A. A bioreactor for combined hypoxia and dynamic compression of engineered human meniscus tissues. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff Park Lodge, Banff, AB, Canada.
2. Rahman S. Oxygen tension modulated in vitro chondrogenesis and in vivo calcification of infrapatellar fat pad–derived mesenchymal stem cells. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff Park Lodge, Banff, AB, Canada.
3. Erkut E. Genipin as a crosslinker to enhance the mechanical properties of 3D bioprinted human nasal cartilage. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff Park Lodge, Banff, AB, Canada.

Poster

1. Lan X (Michelle). 3D bioprinting of engineered nasal cartilage by freeform reversible embedding of suspended hydrogel. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff Park Lodge, Banff, AB, Canada.
2. He J, Wine I, Wu K, Laouar L, Adeeb S, Westover L, Jomha NM. Effect of vitrification on mechanical properties of intact osteochondral tissue. University of Alberta Faculty of Medicine and Dentistry 52nd Annual Summer Students' Research Day, 4 October 2019, Edmonton, AB, Canada.
3. Dong R, Heinrichs L, Shardt N, Wu K, Laouar L, Elliott JAW, Jomha, N. Evaluation of the permeation kinetics of formamide in porcine articular cartilage. University of Alberta Faculty of Medicine and Dentistry 52nd Annual Summer Students' Research Day, 4 October 2019, Edmonton, AB, Canada.
4. Dong R, Heinrichs L, Shardt N, Wu K, Laouar L, Elliott JAW, Jomha N. Evaluation of the permeation kinetics of formamide in porcine articular cartilage. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff, AB, Canada.
5. He J, Wine I, Wu K, Sevick J, Laouar L, Jomha NM, Westover L. Effect of vitrification on mechanical properties of porcine articular tissue. University of Alberta Faculty of Medicine and Dentistry 52nd Annual Summer Students' Research Day, 4 October 2019, Edmonton, AB, Canada.
6. He J, Wine I, Wu K, Sevick J, Laouar L, Jomha NM, Westover L. Effect of vitrification on mechanical properties of porcine articular tissue. 20th Annual Alberta Biomedical Engineering Conference, 25–27 October 2019, Banff, AB, Canada.
7. He J, Wine I, Wu K, Sevick J, Laouar L, Jomha NM, Westover L. Effect of vitrification on mechanical properties of porcine articular tissue. University of Alberta Faculty of Medicine and Dentistry 2nd Annual Excellence in Medical Student Research, 26 November 2019, Edmonton, AB, Canada.
8. Crisol M, Wu K, Laouar L, Elliott JAW, Jomha NM. Antioxidant effects in porcine articular cartilage during exposure to cryoprotective agents. 20th Annual Alberta Biomedical Engineering Conference, 26 October 2019, Banff, AB, Canada.
9. Crisol M, Wu K, Laouar L, Elliott JAW, Jomha NM. Antioxidant effects in porcine articular cartilage during exposure to cryoprotective agents. University of Alberta Faculty of Medicine and Dentistry 52nd Annual Summer Students' Research Day, 4 October 2019, Edmonton, AB, Canada.

Awards

Alexander Szojka
- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Alexander Graham Bell Canada Graduate Scholarship-Doctoral (CGS-D) program

Ryan Chee
- Alberta Innovates Health Solutions Summer Studentship
- Women and Children's Health Research Institute (WCHRI) summer studentship

Clayton Molter
- Natural Sciences and Engineering Research Council of Canada (NSERC)
- Undergraduate Student Research Awards (USRA)

Samia Rahman
- Department of Surgery summer studentship

Esra Erkut
- Summer Temporary Employment Program Award

Kezhou Wu
- Department of Lab Medicine and Pathology Cryo 2007 Travel Award

Kezhou Wu
- Canadian Institutes for Health Research Student Travel Award, used to attend Canadian Student Health Research Forum

Guoju Hong
- Alberta Innovates Graduate Student Scholarship

2019 AWARDS & HONORS

Teacher of the Year (2019)

was awarded to **Dr Nadr Jomha** *and* **Dr Kyle Stamp**

Tom Williams Surgical Research Day Awards

Cooper Johnston Memorial Scholarship was awarded to **Dr Louis Bezuidenhout**

Dr Hastings-Mewburn Postgraduate Scholarship was awarded to **Dr Thomas Goodine**

Gordon Denchfield Thomas Scholarship Award

was awarded to **Dr Teresa Li**

Robert Townsend Scientific Award

was awarded to **Dr Julia Bowes**

Orthopaedic Research Day

Best Clinical Presentation was awarded to **Dr Kirsten Hickie**

Best Literature Review was awarded to **Dr Jillian Karpyshyn**

THANK YOU

We would like to thank the following companies and exhibitors for their support of the 46th Annual Division of Orthopaedic Surgery Research Day

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DePuy Synthes
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Orthopaedic Faculty

Divisional Director: Edward Masson
Research Director: Nadr Jomha
Residency Program Director: Robert Chan
UGME Director: Aleem Lalani

A Badre, R Balyk, J Bergman, M Bouliane, J Bury, R Chan, R Chow, J Cinats, D Dick, S Dulai, D Durand, L Ekert, M Furey, D Glasgow, R Glasgow, G Goplen, R Henderson, B Herman, E Huang, C Hui, H Jiang, N Jomha, F Kortbeek, A Lalani, M Lapner, G Lavoie, M Lavoie, P Leung, J Mahood, A Manolescu E Masson, R McLeod, M Menon, M Moreau,D Otto, C Panaro, P Paul, E Pedersen, A Scharfenberger, B Sharifi, D Sheps, M Sommerfeldt, K Stampe, R Stiegelmar, J Toreson, D Weber, C Weeks

RESEARCH STAFF

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FELLOWS

A Perreault, M Abou-Ghaida, N Khan, C Maroun

ADMINISTRATIVE ASSISTANTS

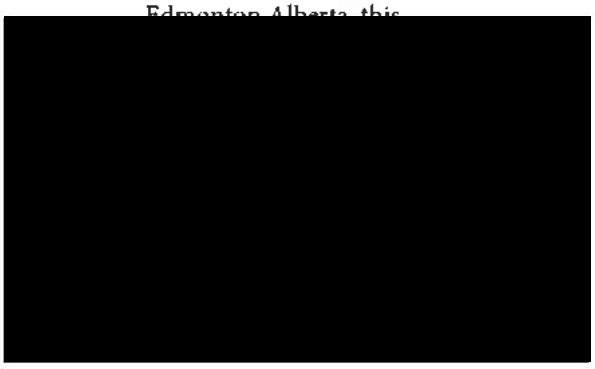
S Dopulos and S Silva Maciel

UNIVERSITY OF ALBERTA
FACULTY OF MEDICINE & DENTISTRY
Department of Surgery

Alberta Health Services

Exhibit "B"

This is **Exhibit "B"** referred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at
Edmonton, Alberta, this



Alberta Health Services

POLICY

TITLE
IMMUNIZATION OF WORKERS FOR COVID-19

SCOPE
Provincial

DOCUMENT #
1189

APPROVAL AUTHORITY
Alberta Health Services President and Chief Executive Officer

INITIAL EFFECTIVE DATE
September 14, 2021

SPONSOR
Workplace Health and Safety

REVISION EFFECTIVE DATE
October 22, 2021

PARENT DOCUMENT TITLE, TYPE, AND NUMBER
Not applicable

SCHEDULED REVIEW DATE
April 22, 2022

NOTE: The first appearance of terms in bold in the body of this document (except titles) are defined terms – please refer to the Definitions section.

If you have any questions or comments regarding the information in this document, please contact Policy Services at policy@ahs.ca. The Policy Services website is the official source of current approved policies, procedures, directives, standards, protocols, and guidelines. Only the electronic version of this document, as hosted on the Policy Services website or www.ahs.ca, is valid.

OBJECTIVES

- To set out **worker** immunization requirements for COVID-19 to protect the health and safety of workers, patients, and the communities that Alberta Health Services (AHS) serves.

PRINCIPLES

AHS is committed to protecting the health and safety of its workers, patients, visitors, and others accessing AHS sites. Immunization against COVID-19 is the most effective means to prevent the spread of COVID-19, to prevent outbreaks in AHS facilities, to preserve workforce capacity to support the health care system, and to protect our workers, patients, visitors, and others accessing AHS sites. Immunization against COVID-19 also supports the AHS Values of Compassion, Accountability, Respect, Excellence, and Safety.

This Policy is in addition to other AHS policy documents supporting worker and patient safety during the COVID-19 pandemic including, but not limited to, the AHS *Use of Masks During COVID-19* Directive, *Attending Work with COVID-19 Symptoms, Positive Test, or Close Contact* Directive, and the *Fit for Work Screening (COVID-19)* Protocol.

This Policy shall be reviewed regularly, and at least every six (6) months, to ensure alignment with public health measures and regulations, and to confirm it adequately covers the health and safety risks that it addresses.

APPLICABILITY

Compliance with this document is required by Alberta Health Services, Alberta Precision Laboratories, Carewest, CapitalCare, and Covenant Health employees, members of the medical and midwifery staffs, students, volunteers, and other persons acting on their behalf. Compliance requirements for other contracted service providers, such as continuing care, will be

communicated directly to the contracted service providers. This document does not apply to physicians with Community Appointments.

ELEMENTS

1. Immunization Requirements

1.1 Effective November 30, 2021, all workers must be **fully immunized** against COVID-19.

1.2 A worker on an approved Leave of Absence must be fully immunized prior to returning to work.

1.3 A worker hired after November 30, 2021 must be fully immunized prior to commencing work.

2. Proof of Immunization Records

2.1 No later than November 15, 2021, workers shall disclose accurate proof of their immunization status to:

a) AHS or an AHS subsidiary, if the worker is an AHS employee, medical staff, midwifery staff, or volunteer;

b) Covenant Health, if the worker is a Covenant Health employee, medical staff, or volunteer;

c) their educational institution, if the worker is a student or instructor; or

d) their employer, if the worker is a contracted service provider.

2.2 Proof of immunization is being collected to protect the health and safety of workers, patients, and other persons accessing AHS sites and to preserve AHS' workforce capacity to support the health care system.

2.3 Proof of immunization records collected under this Policy shall be securely and confidentially retained, accessed, and used as necessary to determine fit for work status of workers, to manage and administer employment and other working relationships with workers, to address accommodation requests, and to comply with all applicable laws, such as the *Occupational Health and Safety Act* (Alberta) and *Regional Health Authorities Act* (Alberta).

2.4 Proof of immunization records are collected under the authority of Section 33(c) of the *Freedom of Information and Protection of Privacy Act* (Alberta) and shall be used, accessed, and disclosed in accordance with the legislation and the AHS *Collection, Access, Use, and Disclosure of Information* Policy.

3. Workplace Accommodation

3.1 Any AHS employee who is unable to be immunized due to a medical reason, or for another protected ground under the *Alberta Human Rights Act*, will be reasonably accommodated, up to the point of undue hardship, in accordance with the AHS *Workplace Accommodation* Policy.

3.2 Employees of AHS subsidiaries, Covenant Health, and applicable contracted service providers, who are unable to be immunized due to a medical reason, or for another protected ground under the *Alberta Human Rights Act*, will be reasonably accommodated, up to the point of undue hardship, in accordance with their applicable workplace accommodation policies.

3.3 Any current AHS employee requesting workplace accommodation shall make a request for the accommodation as soon as reasonably possible, and no later than October 16, 2021, and provide required information in accordance with the AHS *Workplace Accommodation* Policy (or the appropriate accommodation policy of an AHS subsidiary or Covenant Health, if applicable).

3.4 Any current AHS member of the medical or midwifery staff who is not an employee of AHS, an AHS subsidiary, or Covenant Health, and who is unable to be immunized due to a medical reason, may request an exception as soon as reasonably possible and no later than October 16, 2021. A request for an exception shall be made on the *Medical or Midwifery Staff Request for Exception COVID-19 Mandatory Immunization for Workers* form and shall be submitted as directed on the form. The lack of immunization may affect the safe exercise of their Clinical Privileges as described in the *Medical Staff Bylaws* and *Rules* (Rule 3.4.4.2), or may directly impact their ability to practice and patient safety as described in the *Midwifery Staff Bylaws* and *Rules* (Rule 3.3.4), as applicable.

4. Non-Compliance

4.1 With respect to students, instructors, and applicable contracted service providers, failure to comply with this Policy shall result in AHS reviewing the applicable contract or other relevant circumstances and initiating further discussions with the applicable educational institution or contracted service provider and, in this respect, AHS reserves all rights it has at law, equity, or pursuant to any applicable agreement to address such non-compliance.

4.2 In all other cases not outlined in Section 4.1 above, except where a workplace accommodation or exception (for medical or midwifery staff) applies, failure to comply with this Policy shall result in:

a) a meeting being held with the worker to discuss their concerns with vaccination against COVID-19 and provide educational materials on the COVID-19 vaccines; and

b) if the worker remains non-compliant with this Policy, the worker being placed on an unpaid leave of absence for the period of time required to

become fully immunized or, in the case of medical or midwifery staff, Immediate Action being taken as set out in Part 6 of the *Medical Staff* Bylaws or *Midwifery Staff* Bylaws.

DEFINITIONS

Fully immunized means a worker:

a) who has received two doses of a vaccine considered valid by Alberta Health in a two-dose COVID-19 vaccine series or one dose of a vaccine considered valid by Alberta Health in a one-dose COVID-19 vaccine series; and
b) for whom fourteen days have elapsed since the date on which the person received the second dose of the COVID-19 vaccine considered valid by Alberta Health of a two-dose series or one dose of the COVID-19 vaccine considered valid by Alberta Health in a one-dose vaccine series.

Worker means AHS, its subsidiaries and Covenant Health employees, members of the medical and midwifery staffs, students and instructors, volunteers, and applicable contracted service providers (including anyone providing services for AHS on behalf of an applicable contracted service provider).

REFERENCES

- Alberta Health Services Governance Documents:
  - *Attending Work with COVID-19 Symptoms, Positive Test, or Close Contact* Directive (#1188)
  - *Collection, Access, Use, and Disclosure of Information* Policy (#1112)
  - *Fit for Work Screening (COVID-19)* Protocol (#1184-01)
  - *Medical Staff Bylaws* and *Rules*
  - *Midwifery Staff* Bylaws and *Rules*
  - *Use of Masks During COVID-19* Directive (#HCS-267)
  - *Workplace Accommodation* Policy (#1156)
- Alberta Health Services Forms:
  - *Employee Request for Accommodation* Form (#19566)
  - *Got My COVID-19 Immunization* Form
  - *Medical or Midwifery Staff Request for Exception COVID-19 Mandatory Immunization for Workers* Form
- Alberta Health Services Resources:
  - AHS Immunization Information Insite Page
  - AHS Values
- Non-Alberta Health Services Documents:
  - *Alberta Human Rights Act*
  - *Freedom of Information and Protection of Privacy Act* (Alberta)
  - *Occupational Health and Safety Act* (Alberta)
  - *Regional Health Authorities Act* (Alberta)

Exhibit "C"

This is **Exhibit "C"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at Edmonton, Alberta, this

[redacted]

From: [redacted]
Sent:
To:
Subject: 21NO10_Your Kinexus SARS-CoV-2 antibody testing results with your dried blood sample

Eva Chipiuk
Barrister & Solicitor

Klahowya Mr. Nadr Jomha,

Thank you for participating in our clinical study to identify the parts of SARS-CoV-2 proteins that are the most immunogenic in people who become infected with this virus. Your patience in receiving the results of our testing is greatly appreciated. We have been overwhelmed by the response from people like yourself, and with the limited capacity of our company, it has been hard to meet the time-lines that we normally can achieve. The information from our study is helping us to develop more sensitive, accurate, and cheaper serological tests for detection of past infection with SARS-CoV-2 and possible immunity against future infections with this virus and related mutant strains.

To analyze your blood sample, we have used our CCS SARS-CoV-2 Antibody Test, which tracks 41 of the best markers that we have identified, with coverage of 10 of the 26 viral protein encoded by the SARS-CoV-2 genome. These 41 markers were amongst the most immunogenic peptide fragments from the virus's proteins that we have identified out of the original 8000 tested, which were tested on much larger SPOT peptide arrays. If a person possesses a specific antibody that recognizes one of these immunogenic peptides, a strong signal appearing as a dark spot will be generated on the array. The darker the peptide spot, the higher the level of antibody in the serum against that specific peptide sequence. Spot F7 is a control peptide that binds to the secondary detection anti-human IgG antibody used in our test. It ensures that this step of our assay is working. The spots in the top 3 rows A-C correspond to fragments of the SARS-CoV-2 spike protein. This is the virus protein that is made with the current RNA (Pfizer and Moderna) and adenovirus (Johnson & Johnson and AstraZeneca) COVID-19 vaccines. Locations of antibodies that are directed against the other SARS-CoV-2 proteins that are tracked are also shown on the attached jpg figure. When we used dried blood samples instead of serum from fresh blood, we have observed that Spots A6, B7 C6 and D7 are more commonly detected, and it is not clear whether we are monitored blood proteins rather than antibodies in our test. Consequently, the presence of these spots alone are not taken as a sign of SARS-CoV-2 antibody reactivity in the absence of other detectable antibody spots.

Other serological tests from other companies monitor only antibodies against the spike and/or nucleocapsid proteins. The vaccines that are currently being used will specifically induce immunoreactivity towards the spike protein, but unfortunately none of the other SARS-CoV-2 proteins. Actual infection by the virus itself actually confers the best protection against future infections by other SARS-CoV2 mutant strains and related viruses.

It should be appreciated that our tests, and any other tests, cannot not demonstrate that you are necessarily immune to future SARS-CoV-2 infections and development of COVID-19. No commercial test can actually show this. However, the larger your antibody response, the more likely that you are protected. Our test would unlikely be accepted by those organizations that are trying to impose mandatory vaccination for travel or access to public events. They should be used for your personal decision making, in the context of your own unique situation and risks from COVID-19 and the vaccinations. Interestingly, the RNA and likely adenovirus COVID-19 vaccines appear to work poorly against the Delta variant of SARS-CoV-2 according to recent data from the Israel, Iceland and the US, so the long term efficacy of these vaccines has been seriously called into question. Our data seems to indicate the mutations associated with the common variants of concern, including Delta, are irrelevant to immunity, because 1) the parts of the SARS-CoV-2 spike proteins that are the most immunogenic (i.e., antibody producing) are different from the parts with the mutations, and 2) many different parts of the viruses elicit antibodies as you will see in the examples provided. Note that we are only tracking 41 of the possible targets for antibodies. A person with COVID-19 will make hundreds of different antibodies simultaneously.

In the attached jpg, I have provided images of the results of 35 of the tests that we have performed with blood samples from several COVID-19 patients that have been confirmed with the PCR genetic test for SARS-CoV-2. The data from the sera from 10 healthy control individuals is also provided. As you can see in the COVID-19 control

images of our immunoblots, the actual pattern of antibody immunoreactivities varies dramatically between different people that have been infected with the same virus. Such differences are also evident with serum samples from COVID-19 vaccine immunized individuals. Remarkably, we have observed that for people that have had COVID-19, the individual patterns are generally very stable when measured over a year after their initial infection. Our studies have demonstrated that natural immunity can persist for at least 18 months. Normally, antibody levels will wane after a few months, but the persistence of the antibody levels could reflect ongoing re-exposures to the SARS-CoV-2 virus, which act like booster shots.

In fact, many of the people in B.C. that we have tested that are healthy and have no prior indication of infection with SARS-CoV-2 actually already have antibodies that will recognize the virus. It should be appreciated that Health Canada presently suggests that only a tiny portion (less than 5%) of the BC population that is not vaccinated, have protective antibodies against the virus. However, our external collaborative studies seem to indicate that these healthy people that have antibodies with our tests also confer protection from infection by SARS-CoV-2.

Here's your specific results. Our test does seem to indicate that you have several antibodies that can strongly bind to different parts of the spike protein, which supports a previous infection with SARS-CoV-2. You also have some immunoreactivity against a few of the other proteins in the SARS-CoV-2 virus as well, which further strengthenswww this conclusion. It is possible that some of these immunoreactivities arise from pre-existing antibodies that you may have made from exposure to other related coronaviruses, including those that cause the common cold. However, in view of the large number of visible spots and their intensities, it does seem that you have already been infected with SARS-CoV-2.

As time passes on, reductions of these antibody signals is expected if you haven't encountered the virus more recently. This decline will actually happen faster if you have been practising social isolation and using protective measures such as constant hand washing and wearing masks. However, you should still have memory B-lymphocytic cells in your circulation that will rapidly reproduce and produce more antibodies against the virus should you become re-infected.

Some of my thoughts on COVID-19 and vaccinations are discussed in a recent, lengthy interview that I did for "What's Up Canada" that can be accessed with the following link: https://www.facebook.com/WhatsUpCanadians/videos/1392691891123832/

Another more recent interview that I did for Strong and Free Canada with Will Dove can be viewed at: https://strongandfreecanada.org/vlog/7646/

I particularly recommend that you check out the Canadian Covid Care Alliance (CCCA) at their website at www.canadiancovidcarealliance.org, which is continually updated with new materials that are posted weekly and has a newsletter that you can subscribe to.

One of the initiatives that the CCCA is undertaking relates to the issuing of vaccine passports. It has been implemented in some provinces, that people that have not been vaccinated, regardless of whether they have previously developed antibodies from infection with SARS-CoV-2, should be restricted from air and train travel and other activities, or at least segregated. Our studies are already generating data that demonstrates the superiority of natural immunity over vaccination, and there is a wealth of published data that also supports this (https://www.canadiancovidcarealliance.org/media-resources/natural-vs-vaccine-induced-immunity/). Furthermore, most of the over 1500 people that we have tested already have appreciable antibodies against SARS-CoV-2 and do not really need to subject themselves to the risks associated with the vaccines. These antibodies appear to be effective against the more infectious Delta SARS-CoV-2 variant, which appears to produce milder symptoms than other strains. Consequently, the imposition of vaccination is both discriminatory and unnecessary. Another CCCA initiative is concern about the vaccination of children, which may be at greater harm from the current COVID-19 vaccines than from actual infection with SARS-CoV-2.

I would be happy to discuss this with you further should you desire more clarification.

Best wishes from Steven Pelech.

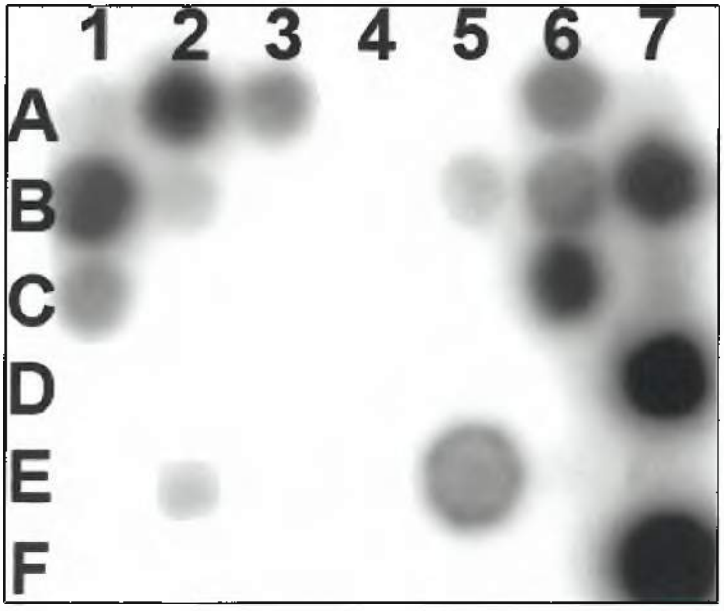
*"Klahowya" is the Chinook jargon greeting that is roughly translated "hello and how are you?" Chinook is a pidgin language that was commonly used for several hundred years as the main form of communication between West Coast natives of North America and European traders and explorers. In view of its origins from the Chinook, Nuuchanuulth, French and English languages, and its historical importance, it is my preferred form of salutation in these politically correct times.

Steven Pelech, Ph.D.
President & Chief Scientific Officer
Kinexus Bioinformatics Corporation

Chair, Scientific and Medical Advisory Committee
Canadian Covid Care Alliance

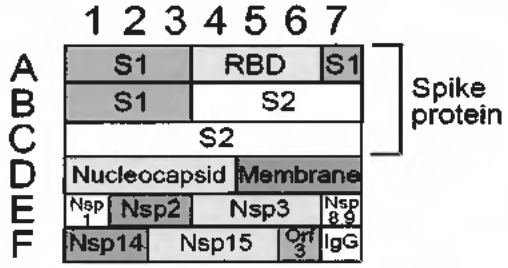
[redacted]

Your SARS-CoV-2 antibodies test results are shown immediately below. Spots A4 to A6 correspond to the ACE-2 receptor binding domain of the Spike protein of the virus and antibodies against this region are suspected to be the most protective. Spots D5-D7 correspond to the SARS-CoV-2 membrane protein, which is one of our best markers for previous SARS-CoV-2 infection. However, an absence of immunoreactivity with these membrane protein markers can also be observed in many COVID-19 recovered patients. The B7 spot is also amongst our most best markers, although this marker can be common in people who have had SARS-CoV-1 and cold coronaviruses in the past. Antibodies against the spike and membrane protein are expected to be able to bind intact virus and tag it for recognition by the immune cells for destruction of the virus.

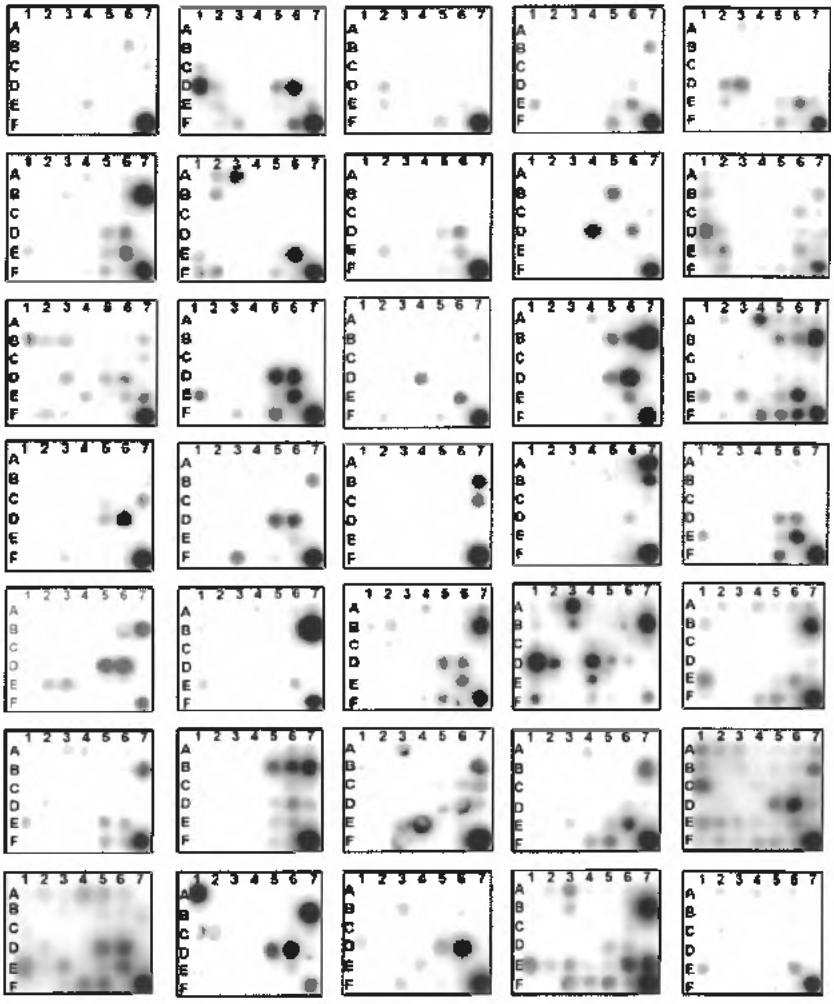


Examples of positive and negative results obtained with serum samples from 35 COVID-19 patients and 10 negative healthy controls with the Kinexus CCS SARS-CoV-2 Antibody Test.

Layout of the different parts of the SARS-CoV-2 proteins that are tested.



Recovered COVID-19 patient sera (confirmed by PCR tests)



Healthy negative control patient sera

Note that Spot F7 is a control peptide that binds IgG class antibodies and ensures that the test reagents are working properly.

Exhibit "D"

This is Exhibit "D" referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at Edmonton Alberta, this

[redacted]

From: [redacted]
Sent:
To:
Cc:

Subject:

Hi Nadr,

Thanks for your email and your resolve to do what you feel is right.

It doesn't seem right that this could take away your means to earn an income. I personally wonder about the legality of the stance ahs is taking. Is there really no clause for exceptions based on moral or religious grounds?

I found this article on cbc that highlights the complexities of this situation. https://www.google.com/amp/s/www.cbc.ca/amp/1.6142584

Nadr, have you considered talking to a lawyer? I can get a cursory opinion from friends and family who work in the legal profession.

If there is anything I can do to help please let me know.

Mark

On Tue., Sep. 21, 2021, 8:09 p.m. [redacted]

Hello All,

It is after extensive thought and reflection that I write this note to you. As you may or may not know, I have chosen not to be vaccinated at this time for personal, medical, professional and philosophical reasons. As you know, as of Nov 1st, those people who are unvaccinated will not be allowed to work on AHS properties. Thus, I will be forced to stop working as a surgeon. This clearly was not an easy decision for me after 24 years of clinical practice.

I have spoke to Dr. Williams and he said I will be furloughed (leave of absence) until I am vaccinated or something else happens. He did say that I can keep my research position although that was before the University changed its policy about rapid testing every week. But I can do that remotely and do not have to attend the lab in general. I am not sure what will happen with that in the future.

My clinical plan for the near future is to rent some space off AHS property for 2 half days per week. I hope that I can continue to see my ongoing patients that do not require plaster room access (like casts, sutures, etc) so as not to burden anyone too much. As those patient declare that they need surgery for nonunions, OA, etc, then I will have to refer them out to you guys. This will work as long as CPSA does not mandate vaccines which looks like they won't in the immediate future. I hope that many of you will be willing take on my active patients that require plaster room follow up. If evenly distributed, it is likely only 1 or 2 patients each for a few weeks.

I really don't know what is going to happen even before Nov 1st but figured I had better put this out there now so that it is not a surprise come Nov 1st. Things can change rapidly as well all know.

I am fine to discuss details if anyone wants to in person. Please let me know in the coming weeks if I can deflect some patients to you after Nov 1st.

Sincerely,

Nadr

[redacted]

From: [redacted]
Sent:
To:
Cc:

Subject: Re: My practice

Nadr: First of all I would like to say that I fully support you in your free choice and have nothing but respect for your informed decision. I think it is deplorable to force people to make decisions against their will to maintain employment or to be allowed to participate in society. Few of us have such courage.
I am more than happy to take any referrals, see patients in my cast clinic, or assist in any other way.

[redacted]

On Sep 21, 2021, at 8:09 PM, [redacted] wrote:

Hello All,

It is after extensive thought and reflection that I write this note to you. As you may or may not know, I have chosen not to be vaccinated at this time for personal, medical, professional and philosophical reasons. As you know, as of Nov 1st, those people who are unvaccinated will not be allowed to work on AHS properties. Thus, I will be forced to stop working as a surgeon. This clearly was not an easy decision for me after 24 years of clinical practice.

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I really don't know what is going to happen even before Nov 1st but figured I had better put this out there now so that it is not a surprise come Nov 1st. Things can change rapidly as well all know.

I am fine to discuss details if anyone wants to in person. Please let me know in the coming weeks if I can deflect some patients to you after Nov 1st.

Sincerely,
Nadr

From: [redacted]
To:
Subject: RE: My practice
Date: September 29, 2021 11:19:04 PM
Attachments: image002.jpg

Thanks Beth.

I will see how it goes.

Nadr

From: [redacted]
Sent: September 23, 2021 5:25 AM
To: [redacted]
Subject: Re: My practice

Nadr,

You will likely have enough support from Angie and Gord but if needed, I can see some of your foot and ankle patients.

Cheers,
Beth

On Tue, 21 Sept 2021 at 20:09, <[redacted] wrote:

> Hello All,
>
> It is after extensive thought and reflection that I write this note to you. As you may or may not know, I have chosen not to be vaccinated at this time for personal, medical, professional and philosophical reasons. As you know, as of Nov 1st, those people who are unvaccinated will not be allowed to work on AHS properties. Thus, I will be forced to stop working as a surgeon. This clearly was not an easy decision for me after 24 years of clinical practice.
>
> I have spoke to Dr. Williams and he said I will be furloughed (leave of absence) until I am vaccinated or something else happens. He did say that I can keep my research position although that was before the University changed its policy about rapid testing every week. But I can do that remotely and do not have to attend the lab in general. I am not sure what will happen with that in the future.
>
> My clinical plan for the near future is to rent some space off AHS property for 2 half days per week. I hope that I can continue to see my ongoing patients that do not require plaster room access (like casts, sutures, etc) so as not to burden anyone too much. As those patient declare that they need surgery for nonunions, OA, etc, then I will have to refer them out to you guys. This will work as long as CPSA does not mandate vaccines which looks like they won't in the immediate

future. I hope that many of you will be willing take on my active patients that require plaster room follow up. If evenly distributed, it is likely only 1 or 2 patients each for a few weeks.

I really don't know what is going to happen even before Nov 1st but figured I had better put this out there now so that it is not a surprise come Nov 1st. Things can change rapidly as well all know.

I am fine to discuss details if anyone wants to in person. Please let me know in the coming weeks if I can deflect some patients to you after Nov 1st.

Sincerely,
Nadr

Exhibit "E"

Exception request

Nadr Jomha [redacted]
Sat 10/16/2021 9:24 PM
To: MD & Midwifery COVID-19 Vaccination Exception Requests [redacted]
Cc: Nadr Jomha [redacted]

1 attachments (1 MB)
Exception request Oct 16, 2021.pdf;

Please find my exception request attached.

Regards,
Nadr

This is Exhibit "E" referred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at

[redacted]

Alberta Health Services

Medical or Midwifery Staff Request for Exception COVID-19 Mandatory Immunization for Workers

In keeping with AHS' mission and values and to protect AHS' workers, patients and others accessing the health system and at all AHS sites, AHS leadership has established the Immunization of Workers for COVID-19 Policy (Policy 1189) (the "Policy"). As of October 31, 2021, Alberta Health Services, Alberta Precision Laboratories, Carewest, CapitalCare, and Covenant Health employees, members of the medical and midwifery staffs, students, volunteers, and other persons acting on their behalf will be required to be fully vaccinated and have provided proof of vaccination to AHS.

This questionnaire may be submitted by any AHS Medical or Midwifery Staff member who is not an AHS, Alberta Precision Lab or Covenant Health employee who wishes to be granted an exception under the Policy. It may also be used by medical residents or fellows who are not AHS employees. If the request includes a medical exception request (Part 2 of this form), it must also be filled in and signed by a regulated Primary Care Provider. If the Medical or Midwifery Staff member is an AHS, Alberta Precision Lab or Covenant Health employee, the employee process must be followed and not this exception request process.

Completed forms should be submitted by email to md.midwife.covidvacc@ahs.ca

Part 1. Medical or Midwifery Staff Member Identification

Last Name: Jomha | First Name: Nadr

Regulatory College: ☑ CPSA ☐ ADAC ☐ Podiatry ☐ Midwifery | Registration Number: [redacted]

Nature of Exception Request

☐ Medical Exception *(Part 2 to be completed by Primary Care Practitioner)*
☑ Other Exception *(Part 3 to be completed the Medical or Midwifery Staff member)*

Part 2: Medical Exception Details

To be completed by the Primary Care Provider providing care to the Medical or Midwifery Staff Member named in Part 1. The Medical or Midwifery Staff member is responsible for any costs the Primary Care Provider may charge to complete this form.

☐ **I acknowledge** that I have reviewed the information on contraindications and recommended precautions for COVID-19 vaccines and links to resources *(pages 4 and 5 of this form).*

Number of years you have known the individual named in Part 1 as a patient of yours? _____

Does the patient have any of the contraindications or recommended precautions to receiving COVID-19 vaccine that are noted in the references provided? ☐ Yes ☐ No

If yes, please specify reason _____

Do you feel that the patient should not receive the COVID-19 vaccine due to a medical condition that **is not** listed as a contraindication or recommended precaution? ☐ Yes ☐ No

If yes, please specify reason _____

If your patient has a medical condition that precludes COVID-19 immunization, then what is the anticipated timeframe?

☐ Permanent ☐ Temporary *(if checked, specify time to resolution)* _____

Has your patient previously received a dose of COVID-19 vaccine? ☐ Yes ☐ No

If yes, details related to vaccine below ▼

Date Vaccine Received *(dd-Mon-yyyy)*	Type of Vaccine
	☐ Pfizer ☐ Moderna ☐ AstraZeneca ☐ Other *(specify)* _____

Were there any adverse reactions after receipt of COVID-19 vaccine? If yes, please provide details *(e.g. timing of reaction in relation to when vaccine was received, nature of the adverse reaction, any required treatment, etc)* and confirm if the Adverse Event Following Immunization (AEFI) was reported to Public Health *(https://www.albertahealthservices.ca/info/Page16187.aspx)*. Please provide any AEFI documentation if available.
Is there any additional information that you feel would be pertinent to your patient's request for an exception on medical grounds to AHS' COVID-19 immunization policy?

Primary Care Provider Name	Relevant Alberta Regulatory College
Signature	Date *(dd-Mon-yyyy)*

Your immunization status information is being collected under the authority of section 33(c) of the Freedom of Information and Protection of Privacy Act (Alberta), and will be used and disclosed as necessary to: (i) manage and administer your working relationship with AHS, Covenant Health, and Alberta Precision Labs, as applicable, including your fitness for work and exception requests, (ii) manage COVID-19 outbreaks, (iii) ensure that there are sufficient healthy staff available to provide health services to Albertans across the province, and (iv) comply with obligations under the Occupational Health and Safety Act (Alberta), the Regional Health Authorities Act (Alberta) and the Public Health Act (Alberta). If you have questions or concerns about the collection, use or disclosure of your information or the completion of this form, please contact an administrator at md.midwife.covidvacc@ahs.ca.

Part 3. Other Reason for Exception Request

To be completed by the Medical or Midwifery Staff member named in Part 1.

If there are any grounds other than medical on which you are requesting an exception under the Policy, please describe those grounds and any relevant, associated context.

I, Nadr Jomha, MD, PhD, FRCS(C) hereby advise that I am exempt by Canadian law and section 7 of the Canadian Charter of Rights and Freedoms, from having the Government of Alberta and Alberta Health Services unlawfully access my personal health information or force me against my will to be injected with any form of mRNA or DNA viral vector injection or any substance that I do not consent to. I make this declaration knowing that it has the full effect of protecting my rights under the Constitution of Canada without fear or coercion.

I DO NOT CONSENT to the access, distribution or release of my personal medical information or status to ANY extent. I believe that any attempt to punish any person on the basis of their refusal to grant consent to a medical procedure constitutes an attempted assault.

I trust this letter provides you more than sufficient information for the approval of my exception request. Please govern yourselves lawfully.

Medical or Midwifery Staff Member Signature	[redacted]	Date *(dd-Mon-yyyy)* 16-Oct-2021

Vaccine Product Monographs and Biological Pages

mRNA Vaccines

- Pfizer-BioNTech:
  - https://covid-vaccine.canada.ca/info/pdf/pfizer-biontech-covid-19-vaccine-pm1-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-pfizer-bio-pg-07-203.pdf
- Moderna:
  - https://covid-vaccine.canada.ca/info/pdf/covid-19-vaccine-moderna-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-moderna-bio-pg-07-204.pdf

Viral Vector Vaccines

- Astra-Zeneca:
  - https://covid-vaccine.canada.ca/info/pdf/astrazeneca-covid-19-vaccine-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-astrazeneca-covishield-bio-pg-07-205.pdf
- COVISHIELD:
  - https://covid-vaccine.canada.ca/info/pdf/covishield-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-astrazeneca-covishield-bio-pg-07-205.pdf
- Janssen: https://covid-vaccine.canada.ca/info/pdf/janssen-covid-19-vaccine-pm-en.pdf

Contraindications and Recommended Precautions for COVID-19 Vaccines

Source: National Advisory Committee on immunizations. Recommendations on the use of COVID-19 Vaccines (https://www.canada.ca/en/public-health/services/immunization/national-advisory-committee-on-immunization-naci/recommendations-use-covid-19-vaccines.html)

1. **An authorized COVID-19 vaccine should not be offered routinely to individuals with a history of severe allergic reaction (e.g., anaphylaxis) after previous administration of a COVID-19 vaccine using a similar platform (mRNA or viral vector)**. If a risk assessment deems that the benefits outweigh the potential risks for the individual, and if informed consent is provided, an authorized COVID-19 vaccine using a different platform may be considered for re-immunization (i.e., individuals with anaphylaxis post mRNA vaccine may be offered a viral vector vaccine and individuals with anaphylaxis post viral vector vaccine may be offered a mRNA vaccine).

   An authorized COVID-19 vaccine should not be routinely offered to individuals who are allergic to any component of the specific COVID-19 vaccine or its container.

   For a comprehensive list of components in the vaccine and packaging, please consult the product leaflet or information contained within the product monograph available through *Health Canada's Drug Product Database* (https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/drug-product-database.html).

   Table 4 (below) lists potential non-medicinal ingredients in authorized, available COVID-19 vaccines that have been associated with allergic reactions in other products. These reactions have occurred rarely and ranged from mild cutaneous reactions to anaphylaxis.

Table 4: Ingredients of authorized COVID-19 vaccines that have been associated with allergic reactions in other products

Vaccine product	Potential allergen included in the vaccine or its container	Other products where the potential allergen may be found*
Pfizer-BioNTech COVID-19 vaccine	polyethylene glycol (PEG) [a] [b] [c]	Over the counter (e.g., cough syrup, laxatives), and prescription medications, medical bowel preparation products for colonoscopy, skin care products, dermal fillers, cosmetics, contact lens care solutions, products such as ultrasound gel [d].
Moderna COVID-19 vaccine	PEG [a] [b] [c]	Over the counter (e.g. cough syrup, laxatives), and prescription medications, medical bowel preparation products for colonoscopy, skin care products, dermal fillers, cosmetics, contact lens care solutions, products such as ultrasound gel [d].
	tromethamine [e] (trometamol or Tris)	Component in contrast media, oral and parenteral medications.
AstraZeneca COVID-19 vaccine	polysorbate 80 [c].	medical preparations (e.g., vitamin oils, tablets, and anticancer agents), cosmetics [d] [f].
Janssen COVID-19 vaccine	polysorbate 80 [c].	medical preparations (e.g., vitamin oils, tablets, and anticancer agents), cosmetics [d] [f].

*N.B. This is not a complete list of products.

[a] Medications that contain PEG are described in Stone CA, et al., DOI:10.1016/j.jaip.2018.12.003

[b] A review of immediate type hypersensitivity reactions to PEG is available in Wenande et al, DOI: 10.1111/cea.12760

[c] There is a potential of cross-reactive hypersensitivity between PEG and polysorbates

[d] PEG is an additive in some food and drinks but allergic reactions to PEG in food or drinks have not been documented.

[e] One case report of anaphylaxis to tromethamine has been described (Lukawska et al, DOI: 10.1016/j.jaip.2018.08.035).

[f] Case reports of anaphylaxis to polysorbate 80 have been described (Badiu et al, DOI: 10.1136/bcr.02.2012.5797, Palacios Castaño et al, DOI: 10.18176/jiaci.0109).

2. Persons who received antiviral monoclonal antibody therapy or convalescent plasma for COVID-19 treatment:
   - There is insufficient evidence on the receipt of both a COVID-19 vaccine and anti-SARS-CoV-2 monoclonal antibodies or convalescent plasma for treatment or prevention. Therefore, timing of administration and potential interference between these two products are currently unknown and expert clinical opinion should be sought on a case-by-case basis.

3. Contraindications or recommended precautions to receiving mRNA COVID-19 vaccines (e.g. Pfizer-BioNTech, Moderna):
   - As a precautionary measure, the second dose in the mRNA COVID-19 vaccination series should be deferred in individuals who experience myocarditis or pericarditis following the first dose of an mRNACOVID-19 vaccine until more information is available.

4. Contraindications or recommended precautions to receiving viral vector COVID-19 vaccines (e.g. AstraZeneca, Janssen):
   - Patients who have experienced venous or arterial thrombosis with thrombocytopenia following vaccination with a viral vector COVID-19 vaccine should not receive a second dose of a viral vector COVID-19 vaccine.
   - As a precautionary measure following the international cases that have been reported, individuals with a history of capillary leak syndrome should not receive the AstraZeneca /COVISHIELD COVID-19 vaccine.

Exhibit "F"

This is **Exhibit "F"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at [redacted] for the Province of Alberta

Eva Chipiuk
Barrister & Solicitor

From: [redacted]
To:
Cc:
Subject: RE: Jomha (SUR) LOA - physician information requested
Date: November 8, 2021 4:23:00 PM

Hello Janice,

Please understand the extreme duress and distress this has caused me personally and professionally. Kindly provide me the curtesy of an appropriate amount of time to digest and respond accordingly. To date, I do not agree with the manner in which this was handled and the actions taken by the Zone and AHS.

I have recently submitted a religious exception request and await the response from that.

Regards,
Nadr

From: Janice [redacted]
Sent: November 3, 2021 8:56 AM
To: [redacted]
Subject: Jomha (SUR) LOA - physician information requested

Hi Dr. Jomha,

Please fill out the attached (LOA) document and return to myself at your earliest convenience.

Thanks,

Janice [redacted]

[redacted]

MedicalAffairs.EdmontonZone-PhysicianResources@ahs.ca

Exhibit "G"

From: Google Forms
To: [redacted]
Subject: University of Alberta Employee Vaccination and/or Rapid Testing Exemption Application (Non-Medical)
Date: November 8, 2021 2:41:58 PM

Thanks for filling out University of Alberta Employee Vaccination and/or Rapid Testing Exemption Application (Non-Medical)

Here's what was received.

This is **Exhibit "G"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at [redacted]

University of Alberta Employee Vaccination and/or Rapid Testing Exemption Application (Non-Medical)

Effective October 4, 2021, individuals may not attend any University of Alberta properties unless they are Fully Vaccinated or Partially Vaccinated and have provided Proof of Vaccination to the University. Individuals who are only Partially Vaccinated must continue with the University's COVID-19 rapid testing requirements until they are Fully Vaccinated. Others will not be entitled to use rapid testing as an alternative, except in cases of approved human rights-based accommodations.. **Note: This means that to be considered Partially Vaccinated by October 4, 2021, the individual must by no later than September 20, 2021 have received at least their first dose of a vaccine that will qualify them to ultimately become Fully Vaccinated. Effective November 1, 2021, individuals may not attend University of Alberta properties unless they are Fully Vaccinated and have provided Proof of Vaccination to the University. Individuals are required to maintain ongoing Fully Vaccinated status in the event that Health Canada updates its requirements for full vaccine protection, in which case the University will update and communicate timing requirements for maintaining Fully Vaccinated status accordingly. **Note: This means that to be considered Fully Vaccinated by November 1, 2021, the individual must by no later than October 18, 2021 have received their second dose of a vaccine that falls within the definition of Fully Vaccinated. The University will take reasonable steps to accommodate individuals who are impacted by operational changes due to COVID-19, including those who cannot or choose not to be vaccinated or participate in rapid testing on the basis of a protected ground under the University's Discrimination, Harassment and Duty to Accommodate Policy. Any employee who wishes to apply for an exemption from

the vaccination or rapid testing Directives on the basis of a non-medical protected ground must complete the Employee Vaccination, and/or Rapid Testing Exemption Application (Non-Medical) form. If you have questions or require assistance, please email your Human Resources Service Partner. Find your HR Service Partner at https://apps.hrs.ualberta.ca/HRSContactForm PLEASE NOTE: University of Alberta faculty or staff members who wish to apply for exemptions to any general safety measures directives should consult the Safety Measures General Directives at https://www.ualberta.ca/covid-19/campus-safety/safety-measures-general-directives/index.html.

Email *

[redacted]

Important Employee Vaccination, and/or Rapid Testing Exemption Application Guidelines

All components of the Employee Vaccination, and/or Rapid Testing Exemption Application (Non-Medical), including information submitted in this form, are subject to the policies and regulations of the University. - Requests for exemptions will be considered upon submission of a complete Employee Vaccination, and/or Rapid Testing Exemption Application (Non-Medical) form. - Incomplete applications will not be reviewed. - Application supporting documentation must be completed in English or French. - Employees are not approved for an exemption until they receive written notification of approval through their University email account. - The University reserves the right to make appropriate inquiries to verify the authenticity of applications, including review by applicable specialists for applications made on protected grounds under the University's Discrimination, Harassment or Duty to Accommodate Policy. The University may require that an employee applying for an exemption provide additional information in order to consider and decide on a request. - The duration of the exemption is at the sole determination of the University. Employees approved for an exemption may request an extension to the exemption duration, if required. - Where employees are approved for exemptions, the University will make efforts to reasonably accommodate. As part of a reasonable accommodation plan, employees may be required to comply with other measures, modifications, or adjustments, or conditions may be required in order to protect the health and safety of the university community. To continue to protect the health and safety of the campus community, at the sole discretion of the University, employees approved for exemptions to the directives may be accommodated through measures other than being granted access to campus. - Decisions may be appealed using the process outlined in the University's Duty to Accommodate Procedure. - Employees are permitted to reapply if new documentation and/or information becomes available, but employees are responsible for ensuring that they provide all available, relevant information at the time of application. - Approved exemptions apply only to requests for exemption from the University's directives. If you are seeking accommodations for other purposes, you will be required to make a separate application in accordance with existing accommodation registration procedures. If you have a previously approved accommodation, you must still submit the Employee Vaccination, and/or Rapid Testing Exemption Application (Non-Medical) form if you wish to be considered for an exemption from the directives. - Employees approved for exemptions from the directives will be notified by email and are advised to save the email notification in order that they may provide the email notification as proof of the exemption.

IMPORTANT INFORMATION ABOUT COMPLETING THIS FORM

-If you need to access the form in an alternate format or have questions, please contact your HR Service Partner. Find your HR Service Partner at https://apps.hrs.ualberta.ca/HRSContactForm - Any supporting documents uploaded in your application form must be PDFs. - You need to provide your University of Alberta Employee ID Number in the form. - Plan to spend about 5-10 minutes completing the form. - Please remember to click the SUBMIT button at the end of the form to submit your form. - A copy of your completed form will be automatically emailed to you. - Check your University of Alberta email account regularly for communications about your application.

RELATED LINKS

COVID-19 information for the University Community: https://www.ualberta.ca/covid-19/index.html Declaring Vaccination Status and Rapid Testing: https://www.ualberta.ca/covid-19/updates/2021/08/declaring-vaccination-status-rapid-testing.html Discrimination, Harassment and Duty to Accommodate Procedure: https://policiesonline.ualberta.ca/PoliciesProcedures/Policies/Discrimination-Harassment-and-Duty-to-Accommodate-Policy.pdf Duty to Accommodate Procedure: https://policiesonline.ualberta.ca/PoliciesProcedures/Procedures/Duty-to-Accommodate-Procedure.pdf Safety Measures General Directives: https://www.ualberta.ca/covid-19/campus-safety/safety-measures-general-directives/index.html

Privacy Notice

PLEASE NOTE: The personal information you provide on this form is confidential. The personal information requested is collected under the authority of Section 33(c) of the Alberta Freedom of Information and Protection of Privacy (FOIPP) Act and will be protected under Part 2 of that Act. It will be used for registration and administrative purposes in the above program offered by the University of Alberta. This information may be shared as needed to facilitate Employee Vaccination, and/or Rapid Testing Exemption Application (Non-Medical) with other University of Alberta faculties, departments, or units. For further information, contact Marj Cayford, Director HR Service Partnerships, 2-60 University Terrace, Edmonton, Alberta, T6G 1K4; email: hrs.covid19@ualberta.ca

Demographic Information

Please provide the folllowing demographic information.

Last Name *

Jomha

First Name *

Nadr

Please include your University of Alberta Email Address *

[redacted]

Telephone number (including area code) * *

[redacted]

In which Unit/Department/Faculty do you work? *

Surgery/Faculty of Medicine and Dentistry

Please include your Employee ID Number *

[redacted]

Non-Medical Exception Based on a Protected Ground

Are you applying for an exemption based on a protected ground in the University's Discrimination, Harassment and Duty to Accommodate Policy? * *

- ◯ Yes

◉ No

Upload Your Protect Ground Information

Please attach/upload your documentation below in PDF format. You are able to attach up to five (5) individual files. Documents that are not clearly readable will not be accepted. If you cannot scan your documentation or access electronic copies of your documentation, you can use a smartphone to take a picture of each individual page of your documentation and convert the images into a PDF document using applications/programs such as iScanner, Cam Scanner, and Adobe. Please use the following format to name all PDFs: last name_first initial_yyyymmdd (e.g., Smith_J_20200125).

File Upload

Submitted files

- ☐ Imam letter Nov 8, 21 - Nadr Jomha.pdf
- ☐ Covid exemption letter Nov 8 21 - Nadr Jomha.pdf

On the basis of which protected grounds are you applying for an exemption? Please select all that apply. *

- ☐ Race
- ■ Religious beliefs
- ☐ Colour
- ☐ Ancestry
- ☐ Place of origin
- ☐ Gender, gender identity and gender expression
- ☐ Marital status
- ☐ Family status

- [ ] Source of income
- [ ] Sexual orientation
- [ ] Age
- [ ] Political beliefs

Please explain in detail how and why this protected ground makes you unable to be vaccinated and/or complete rapid testing. *

It is based on my deeply held religious and cultural beliefs that I should have choice after informed consent of whether to take a medical treatment like a vaccine. Please see two attached letters.

Please provide any other relevant information that is important for understanding your exemption application.

I intend to continue to work remotely until the situation is considered more amenable for unvaccinated people to return. I will pose no risk to University staff or students.

Please click the SUBMIT button below to submit your responses

A copy of your responses will be sent to your University of Alberta email account. PLEASE NOTE: If you would like to review or change any of your responses, before submitting the form, use the Back button to return to the previous page(s). Once you submit the form, you will not be able to return to or edit your form.

Alberta Health Services

Medical or Midwifery Staff Request for Exception COVID-19 Mandatory Immunization for Workers

In keeping with AHS' mission and values and to protect AHS' workers, patients and others accessing the health system and at all AHS sites, AHS leadership has established the Immunization of Workers for COVID-19 Policy (Policy 1189) (the "Policy"). As of October 31, 2021, Alberta Health Services, Alberta Precision Laboratories, Carewest, CapitalCare, and Covenant Health employees, members of the medical and midwifery staffs, students, volunteers, and other persons acting on their behalf will be required to be fully vaccinated and have provided proof of vaccination to AHS.

This questionnaire may be submitted by any AHS Medical or Midwifery Staff member who is not an AHS, Alberta Precision Lab or Covenant Health employee who wishes to be granted an exception under the Policy. It may also be used by medical residents or fellows who are not AHS employees. If the request includes a medical exception request (Part 2 of this form), it must also be filled in and signed by a regulated Primary Care Provider. If the Medical or Midwifery Staff member is an AHS, Alberta Precision Lab or Covenant Health employee, the employee process must be followed and not this exception request process.

Completed forms should be submitted by email to md.midwife.covidvacc@ahs.ca

Part 1. Medical or Midwifery Staff Member Identification

Last Name: Jomha

First Name: Nadr

Regulatory College

☑ CPSA ☐ ADAC ☐ Podiatry ☐ Midwifery

[redacted]ion Number [redacted]

Nature of Exception Request

☐ Medical Exception *(Part 2 to be completed by Primary Care Practitioner)*

☑ Other Exception *(Part 3 to be completed the Medical or Midwifery Staff member)*

Part 2: Medical Exception Details

To be completed by the Primary Care Provider providing care to the Medical or Midwifery Staff Member named in Part 1. The Medical or Midwifery Staff member is responsible for any costs the Primary Care Provider may charge to complete this form.

☐ **I acknowledge** that I have reviewed the information on contraindications and recommended precautions for COVID-19 vaccines and links to resources *(pages 4 and 5 of this form).*

Number of years you have known the individual named in Part 1 as a patient of yours? _____

Does the patient have any of the contraindications or recommended precautions to receiving COVID-19 vaccine that are noted in the references provided? ☐ Yes ☐ No

If **yes**, please specify reason _____

Do you feel that the patient should not receive the COVID-19 vaccine due to a medical condition that **is not** listed as a contraindication or recommended precaution? ☐ Yes ☐ No

If **yes**, please specify reason _____

If your patient has a medical condition that precludes COVID-19 immunization, then what is the anticipated timeframe?

☐ Permanent ☐ Temporary *(if checked, specify time to resolution)* _____

Has your patient previously received a dose of COVID-19 vaccine? ☐ Yes ☐ No

If yes, details related to vaccine below ▼

Date Vaccine Received *(dd-Mon-yyyy)*	Type of Vaccine
	☐ Pfizer ☐ Moderna ☐ AstraZeneca ☐ Other *(specify)* _____

Were there any adverse reactions after receipt of COVID-19 vaccine? If yes, please provide details *(e.g. timing of reaction in relation to when vaccine was received, nature of the adverse reaction, any required treatment, etc)* and confirm if the Adverse Event Following Immunization (AEFI) was reported to Public Health *(https://www.albertahealthservices.ca/info/Page16187.aspx).* Please provide any AEFI documentation if available.
Is there any additional information that you feel would be pertinent to your patient's request for an exception on medical grounds to AHS' COVID-19 immunization policy?

Primary Care Provider Name	Relevant Alberta Regulatory College
Signature	Date *(dd-Mon-yyyy)*

Your immunization status information is being collected under the authority of section 33(c) of the Freedom of Information and Protection of Privacy Act (Alberta), and will be used and disclosed as necessary to: (i) manage and administer your working relationship with AHS, Covenant Health, and Alberta Precision Labs, as applicable, including your fitness for work and exception requests, (ii) manage COVID-19 outbreaks, (iii) ensure that there are sufficient healthy staff available to provide health services to Albertans across the province, and (iv) comply with obligations under the Occupational Health and Safety Act (Alberta), the Regional Health Authorities Act (Alberta) and the Public Health Act (Alberta). If you have questions or concerns about the collection, use or disclosure of your information or the completion of this form, please contact an administrator at md.midwife.covidvacc@ahs.ca.

Part 3. Other Reason for Exception Request

To be completed by the Medical or Midwifery Staff member named in Part 1.

If there are any grounds other than medical on which you are requesting an exception under the Policy, please describe those grounds and any relevant, associated context.

My request for non-medical exemption is based on the Alberta Human Rights Act which states in its preamble "WHEREAS recognition of the inherent dignity and the equal and inalienable rights of all persons is the foundation of freedom, justice and peace in the world" followed by:

"Discrimination re goods, services, accommodation, facilities

4 No person shall

…

(b) discriminate against any person or class of persons with respect to any goods, services, accommodation or facilities that are customarily available to the public, because of the race, religious beliefs, colour, gender, gender identity, gender expression, physical disability, mental disability, age, ancestry, place of origin, marital status, source of income, family status or sexual orientation of that person or class of persons or of any other person or class of persons."

And;

"Discrimination re employment practices

7(1) No employer shall

(a) refuse to employ or refuse to continue to employ any person, or

(b) discriminate against any person with regard to employment or any term or condition of employment, because of the race, religious beliefs, colour, gender, … of that person or of any other person."

My request for exemption is my deeply held Islamic religious belief that my body is sacred and that my body and mind are solely owned by God and me and I shall not willingly do anything that is against my inner sanctity. I have held this belief since it was instilled in me as a child but honed over the past 30+ years through regular prayer, fasting, abstinence from toxic substances, compassionate life practice and deep thought. This relates to the vaccine in the following ways.

1. I view my body as sacred and I strive to only put pure, natural substances into it. It is a basic Islamic principle that your body is sacred and a gift from God which should be treated with the utmost respect. I treat my body as such. I do not smoke, drink alcohol or do any drugs. I eat healthy and exercise regularly. The vaccines are designed to engineer my own cells to produce a spike protein which has conclusive been shown to be toxic in published literature. I will not knowingly and willing introduce toxic substances into my body as I have not done for the past 55 years.

2. My mind is mine to control. Autonomy is considered as the essential ethical principle by many ethicists and this autonomy is sanctified in Islam. Further, it is an established Islamic principle that no one is allowed to usurp another person's rights without their permission. This leads to the issue of informed consent which is the bedrock of modern medical practice. Informed consent involves discussion of all real and possible benefits and risks of a treatment. It also includes the option to accept or decline treatment.

In conclusion, supported by my Islamic imam (letter attached) and based on my deeply held and long-lived belief in the Islamic principles of sanctity of body, autonomy and informed consent, I request an exemption from taking the vaccine.

I DO NOT CONSENT to the access, distribution or release of my personal medical information or status to ANY extent.

Medical or Midwifery Staff Member Signature	[redacted]	Date *(dd-Mon-yyyy)* 08-Nov-2021

Vaccine Product Monographs and Biological Pages

mRNA Vaccines

- Pfizer-BioNTech:
  - https://covid-vaccine.canada.ca/info/pdf/pfizer-biontech-covid-19-vaccine-pm1-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-pfizer-bio-pg-07-203.pdf
- Moderna:
  - https://covid-vaccine.canada.ca/info/pdf/covid-19-vaccine-moderna-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-moderna-bio-pg-07-204.pdf

Viral Vector Vaccines

- Astra-Zeneca:
  - https://covid-vaccine.canada.ca/info/pdf/astrazeneca-covid-19-vaccine-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-astrazeneca-covishield-bio-pg-07-205.pdf
- COVISHIELD:
  - https://covid-vaccine.canada.ca/info/pdf/covishield-pm-en.pdf
  - https://www.albertahealthservices.ca/assets/info/hp/cdc/if-hp-cdc-ipsm-covid-19-astrazeneca-covishield-bio-pg-07-205.pdf
- Janssen: https://covid-vaccine.canada.ca/info/pdf/janssen-covid-19-vaccine-pm-en.pdf

Contraindications and Recommended Precautions for COVID-19 Vaccines

Source: National Advisory Committee on immunizations. Recommendations on the use of COVID-19 Vaccines (https://www.canada.ca/en/public-health/services/immunization/national-advisory-committee-on-immunization-naci/recommendations-use-covid-19-vaccines.html)

1. **An authorized COVID-19 vaccine should not be offered routinely to individuals with a history of severe allergic reaction (e.g., anaphylaxis) after previous administration of a COVID-19 vaccine using a similar platform (mRNA or viral vector)**. If a risk assessment deems that the benefits outweigh the potential risks for the individual, and if informed consent is provided, an authorized COVID-19 vaccine using a different platform may be considered for re-immunization (i.e., individuals with anaphylaxis post mRNA vaccine may be offered a viral vector vaccine and individuals with anaphylaxis post viral vector vaccine may be offered a mRNA vaccine).

   An authorized COVID-19 vaccine should not be routinely offered to individuals who are allergic to any component of the specific COVID-19 vaccine or its container.

   For a comprehensive list of components in the vaccine and packaging, please consult the product leaflet or information contained within the product monograph available through *Health Canada's Drug Product Database* (https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/drug-product-database.html).

   Table 4 (below) lists potential non-medicinal ingredients in authorized, available COVID-19 vaccines that have been associated with allergic reactions in other products. These reactions have occurred rarely and ranged from mild cutaneous reactions to anaphylaxis.

Table 4: Ingredients of authorized COVID-19 vaccines that have been associated with allergic reactions in other products

Vaccine product	Potential allergen included in the vaccine or its container	Other products where the potential allergen may be found*
Pfizer-BioNTech COVID-19 vaccine	polyethylene glycol (PEG) [a] [b] [c]	Over the counter (e.g., cough syrup, laxatives), and prescription medications, medical bowel preparation products for colonoscopy, skin care products, dermal fillers, cosmetics, contact lens care solutions, products such as ultrasound gel [d].
Moderna COVID-19 vaccine	PEG [a] [b] [c]	Over the counter (e.g. cough syrup, laxatives), and prescription medications, medical bowel preparation products for colonoscopy, skin care products, dermal fillers, cosmetics, contact lens care solutions, products such as ultrasound gel [d].
	tromethamine [e]. (trometamol or Tris)	Component in contrast media, oral and parenteral medications.
AstraZeneca COVID-19 vaccine	polysorbate 80 [c].	medical preparations (e.g., vitamin oils, tablets, and anticancer agents), cosmetics [d] [f].
Janssen COVID-19 vaccine	polysorbate 80 [c].	medical preparations (e.g., vitamin oils, tablets, and anticancer agents), cosmetics [d] [f].

*N.B. This is not a complete list of products.

[a] Medications that contain PEG are described in Stone CA, et al., DOI:10.1016/j.jaip.2018.12.003

[b] A review of immediate type hypersensitivity reactions to PEG is available in Wenande et al, DOI: 10.1111/cea.12760

[c] There is a potential of cross-reactive hypersensitivity between PEG and polysorbates

[d] PEG is an additive in some food and drinks but allergic reactions to PEG in food or drinks have not been documented.

[e] One case report of anaphylaxis to tromethamine has been described (Lukawska et al, DOI: 10.1016/j.jaip.2018.08.035).

[f] Case reports of anaphylaxis to polysorbate 80 have been described (Badiu et al, DOI: 10.1136/bcr.02.2012.5797, Palacios Castaño et al, DOI: 10.18176/jiaci.0109).

2. Persons who received antiviral monoclonal antibody therapy or convalescent plasma for COVID-19 treatment:
   - There is insufficient evidence on the receipt of both a COVID-19 vaccine and anti-SARS-CoV-2 monoclonal antibodies or convalescent plasma for treatment or prevention. Therefore, timing of administration and potential interference between these two products are currently unknown and expert clinical opinion should be sought on a case-by-case basis.

3. Contraindications or recommended precautions to receiving mRNA COVID-19 vaccines (e.g. Pfizer-BioNTech, Moderna):
   - As a precautionary measure, the second dose in the mRNA COVID-19 vaccination series should be deferred in individuals who experience myocarditis or pericarditis following the first dose of an mRNACOVID-19 vaccine until more information is available.

4. Contraindications or recommended precautions to receiving viral vector COVID-19 vaccines (e.g. AstraZeneca, Janssen):
   - Patients who have experienced venous or arterial thrombosis with thrombocytopenia following vaccination with a viral vector COVID-19 vaccine should not receive a second dose of a viral vector COVID-19 vaccine.
   - As a precautionary measure following the international cases that have been reported, individuals with a history of capillary leak syndrome should not receive the AstraZeneca /COVISHIELD COVID-19 vaccine.

Universal Muslim Association
519 Lessard Drive, Edmonton, Alberta
Canada T6M 1A9
Telephone & Fax: (403) 444-4408

جمعية المسلمين العالمية
٥١٩ طريق لسارد - إدمنتن - ألبرتا - كندا
رقم الهاتف والفكس : ٤٤٠٨ - ٤٤٤ (٤٠٣)

To Whom It May Concern

November 05, 2021

Assertion of Religious Exemption to Covid-19 Vaccination in support of Dr. Nadr M. Jomha

Since the outbreak of Covid-19, the vaccine issue has occupied the religious agenda of many Muslim scholars and institutions in North America. Taking the vaccine is widely-viewed by most of Islamic institutions, including the Canadian Council of Imams (clergies), to be recommended; not mandatory.

Being religiously recommended means that a Muslim has the choice to do it or not based on his free will; a thing which can not be compromised in Islam. As Muslims, we believe in having the full choice to do or not to do an act as long as we are aware of its positive impacts and negative consequences.

Compulsion and coercion are abhorred prohibited practices in Islam. A Muslim is not expected/supposed to practice them himself/herself with others, or let others do the same to him/her. Ideological compulsion to embrace a specific thought, creed, way of thinking, way of living, theoretical orientation, or a medical treatment/immunization choice are all strictly prohibited practices for a Muslim to tolerate or accept.

Based on the above, we conclude that in Islam, a Muslim is given the full choice and consent to choose to be vaccinated or not, treated from a specific disease or not. With the above in mind, it is, therefore, the absolute religious choice of Dr. Jomha, Nadr Mohamed, Professor at the Department of Surgery, Division of Orthopedc Surgery, University of Alberta to get any of the Covid-19 vaccines or not. Our religious view is that your esteemed institution(s) goes by the will of our community member (Dr. Jomha) by allowing him the exemption from the Covid-19 vaccines based on the religiously-outlined reasons and justifications above.

[redacted]

Exhibit "H"

Alberta Health Services

Healthy Albertans. Healthy Communities. Together.

Canada's Top 100 Employers 2018

This is **Exhibit "H"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at [redacted]

CONFIDENTIAL

November 18, 2021

Dr. Nadr Jomha
[redacted]

Sent via email: [redacted]

Dear Dr. Jomha:

Re: Request for Exception to Immunization of Workers for COVID-19 Policy, File #1920

I am in receipt of your request for an exception, dated November 8, 2021, to the Immunization of Workers for COVID-19 Policy (Policy). I also acknowledge receiving the letter, written by your Imam and dated November 5, 2021, and submitted by you on November 8, 2021. All exception requests for members of the Medical Staff are reviewed by the Exception Review Panel, which makes a recommendation to me in my role as Zone Medical Director. Please find enclosed with this letter a copy of the report from the Exception Review Panel dated November 15, 2021.

As set out in the enclosed report, the Exception Review Panel has recommended that your exception request for non-medical reasons be denied.

I agree with the recommendation of the Exception Review Panel. While I understand that you have expressed a religious belief against receiving a Covid-19 vaccine, Alberta Health Services' (AHS) foremost concern is to ensure the safety and wellbeing of its staff and the patients under its care. As a result, AHS will not be granting you an exception as mandatory vaccination is necessary to protect the patients and staff at its facilities and to ensure the continued delivery of healthcare in a safe manner.

As your request for an exception to the Policy is being denied, in accordance with section 3.4.4.3 of the Medical Staff Rules (Rules) I have determined that further action or investigation is required by my office. In accordance with section 3.4.4.5 of the Rules, my office will proceed to schedule a brief online or telephone meeting with you to discuss whether you intend to become fully immunized, and the path forward.

In accordance with section 4 of the Policy, at this meeting, we can also discuss any concerns you may have regarding the COVID-19 vaccination and any information that would assist you in making your decision. Please let me know if you would like me to facilitate a meeting.

Office of Edmonton Zone Medical Director
11th Floor, South Tower, Seventh Street Plaza
Edmonton, AB T5J 3E4
www.ahs.ca

If you are unable to meet the deadline of **November 30, 2021**, to become fully immunized, AHS will take steps in accordance with the non-compliance section of the Policy.

Sincerely,

[redacted]

David Zygun, MD MSc. FRCPC
Edmonton Zone Medical Director
Alberta Health Services

Encl. 1. Exception Review Panel Report dated November 15, 2021

Alberta Health Services

Edmonton Zone
RECOMMENDATION by the AHS MEDICAL and MIDWIFERY STAFF EXCEPTION REVIEW PANEL on an EXCEPTION REQUEST of the IMMUNIZATION OF WORKERS FOR COVID-19 POLICY 1189

CONFIDENTIAL

Name of Medical Staff Member: Dr. Nadr Jomha

[redacted]

Date: November 15, 2021

I. Nature of the Request

On November 8, 2021 Dr. Nadr Jomha submitted a request for an exception of the AHS Immunization of Workers for COVID-19 Policy 1189 (Policy), in accordance with paragraph 3.4 of the Policy. The request for exception was for Non-Medical Reasons.

II. Supporting Documentation Provided

On November 8, 2021 Dr. Nadr Jomha submitted the following documents in support of the exception request:

a. AHS religious exception Nov 8, 21.pdf
b. Imram letter Nov 8, 21.pdf

III. Recommendation

The applicant was not compliant with the exception deadline of October 16, 2021, however the Exception Review Panel still considered the request.

In considering the request dated November 8 2021 and the documents provided, the Panel recommends that an exception on the basis of Non-Medical Reasons **not be approved** by the Edmonton Zone Medical Director.

IV. Reasons

The applicant has applied for a **non-medical exception** to receiving the COVID-19 vaccination. The request was reviewed by the members of the Physician & Midwifery COVID-19 Vaccination Exemption Review Panel who reached a unanimous decision that the exception **is not recommended.**

AHS is committed to protecting the health and safety of its workers, patients, visitors, and others accessing AHS sites. Immunization against COVID-19 is the most effective means to prevent the spread of COVID-19, to prevent outbreaks in AHS facilities, to preserve workforce capacity to support the health care system, and to protect our workers, patients, visitors, and others accessing AHS sites. Immunization against COVID-19 also supports AHS' Values of Compassion, Accountability, Respect, Excellence, and Safety.

On September 14, 2021, AHS implemented the Policy to address immunization requirements for COVID-19 as a measure to protect the health and safety of workers, patients, and the communities AHS serves. The Policy applies to all AHS employees and members of the Medical and Midwifery Staff, except as otherwise indicated.

The Policy requires that all workers (as defined the Policy) must be fully immunized against COVID-19 by October 31, 2021. Fully immunized means having received two doses of a vaccine considered valid by Alberta Health in a two dose COVID-19 vaccine series or one dose of a vaccine considered valid by Alberta Health in a one dose COVID-19 vaccine series; and for whom fourteen days have elapsed since the date on which the person received the second dose of the COVID-19 vaccine considered valid by Alberta Health of a two dose series or one dose of the COVID-19 vaccine considered valid by Alberta Health in a one dose vaccine series.

The Policy contemplates that there may be instances in which a member of the Medical Staff is unable to be immunized due to a medical reason. In such instances, and upon the request of the individual, this Panel has evaluated the exception request.

V. Next Steps

This recommendation will be provided to Dr. David Zygun, Edmonton Zone Medical Director.

Exhibit "I"

This is **Exhibit "I"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at

[redacted]

From: [redacted]
To:
Cc:
Subject: RE: Jomha (SUR) LOA - physician information requested
Date: November 8, 2021 4:23:00 PM

Hello Janice,

Please understand the extreme duress and distress this has caused me personally and professionally. Kindly provide me the curtesy of an appropriate amount of time to digest and respond accordingly. To date, I do not agree with the manner in which this was handled and the actions taken by the Zone and AHS.

I have recently submitted a religious exception request and await the response from that.

Regards,
Nadr

From: [redacted]
Sent: November 3, 2021 8:56 AM
To: [redacted]
[redacted] physician information requested

Hi Dr. Jomha,

Please fill out the attached (LOA) document and return to myself at your earliest convenience.

Thanks,

Janice [redacted]

From: [redacted]
To:
Cc:
Subject: RE: Notice of Concern
Date: November 23, 2021 10:16:57 PM
Attachments: ~WRD0000.jpg
image001.png
Sensitivity: Confidential

Dear Dr. Zygun and Dr. Manns,

Please understand the extreme duress and distress this has caused me personally and professionally. I have many career and family considerations that are extremely difficult to consider and deal with. Kindly provide me the courtesy of an appropriate amount of time to digest and respond accordingly. To date, I do not agree with the manner in which this was handled and the actions taken by yourself and AHS.

Sincerely,
Nadr

From: [redacted]
Sent: November 23, 2021 2:33 PM
To: [redacted]
Cc:
Subject: Notice of Concern
Importance: High
Sensitivity: Confidential

David Zygun [redacted] has sent you a protected message. You will need to obtain a single-use code to read the message: 1. Select Read the message. 2. You'll be redirected to a page where you can sign in and receive a one-time passcode. 3. Check your email for the one-time passcode. Enter the code in the browser window, then select Continue to read your message.

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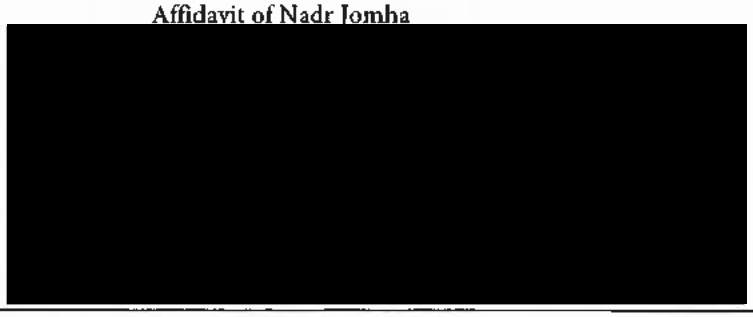
Exhibit "J"



Article

SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans

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Long-lived bone marrow plasma cells (BMPCs) are a persistent and essential source of protective antibodies[1–7]. Individuals who have recovered from COVID-19 have a substantially lower risk of reinfection with SARS-CoV-2[8–10]. Nonetheless, it has been reported that levels of anti-SARS-CoV-2 serum antibodies decrease rapidly in the first few months after infection, raising concerns that long-lived BMPCs may not be generated and humoral immunity against SARS-CoV-2 may be short-lived[11–13]. Here we show that in convalescent individuals who had experienced mild SARS-CoV-2 infections ($n = 77$), levels of serum anti-SARS-CoV-2 spike protein (S) antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection. Anti-S antibody titres correlated with the frequency of S-specific plasma cells in bone marrow aspirates from 18 individuals who had recovered from COVID-19 at 7 to 8 months after infection. S-specific BMPCs were not detected in aspirates from 11 healthy individuals with no history of SARS-CoV-2 infection. We show that S-binding BMPCs are quiescent, which suggests that they are part of a stable compartment. Consistently, circulating resting memory B cells directed against SARS-CoV-2 S were detected in the convalescent individuals. Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans.

Reinfections by seasonal coronaviruses occur 6 to 12 months after the previous infection, indicating that protective immunity against these viruses may be short-lived[14,15]. Early reports documenting rapidly declining antibody titres in the first few months after infection in individuals who had recovered from COVID-19 suggested that protective immunity against SARS-CoV-2 might be similarly transient[11–13]. It was also suggested that infection with SARS-CoV-2 could fail to elicit a functional germinal centre response, which would interfere with the generation of long-lived plasma cells[3–5,7,16]. More recent reports analysing samples that were collected approximately 4 to 6 months after infection indicate that SARS-CoV-2 antibody titres decline more slowly than in the initial months after infection[8,17–21]. Durable serum antibody titres are maintained by long-lived plasma cells—non-replicating, antigen-specific plasma cells that are detected in the bone marrow long after the clearance of the antigen[1–7]. We sought to determine whether they were detectable in convalescent individuals approximately 7 months after SARS-CoV-2 infection.

Biphasic decay of anti-S antibody titres

(49% female, 51% male, median age 49 years), the majority of whom had experienced mild illness (7.8% hospitalized, Extended Data Tables 1, 2). Follow-up blood samples were collected three times at approximately three-month intervals. Twelve convalescent participants received either the BNT162b2 (Pfizer) or the mRNA-1273 (Moderna) SARS-CoV-2 vaccine between the last two time points; these post-vaccination samples were not included in our analyses. In addition, bone marrow aspirates were collected from 18 of the convalescent individuals at 7 to 8 months after infection and from 11 healthy volunteers with no history of SARS-CoV-2 infection or vaccination. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent individuals and from 1 additional convalescent donor approximately 11 months after infection (Fig. 1a, Extended Data Tables 3, 4). We first performed a longitudinal analysis of circulating anti-SARS-CoV-2 serum antibodies. Whereas anti-SARS-CoV-2 spike protein (S) IgG antibodies were undetectable in blood from control individuals, 74 out of the 77 convalescent individuals had detectable serum titres approximately 1 month after the onset of symptoms. Between 1 and 4 months after symptom onset, overall anti-S IgG titres decreased from a mean $\log_e$-transformed half-maximal

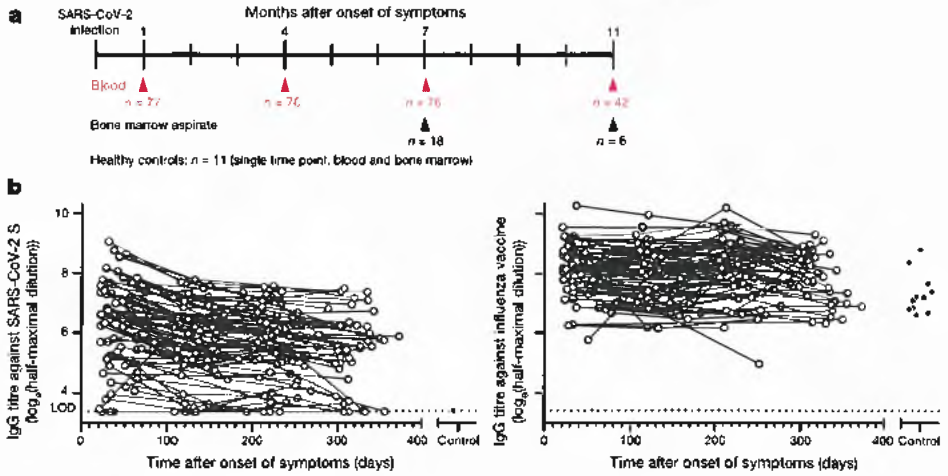


Fig. 1 | SARS-CoV-2 infection elicits durable serum anti-S antibody titres. **a**, Study design. Seventy-seven convalescent individuals who had experienced mild SARS-CoV-2 infections (aged 21–69 years) were enrolled and blood was collected approximately 1 month, 4 months, 7 months and 11 months after the onset of symptoms. Bone marrow aspirates were collected from 18 of the convalescent individuals 7 to 8 months after infection and from 11 healthy volunteers (aged 23–60 years) with no history of SARS-CoV-2 infection. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent donors and 1 additional convalescent donor approximately 11 months after infection. **b**, Blood IgG titres against SARS-CoV-2 S (left) and influenza virus vaccine (right) measured by enzyme-linked immunosorbent assay (ELISA) in convalescent individuals (white circles) at the indicated time after onset of symptoms, and in control individuals (black circles). The dotted lines indicate the limit of detection (LOD). Mean titres and pairwise differences at each time point were estimated using a linear mixed model analysis.

of decline slowed, and mean titres decreased from 5.7 to 5.3 (mean difference 0.44 ± 0.10, $P < 0.001$; Fig. 1a). In contrast to the anti-S antibody titres, IgG titres against the 2019–2020 inactivated seasonal influenza virus vaccine were detected in all control individuals and individuals who were convalescing from COVID-19, and declined much more gradually, if at all over the course of the study, with mean titres decreasing from 8.0 to 7.9 (mean difference 0.16 ± 0.06, $P = 0.042$) and 7.9 to 7.8 (mean difference 0.02 ± 0.08, $P = 0.997$) across the 1-to-4-month and 4-to-11-month intervals after symptom onset, respectively (Fig. 1b).

Induction of S-binding long-lived BMPCs

The relatively rapid early decline in the levels of anti-S IgG, followed by a slower decrease, is consistent with a transition from serum antibodies being secreted by short-lived plasmablasts to secretion by a smaller but more persistent population of long-lived plasma cells generated later in the immune response. The majority of this latter population resides in the bone marrow[1–6]. To investigate whether individuals who had recovered from COVID-19 developed a virus-specific long-lived BMPC compartment, we examined bone marrow aspirates obtained approximately 7 and 11 months after infection for anti-SARS-CoV-2 S-specific BMPCs. We magnetically enriched BMPCs from the aspirates and then quantified the frequencies of those secreting IgG and IgA directed against the 2019–2020 influenza virus vaccine, the tetanus–diphtheria vaccine and SARS-CoV-2 S by enzyme-linked immunosorbent spot assay (ELISpot) (Fig. 2a). Frequencies of influenza- and tetanus–diphtheria-vaccine-specific BMPCs were comparable between control individuals and convalescent individuals. IgG- and IgA-secreting S-specific BMPCs were detected in 15 and 9 of the 19 convalescent individuals, respectively, but not in any of the 11 control individuals (Fig. 2b). Notably, none of the control individuals or convalescent individuals BMPC frequencies, anti-S IgG titres in the 5 convalescent individuals remained consistent between 7 and 11 months after symptom onset. IgG titres measured against the receptor-binding domain (RBD) of the S protein—a primary target of neutralizing antibodies—were detected in 4 of the 5 convalescent individuals and were also stable between 7 and 11 months after symptom onset (Fig. 2d). Frequencies of anti-S IgG BMPCs showed a modest but significant correlation with circulating anti-S IgG titres at 7–8 months after the onset of symptoms in convalescent individuals, consistent with the long-term maintenance of antibody levels by these cells ($r = 0.48$, $P = 0.046$). In accordance with previous reports[22–24], frequencies of influenza-vaccine-specific IgG BMPCs and antibody titres exhibited a strong and significant correlation ($r = 0.67$, $P < 0.001$; Fig. 2e). Nine of the aspirates from control individuals and 12 of the 18 aspirates that were collected 7 months after symptom onset from convalescent individuals yielded a sufficient number of BMPCs for additional analysis by flow cytometry. We stained these samples intracellularly with fluorescently labelled S and influenza virus haemagglutinin (HA) probes to identify and characterize antigen-specific BMPCs. As controls, we also intracellularly stained peripheral blood mononuclear cells (PBMCs) from healthy volunteers one week after vaccination against SARS-CoV-2 or seasonal influenza virus (Fig. 3a, Extended Data Fig. 1a–c). Consistent with the ELISpot data, low frequencies of S-binding BMPCs were detected in 10 of the 12 samples from convalescent individuals, but not in any of the 9 control samples (Fig. 3b). Although both recently generated circulating plasmablasts and S- and HA-binding BMPCs expressed BLIMP-1, the BMPCs were differentiated by their lack of expression of Ki-67—indicating a quiescent state—as well as by higher levels of CD38 (Fig. 3c).

Robust S-binding memory B cell response

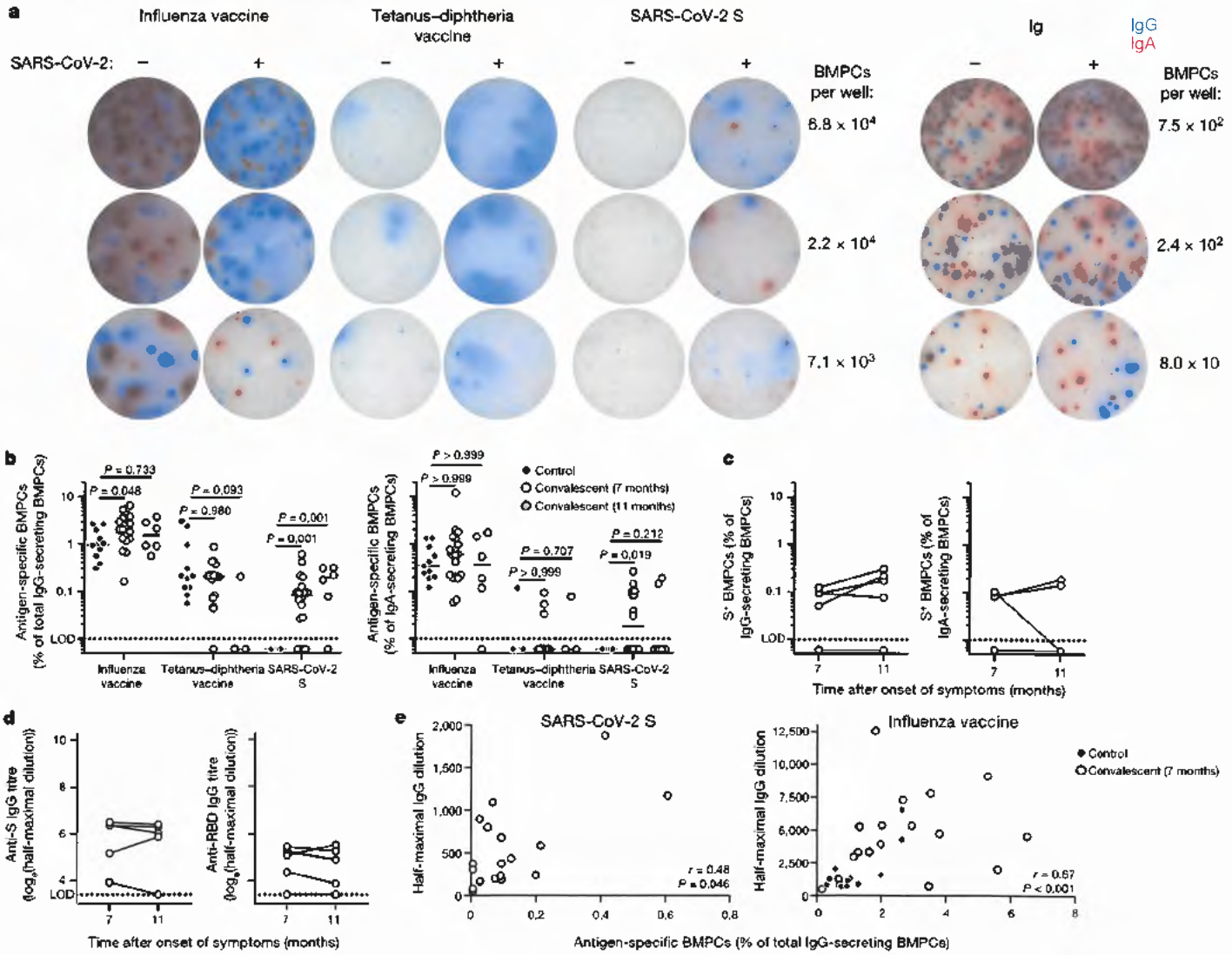


Fig. 2 | SARS-CoV-2 infection elicits S-binding long-lived BMPCs. **a**, Representative images of ELISpot wells coated with the indicated antigens or anti-immunoglobulin (Ig) and developed in blue and red for IgG and IgA, respectively, after incubation of magnetically enriched BMPCs from control individuals and convalescent individuals. **b**, Frequencies of BMPCs secreting IgG (left) or IgA (right) antibodies specific for the indicated antigens, indicated as percentages of total IgG- or IgA-secreting BMPCs in control individuals (black circles) or convalescent individuals 7 months (white circles) or 11 months (grey circles) after symptom onset. Horizontal lines indicate the median. *P* values from two-sided Kruskal–Wallis tests with Dunn's correction for multiple comparisons between control individuals and convalescent individuals. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control). **c**, Paired frequencies of S-binding BMPCs among IgG-secreting (left) and IgA-secreting (right) BMPCs from convalescent individuals 7 months and 11 months after symptom onset. **d**, Paired anti-S (left) and anti-RBD (right) IgG serum antibody titres from convalescent individuals 7 months and 11 months after symptom onset. Data in **c** and **d** (left) are also shown in **b** and Fig. 1b, respectively. Each symbol represents one sample ($n = 5$). Dotted lines indicate the limit of detection. **e**, Frequencies of BMPCs secreting IgG antibodies specific for SARS-CoV-2 S (left) and influenza virus vaccine (right) plotted against respective IgG titres in paired blood samples from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). *P* and *r* values from two-sided Spearman's correlations. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control).

co-stained the cells with fluorescently labelled influenza virus HA probes (Fig. 4a, Extended Data Fig. 1d). S-binding memory B cells were identified in convalescent individuals in the first sample that was collected approximately one month after the onset of symptoms, with comparable frequencies to influenza HA-binding memory B cells (Fig. 4b). S-binding memory B cells were maintained for at least 7 months after symptom

SARS-CoV-2 S-specific BMPCs in bone marrow aspirates from 15 out of 19 convalescent individuals, and in none from the 11 control participants. The frequencies of anti-S IgG BMPCs modestly correlated with serum IgG titres at 7–8 months after infection. Phenotypic analysis by flow cytometry showed that S-binding BMPCs were quiescent, and their frequencies were largely consistent in 5 paired aspirates collected at 7

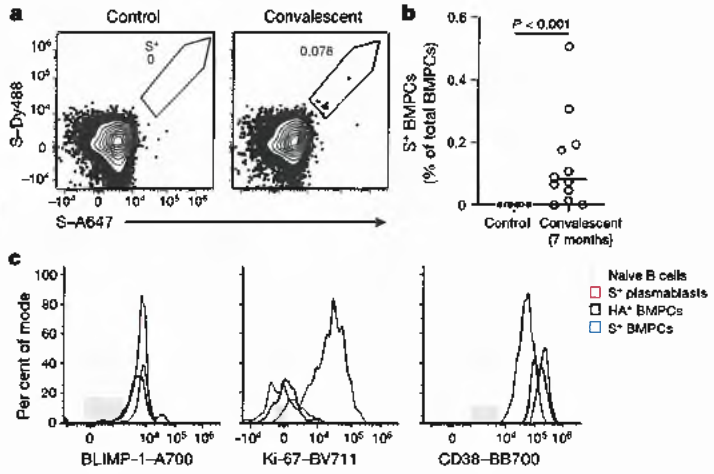
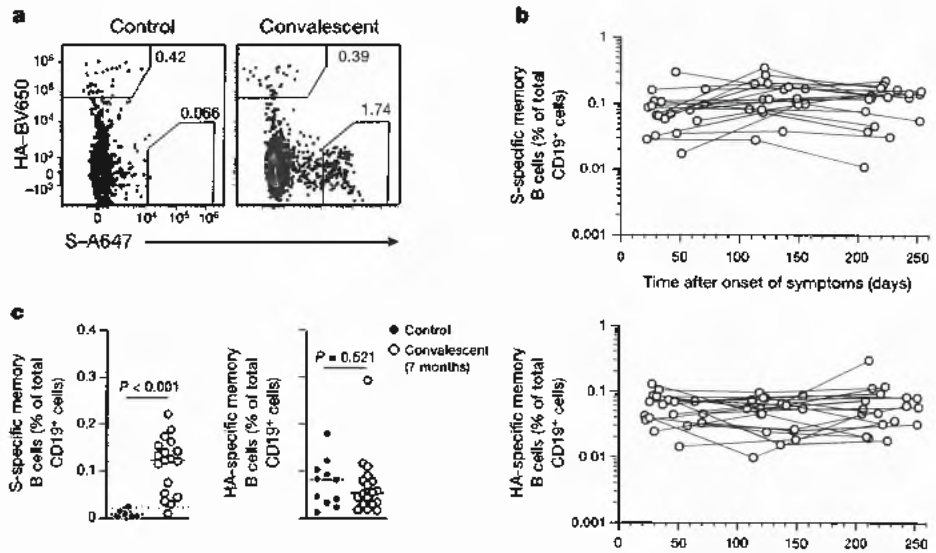


Fig. 3 | SARS-CoV-2 S-binding BMPCs are quiescent and distinct from circulating plasmablasts. **a**, Representative plots of intracellular S staining in $CD20^{lo}CD38^{+}IgD^{lo}CD19^{+/lo}CD3^{-}$ live singlet BMPCs (gating in Extended Data Fig. 1a) from magnetically enriched BMPCs from control individuals (left) or convalescent individuals 7 months after symptom onset (right). **b**, Frequencies of S-binding BMPCs in total BMPCs from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). Horizontal lines indicate the median. *P* value from two-sided Mann–Whitney *U* test. Each symbol represents one sample ($n = 12$ convalescent, $n = 9$ control). **c**, Histograms of BLIMP-1 (left), Ki-67 (centre), and CD38 (right) staining in S^{+} (blue) and HA^{+} (black) BMPCs from magnetically enriched BMPCs 7 months after symptom onset, and in S^{+} plasmablasts (red) and naive B cells (grey) from healthy donor PBMCs 1 week after SARS-CoV-2 S immunization.

results are consistent with SARS-CoV-2 infection eliciting a canonical T-cell-dependent B cell response, in which an early transient burst of extrafollicular plasmablasts generates a wave of serum antibodies that decline relatively quickly. This is followed by more stably maintained levels of serum antibodies that are supported by long-lived BMPCs.

Although this overall trend captures the serum antibody dynamics of the majority of participants, we observed that in three participants, anti-S serum antibody titres increased between 4 and 7 months after the onset of symptoms, after having initially declined between 1 and 4 months. This could be stochastic noise, could represent increased net binding affinity as early plasmablast-derived antibodies are replaced by those from affinity-matured BMPCs, or could represent increases in antibody concentration from re-encounter with the virus (although none of the participants in our cohort tested positive a second time). Although anti-S IgG titres in the convalescent cohort were relatively stable in the interval between 4 and 11 months after symptom onset, they did measurably decrease, in contrast to anti-influenza virus vaccine titres. It is possible that this decline reflects a final waning of early plasmablast-derived antibodies. It is also possible that the lack of decline in influenza titres was due to boosting through exposure to influenza antigens. Our data suggest that SARS-CoV-2 infection induces a germinal centre response in humans because long-lived BMPCs are thought to be predominantly germinal-centre-derived[7]. This is consistent with a recent study that reported increased levels of somatic hypermutation in memory B cells that target the RBD of SARS-CoV-2 S in convalescent individuals at 6 months compared to 1 month after infection[20].

To our knowledge, the current study provides the first direct evidence for the induction of antigen-specific BMPCs after a viral infection in humans. However, we do acknowledge several limitations. Although we detected anti-S IgG antibodies in serum at least 7 months after infection in all 19 of the convalescent donors from whom we obtained bone marrow aspirates, we failed to detect S-specific BMPCs in 4 donors. Serum anti-S antibody titres in those four donors were low, suggesting that S-specific BMPCs may potentially be present at very low frequencies that are below the limit of detection of the assay. Another limitation is that we do not know the fraction of the S-binding BMPCs detected in our study that encodes neutralizing antibodies. SARS-CoV-2 S protein is the main target of neutralizing antibodies[17,25–30] and a correlation between serum anti-S IgG binding and neutralization titres has been documented[17,31]. Further studies will be required to determine the



epitopes that are targeted by BMPCs and memory B cells, as well as their clonal relatedness. Finally, although our data document a robust induction of long-lived BMPCs after infection with SARS-CoV-2, it is critical to note that our convalescent individuals mostly experienced mild infections. Our data are consistent with a report showing that individuals who recovered rapidly from symptomatic SARS-CoV-2 infection generated a robust humoral immune response[32]. It is possible that more-severe SARS-CoV-2 infections could lead to a different outcome with respect to long-lived BMPC frequencies, owing to dysregulated humoral immune responses. This, however, has not been the case in survivors of the 2014 Ebola virus outbreak in West Africa, in whom severe viral infection induced long-lasting antigen-specific serum IgG antibodies[33].

Long-lived BMPCs provide the host with a persistent source of preformed protective antibodies and are therefore needed to maintain durable immune protection. However, the longevity of serum anti-S IgG antibodies is not the only determinant of how durable immune-mediated protection will be. Isotype-switched memory B cells can rapidly differentiate into antibody-secreting cells after re-exposure to a pathogen, offering a second line of defence[34]. Encouragingly, the frequency of S-binding circulating memory B cells at 7 months after infection was similar to that of B cells directed against contemporary influenza HA antigens. Overall, our data provide strong evidence that SARS-CoV-2 infection in humans robustly establishes the two arms of humoral immune memory: long-lived BMPCs and memory B cells. These findings provide an immunogenicity benchmark for SARS-CoV-2 vaccines and a foundation for assessing the durability of primary humoral immune responses that are induced in humans after viral infections.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03647-4.

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Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded during outcome assessment.

Sample collection, preparation and storage

All studies were approved by the Institutional Review Board of Washington University in St Louis. Written consent was obtained from all participants. Seventy-seven participants who had recovered from SARS-CoV-2 infection and eleven control individuals without a history of SARS-CoV-2 infection were enrolled (Extended Data Tables 1, 4). Blood samples were collected in EDTA tubes and PBMCs were enriched by density gradient centrifugation over Ficoll 1077 (GE) or Lymphopure (BioLegend). The remaining red blood cells were lysed with ammonium chloride lysis buffer, and cells were immediately used or cryopreserved in 10% dimethyl sulfoxide in fetal bovine serum (FBS). Bone marrow aspirates of approximately 30 ml were collected in EDTA tubes from the iliac crest of 18 individuals who had recovered from COVID-19 and the control individuals. Bone marrow mononuclear cells were enriched by density gradient centrifugation over Ficoll 1077, and the remaining red blood cells were lysed with ammonium chloride buffer (Lonza) and washed with phosphate-buffered saline (PBS) supplemented with 2% FBS and 2 mM EDTA. Bone marrow plasma cells were enriched from bone marrow mononuclear cells using the CD138 Positive Selection Kit II (Stemcell) and immediately used for ELISpot or cryopreserved in 10% dimethyl sulfoxide in FBS.

Antigens

Recombinant soluble spike protein (S) and its receptor-binding domain (RBD) derived from SARS-CoV-2 were expressed as previously described[35]. In brief, mammalian cell codon-optimized nucleotide sequences coding for the soluble version of S (GenBank: MN908947.3, amino acids (aa) 1–1,213) including a C-terminal thrombin cleavage site, T4 foldon trimerization domain and hexahistidine tag cloned into the mammalian expression vector pCAGGS. The S protein sequence was modified to remove the polybasic cleavage site (RRAR to A) and two stabilizing mutations were introduced (K986P and V987P, wild-type numbering). The RBD, along with the signal peptide (aa 1–14) plus a hexahistidine tag were cloned into the mammalian expression vector pCAGGS. Recombinant proteins were produced in Expi293F cells (Thermo Fisher Scientific) by transfection with purified DNA using the ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific). Supernatants from transfected cells were collected 3 (for S) or 4 (for RBD) days after transfection, and recombinant proteins were purified using Ni-NTA agarose (Thermo Fisher Scientific), then buffer-exchanged into PBS and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore). For flow cytometry staining, recombinant S was labelled with Alexa Fluor 647- or DyLight 488-NHS ester (Thermo Fisher Scientific); excess Alexa Fluor 647 and DyLight 488 were removed using 7-kDa and 40-kDa Zeba desalting columns, respectively (Pierce). Recombinant HA from A/Michigan/45/2015 (aa 18–529, Immune Technology) was labelled with DyLight 405-NHS ester (Thermo Fisher Scientific); excess DyLight 405 was removed using 7-kDa Zeba desalting columns. Recombinant HA from A/Brisbane/02/2018 (aa 18–529) and B/Colorado/06/2017 (aa 18–546) (both Immune Technology) were biotinylated using the EZ-Link S-binding IgG- and IgA-secreting cells present in BMPC and PBMC samples using IgG/IgA double-colour ELISpot Kits (Cellular Technology) according to the manufacturer's instructions. ELISpot plates were analysed using an ELISpot counter (Cellular Technology).

ELISA

Assays were performed in 96-well plates (MaxiSorp, Thermo Fisher Scientific) coated with 100 µl of Flucelvax 2019/2020 or recombinant S in PBS, and plates were incubated at 4 °C overnight. Plates were then blocked with 10% FBS and 0.05% Tween-20 in PBS. Serum or plasma were serially diluted in blocking buffer and added to the plates. Plates were incubated for 90 min at room temperature and then washed 3 times with 0.05% Tween-20 in PBS. Goat anti-human IgG–HRP (Jackson ImmunoResearch, 1:2,500) was diluted in blocking buffer before adding to wells and incubating for 60 min at room temperature. Plates were washed 3 times with 0.05% Tween-20 in PBS, and then washed 3 times with PBS before the addition of *o*-phenylenediamine dihydrochloride peroxidase substrate (Sigma-Aldrich). Reactions were stopped by the addition of 1 M HCl. Optical density measurements were taken at 490 nm. The half-maximal binding dilution for each serum or plasma sample was calculated using nonlinear regression (GraphPad Prism v.8). The limit of detection was defined as 1:30.

Statistics

Spearman's correlation coefficients were estimated to assess the relationship between 7-month anti-S and anti-influenza virus vaccine IgG titres and the frequencies of BMPCs secreting IgG specific for S and for influenza virus vaccine, respectively. Means and pairwise differences of antibody titres at each time point were estimated using a linear mixed model analysis with a first-order autoregressive covariance structure. Time since symptom onset was treated as a categorical fixed effect for the 4 different sample time points spaced approximately 3 months apart. *P* values were adjusted for multiple comparisons using Tukey's method. All analyses were conducted using SAS v.9.4 (SAS Institute) and Prism v.8.4 (GraphPad), and *P* values of less than 0.05 were considered significant.

Flow cytometry

Staining for flow cytometry analysis was performed using cryo-preserved magnetically enriched BMPCs and cryo-preserved PBMCs. For BMPC staining, cells were stained for 30 min on ice with CD45-A532 (HI30, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2, BD Horizon, 1:500), CD19-PE (HIB19, 1:200), CXCR5-PE-Dazzle 594 (J252D4, 1:50), CD71-PE-Cy7 (CY1G4, 1:400), CD20-APC-Fire750 (2H7, 1:400), CD3-APC-Fire810 (SK7, 1:50) and Zombie Aqua (all BioLegend) diluted in Brilliant Stain buffer (BD Horizon). Cells were washed twice with 2% FBS and 2 mM EDTA in PBS (P2), fixed for 1 h using the True Nuclear permeabilization kit (BioLegend), washed twice with perm/wash buffer, stained for 1h with DyLight 405-conjugated recombinant HA from A/Michigan/45/2015, DyLight 488- and Alexa 647-conjugated S, Ki-67-BV711 (Ki-67, 1:200, BioLegend) and BLIMP-1-A700 (646702, 1:50, R&D), washed twice with perm/wash buffer, and resuspended in P2. For memory B cell staining, PBMCs were stained for 30 min on ice with biotinylated recombinant HAs diluted in P2, washed twice, then stained for 30 min on ice with Alexa 647-conjugated S, IgA-FITC (M24A, Millipore, 1:500), IgG-BV480 (goat polyclonal, Jackson ImmunoResearch, 1:100), IgD-SB702 (IA6-2, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2,

PBMCs were included from convalescent individuals and control individuals.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Relevant data are available from the corresponding author upon reasonable request.

35. Stadlbauer, D. et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr. Protoc. Microbiol.* **57**, e100 (2020).

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Author contributions A.H.E. conceived and designed the study. J.S.T. and A.H.E. designed experiments and composed the manuscript. A.H., M.K.K., I.P., J.A.O. and R.M.P. wrote and maintained the Institutional Review Board protocol, recruited and phlebotomized participants and coordinated sample collection. J.S.T., W.K., E.K., A.J.S. and L.H. processed specimens. A.J.S. expressed S and RBD proteins. J.S.T., W.K. and E.K. performed ELISA and ELISpot. J.S.T. performed flow cytometry. J.S.T., A.M.R., C.W.G. and A.H.E. analysed data. All authors reviewed the manuscript.

Competing interests The Ellebedy laboratory received funding under sponsored research agreements that are unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie. J.S.T., A.J.S. and A.H.E. are recipients of a licensing agreement with Abbvie that is unrelated to the data presented in the current study. A.H.E. is a consultant for Mubadala Investment Company and the founder of ImmuneBio Consulting. All other authors declare no competing interests.

Additional information
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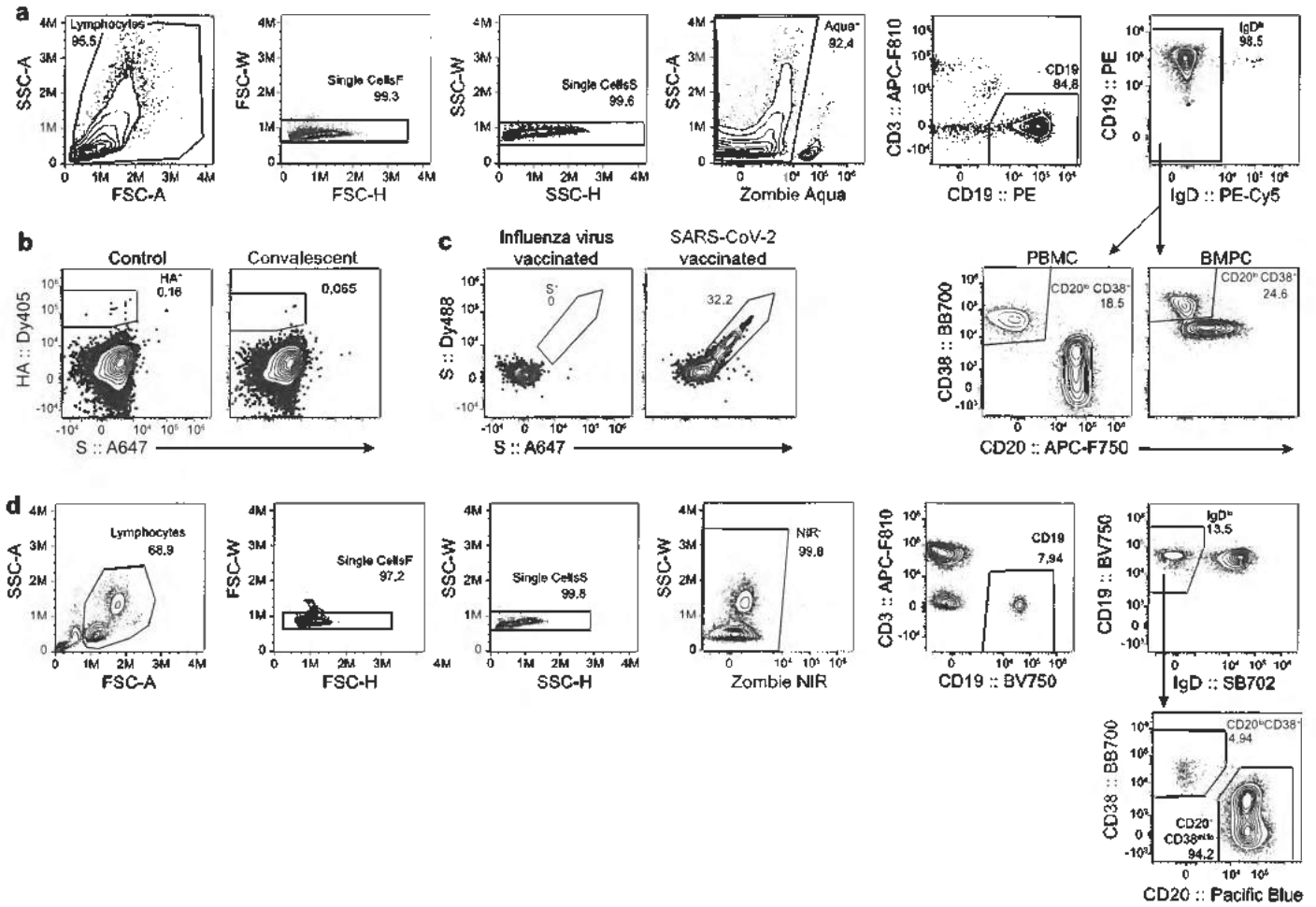

Extended Data Fig. 1 | Flow cytometry identification of SARS-CoV-2-elicited plasma cells and memory B cells. a, **d**, Flow cytometry gating strategies for BMPCs in magnetically enriched BMPCs and plasmablasts in PBMCs (**a**) and isotype-switched memory B cells and plasmablasts in PBMCs (**d**). **b**, Representative plots of intracellular SARS-CoV-2 S and influenza virus HA staining in BMPCs from samples from control individuals (left) and individuals who were convalescing from COVID-19 (right) 7 months after symptom onset. **c**, Representative plots of intracellular S staining in plasmablasts in PBMCs one week after vaccination against seasonal influenza virus or SARS-CoV-2.

Extended Data Table 1 | Demographics of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
Age (median [range])	49 (21-69)	52 (30-69)
Sex		
Female	38 (49.4)	7 (36.8)
Male	39 (50.6)	12 (63.2)
Race		
White	70 (90.9)	18 (94.7)
Black	1 (1.3)	0 (0)
Asian	4 (5.2)	0 (0)
Other	2 (2.6)	1 (5.3)
Comorbidities		
Asthma	13 (16.9)	3 (15.8)
Lung disease	0 (0)	0 (0)
Heart disease	3 (3.9)	0 (0)
Hypertension	13 (16.9)	6 (31.6)
Diabetes mellitus	3 (3.9)	3 (15.8)
Cancer	10 (13)	3 (15.8)
Autoimmune disease	4 (5.2)	2 (10.5)
Hyperlipidemia	8 (10.4)	2 (10.5)
Hypothyroidism	5 (6.5)	3 (15.8)
Gastroesophageal reflux disease	5 (6.5)	2 (10.5)
Other	26 (33.8)	10 (52.6)
Solid organ transplant	1 (1.3)	1 (5.3)
Obesity	1 (1.3)	0 (0)

Extended Data Table 2 | Symptoms of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
First symptom		
Cough	12 (15.6)	3 (15.8)
Diarrhea	1 (1.3)	0 (0)
Dyspnea	2 (2.6)	1 (5.3)
Fatigue	7 (9.1)	0 (0)
Fever	22 (28.6)	9 (47.4)
Headache	8 (10.4)	2 (10.5)
Loss of taste	3 (3.9)	2 (10.5)
Malaise	4 (5.2)	1 (5.3)
Myalgias	9 (11.7)	0 (0)
Nasal congestion	2 (2.6)	0 (0)
Nausea	1 (1.3)	0 (0)
Night sweats	1 (1.3)	0 (0)
Sore throat	5 (6.5)	1 (5.3)
Symptom present during disease		
Fever	65 (84.4)	17 (89.5)
Cough	54 (70.1)	14 (73.7)
Dyspnea	31 (40.3)	11 (57.9)
Nausea	19 (24.7)	4 (21.1)
Vomiting	9 (11.7)	3 (15.8)
Diarrhea	39 (50.6)	10 (52.6)
Headaches	47 (61)	12 (63.2)
Loss of taste	42 (54.5)	11 (57.9)
Loss of smell	42 (54.5)	10 (52.6)
Fatigue	38 (49.4)	7 (36.8)
Malaise	6 (7.8)	1 (5.3)
Myalgias or body aches	34 (44.2)	8 (42.1)
Sore throat	12 (15.6)	1 (5.3)
Chills	25 (32.5)	6 (31.6)
Nasal congestion	6 (7.8)	0 (0)
Other	32 (41.6)	7 (36.8)
Duration of symptoms in days (median [range])	14 (1-43)	13 (6-30)
Days from symptom onset to positive SARS-CoV-2 PCR test (median [range])	6 (0-36)	6 (1-31)
Days from symptom onset to 1-month blood sample collection (median [range])	41 (21-84)	34 (22-71)
Hospitalization	6 (7.8)	1 (5.3)
COVID medications		
Hydroxychloroquine	2 (2.6)	0 (0)
Chloroquine	1 (1.3)	0 (0)
Azithromycin	14 (18.2)	6 (31.6)
Lopinavir/ritonavir	0 (0)	0 (0)
Remdesivir	0 (0)	0 (0)
Convalescent plasma	0 (0)	0 (0)
None	61 (79.2)	12 (63.2)
Other	2 (2.6)	1 (5.3)

Extended Data Table 3 | Symptoms and follow up samples (months 4–11) of convalescent individuals

	Month 4		Month 7		Month 11	
	Total N= 76 N (%)	**Bone marrow biopsy N=19 N (%)**	**Total N= 76 N (%)**	**Bone marrow biopsy N=18 N (%)**	**Total N= 42 N (%)**	**Bone marrow biopsy N=12 N (%)**
Days from positive SARS-CoV-2 PCR test to follow up visit (median [range])	125 (102-192)	117 (105-150)	222 (191-275)	213 (200-247)	308 (283-369)	303 (283-325)
Days from symptom onset to blood sample collection (median [range])	131 (106-193)	124 (108-155)	227 (194-277)	222 (205-253)	314 (288-373)	309 (297-343)
Any symptom present at follow up visit	25 (32.9)	8 (42.1)	33 (43)	10 (55.6)	20 (47.6)	6 (50)
Fever	0 (0)	0 (0)	2 (2.6)	0 (0)	1 (2.4)	0 (0)
Cough	1 (1.3)	1 (5.3)	0 (0)	0 (0)	1 (2.4)	0 (0)
Dyspnea	7 (9.2)	2 (10.5)	6 (7.9)	3 (16.7)	6 (14.3)	3 (25)
Nausea	1 (1.3)	0 (0)	1 (1.3)	0 (0)	0 (0)	0 (0)
Vomiting	1 (1.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea	2 (2.6)	1 (5.3)	1 (1.3)	0 (0)	0 (0)	0 (0)
Headaches	1 (1.3)	0 (0)	3 (3.9)	0 (0)	2 (4.8)	0 (0)
Loss or altered taste	8 (10.5)	0 (0)	9 (11.8)	1 (5.6)	5 (11.9)	1 (8.3)
Loss or altered smell	13 (17.1)	2 (10.5)	12 (15.8)	2 (11.1)	8 (19)	2 (16.7)
Fatigue	9 (11.8)	4 (21.1)	13 (17.1)	5 (27.8)	8 (19)	3 (25)
Forgetfulness/brain fog	8 (10.5)	6 (31.6)	12 (15.8)	6 (33.3)	10 (23.8)	4 (33.3)
Hair loss	5 (6.6)	1 (5.3)	3 (3.9)	1 (5.6)	2 (4.8)	0 (0)
Other	7 (9.2)	3 (15.8)	12 (15.8)	1 (5.6)	10 (23.8)	1 (8.3)
Joint pain	3 (3.9)	1 (5.3)	7 (9.2)	1 (5.3)	3 (7.1)	0 (0)

Extended Data Table 4 | Healthy control demographics

Variable	Total N= 11 N (%)
Age (median [range])	38 (23-53)
Sex	
Female	3 (27.3)
Male	8 (72.7)
Race	
White	9 (71.8)
Black	1 (9.1)
Asian	1 (9.1)

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Corresponding author(s): Ali H. Ellebedy

Last updated by author(s): May 26, 2021

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data were acquired using SpectroFlo software v2.2.
Data analysis	Flow cytometry data were analyzed using FlowJo v10 and Prism v8 ELISA and ELISpot data were analyzed using Prism v8 and SAS 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All relevant data are available from the corresponding author upon reasonable request.

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to determine sample size. 77 convalescent patients and 11 control participants were enrolled based on recruitment; these numbers provided sufficient power to determine differences in SARS-CoV-2 responses between the groups.
Data exclusions	No data were excluded
Replication	Samples were collected from 77 convalescent patients and 11 control participants. ELISA for each participant at each timepoint was performed once with two technical replicates. ELISpot and flow cytometry experiments were performed once for each sample at each timepoint.
Randomization	Different experimental groups were not assigned.
Blinding	No blinding was done in this study; subjective measurements were not made.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	IgG-HRP (goat polyclonal, Jackson ImmuoResearch 109-035-088), IgG-BV480 (goat polyclonal, Jackson Immunoresearch 109-685-098), IgD-SB702 (IA6-2, Thermo 67-9868-42), IgA-FITC (M24A, Millipore CBL114F), CD45-A532 (HI30, Thermo 58-0459-42), CD38-BB700 (HIT2, BD Horizon 566445), Blimp1-A700 (646702, R&D IC36081N), CD20-Pacific Blue (2H7, 302320), CD4-BV570 (OKT4, 317445), CD24-BV605 (ML5, 311124), streptavidin-BV650 (405232), Ki-67-BV711 (Ki-67, 350516), CD19-BV750 (HIB19, 302262), CD19-PE (HIB19, 302254), CD71-PE (CY1G4, 334106), CXCR5-PE-Dazzle 594 (J252D4, 356928), CD27-PE-Cy7 (O323, 302838), CD71-PE-Cy7 (CY1G4, 334112), CD20-APC-Fire750 (2H7, 302358), IgM-APC-Fire750 (MHM-88, 314546), CD3-APC-Fire810 (SK7, 344858); all Biolegend.
Validation	Commercial antibodies were validated by their respective manufacturers per their associated data sheets and titrated in the lab for their respective assay (ELISA or flow cytometry) by serial dilution

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293F (Thermo)
Authentication	The cell line was not authenticated
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination. Growth rates were consistent with manufacturer's published data.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Human research participants

Policy information about studies involving human research participants

Population characteristics	77 SARS-CoV-2 convalescent study participants were recruited, ages 21-69, 49.4% female, 50.6% male 11 healthy control participants with no history of SARS-CoV-2 infection were recruited, ages 23-53, 27.3% female, 72.7% male
Recruitment	Study participants were recruited from the St. Louis metropolitan area by the Washington University Clinical Trials Unit. Potential self-selection and recruiting biases are unlikely to affect the parameters we measured.
Ethics oversight	The study was approved by the Washington University IRB

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Peripheral blood and bone marrow mononuclear cells were isolated from EDTA anticoagulated blood and bone marrow aspirates, respectively using density gradient centrifugation, and remaining RBCs were lysed with ammonium chloride lysis buffer. Bone marrow plasma cells were magnetically enriched from bone marrow mononuclear cells and immediately used for ELISpot or cryopreserved in 10% dimethylsufoxide in FBS for flow cytometric analysis. PBMCs were immediately used or cryopreserved in 10% DMSO in FBS.
Instrument	Cytek Aurora
Software	Flow cytometry data were acquired using Cytek SpectroFlo software, and analyzed using FlowJo (Treestar) v10.
Cell population abundance	Cells were not sorted
Gating strategy	Gating strategies are shown in extended data figure

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Exhibit "K"

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ORIGINAL ARTICLE

WILEY

SARS-CoV-2 re-infection risk in Austria

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Correction added on 26 February 2021, after first online publication: Author spelling is corrected to John PA Ioannidis

Abstract

Background: A key question concerning coronavirus disease 2019 (COVID-19) is how effective and long lasting immunity against this disease is in individuals who were previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We aimed to evaluate the risk of SARS-CoV-2 re-infections in the general population in Austria.

Methods: This is a retrospective observational study using national SARS-CoV-2 infection data from the Austrian epidemiological reporting system. As the primary outcome, we aim to compare the odds of SARS-CoV-2 re-infections of COVID-19 survivors of the first wave (February to April 30, 2020) versus the odds of first infections in the remainder general population by tracking polymerase chain reaction (PCR)-confirmed infections of both groups during the second wave from September 1 to November 30, 2020. Re-infection counts are tentative, since it cannot be excluded that the positive PCR in the first and/or second wave might have been a false positive.

Results: We recorded 40 tentative re-infections in 14 840 COVID-19 survivors of the first wave (0.27%) and 253 581 infections in 8 885 640 individuals of the remaining general population (2.85%) translating into an odds ratio (95% confidence interval) of 0.09 (0.07 to 0.13).

Conclusions: We observed a relatively low re-infection rate of SARS-CoV-2 in Austria. Protection against SARS-CoV-2 after natural infection is comparable with the highest available estimates on vaccine efficacies. Further well-designed research on this issue is urgently needed for improving evidence-based decisions on public health measures and vaccination strategies.

KEYWORDS

COVID-19, epidemiology, PCR, re-infection, Risk, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is a major public health crisis.[1,2] A key question concerning measures against COVID-19 is the strength and durability of immunity against this disease in individuals previously infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3-10] Vaccination strategies, considerations regarding herd immunity, and overall simulations for the pandemic depend on the efficacy and the time course of immunity against COVID-19.[5]

Data on immune responses to COVID-19 are limited by knowledge gaps regarding their dynamics over time and their clinical significance with reference to protection against re-infections.[3-10] There is evidence for re-infections from numerous case reports, but it is occasionally challenging to differentiate true re-infections from prolonged viral shedding that may last for up to about 4 months.[5,11,12] Notably, a study of 12 541 healthcare workers in the UK recently found major protection against re-infection for those who had anti-SARS-CoV-2 antibodies determined by anti-spike and anti-nucleocapsid assays versus those who did not.[13] After a follow-up of up to 31 weeks, they calculated a rate ratio of 0.11 (95% confidence interval (CI): 0.03 to 0.44; P =.002) for re-infections in seropositive healthcare workers versus first infections in healthcare workers with negative antibody status.[13] Similarly, another recent study among healthcare workers from the UK reported no re-infection case in 1038 individuals with evidence of previous SARS-CoV-2 infection based on PCR tests and/or antibody status.[10] While these studies suggest a high protection against SARS-CoV-2 re-infections in healthcare workers, the risk of re-infections in the general population remains uncertain.

Austria was hit very early in this pandemic with a first wave occurring from 22 February to 30 April 2020 (all further dates refer to the year 2020). Data on the re-infection rate during the second wave from September 1 to November 30 can therefore provide, as a rough estimate, evidence on the immunity against SARS-CoV-2 over more than half a year.[14,15] Therefore, we investigated data from the Austrian epidemiological reporting system (ERS) provided by the Austrian Agency for Health and Food Safety (AGES).[15] As the primary outcome, we compared the odds for SARS-CoV-2 re-infections in COVID-19 survivors versus first infections in the remainder general population during the second infection wave. In addition, we also evaluate data on hospitalization status during both infection waves and on COVID-19 deaths during the second wave, in order to obtain measures of disease severity.

Key messages

- In this study in the whole general population in Austria with a follow-up of over half a year, those individuals with a previous SARS-CoV-2 infection had a significant reduction by 91% for the odds of a re-infection versus the odds of a first infection in the remainder general population.
- Protection against SARS-CoV-2 after natural infection is comparable with the highest available estimates on vaccine efficacies.

among others data on hospitalization status and COVID-19 deaths.[15] Ethical approval for this study was obtained from the ethics committee at the Medical University of Graz, Graz, Austria.

Patients who had a positive polymerase chain reaction (PCR) test during both, the first and second infection wave are referred to here as patients with 'tentative re-infections'. We use the term 'tentative' re-infection because a certain number of these cases might reflect false-positive results in the testing during the first and/or second wave. This is based on the consideration that the specificity (with 95% confidence region) of PCR tests (nucleic acid amplification tests) for SARS-CoV-2 is less than 100%, with 98.1% (95.9 to 99.2%) according to a recent meta-analysis.[16]

The group size of 'COVID-19 survivors' was calculated as all individuals who had a positive PCR test result for SARS-CoV-2 minus all reported COVID-19 deaths from February 22 to April 30. The control group ('general population group') are the remainder Austrian residents that we calculated as the reported Austrian population on January 1 with 8 901 064 individuals (the closest approximation for the population size) minus all patients tested SARS-CoV-2 positive during the first wave.[17] In Austria, population changes from year to year are usually significantly less than 1%.[17] The observation period for tracking SARS-CoV-2 infections was from September 1 to November 30 (the pre-specified date for our analyses), corresponding to what we term the second wave. Automated matching of records in the first and second wave to detect tentative re-infections was done by using IDs consisting of the first two initials of the first name, the first three initials of the surname and the date of birth (eg ST.PIL.15.12.1979). All entries with the identical ID were then carefully and manually checked including data such as

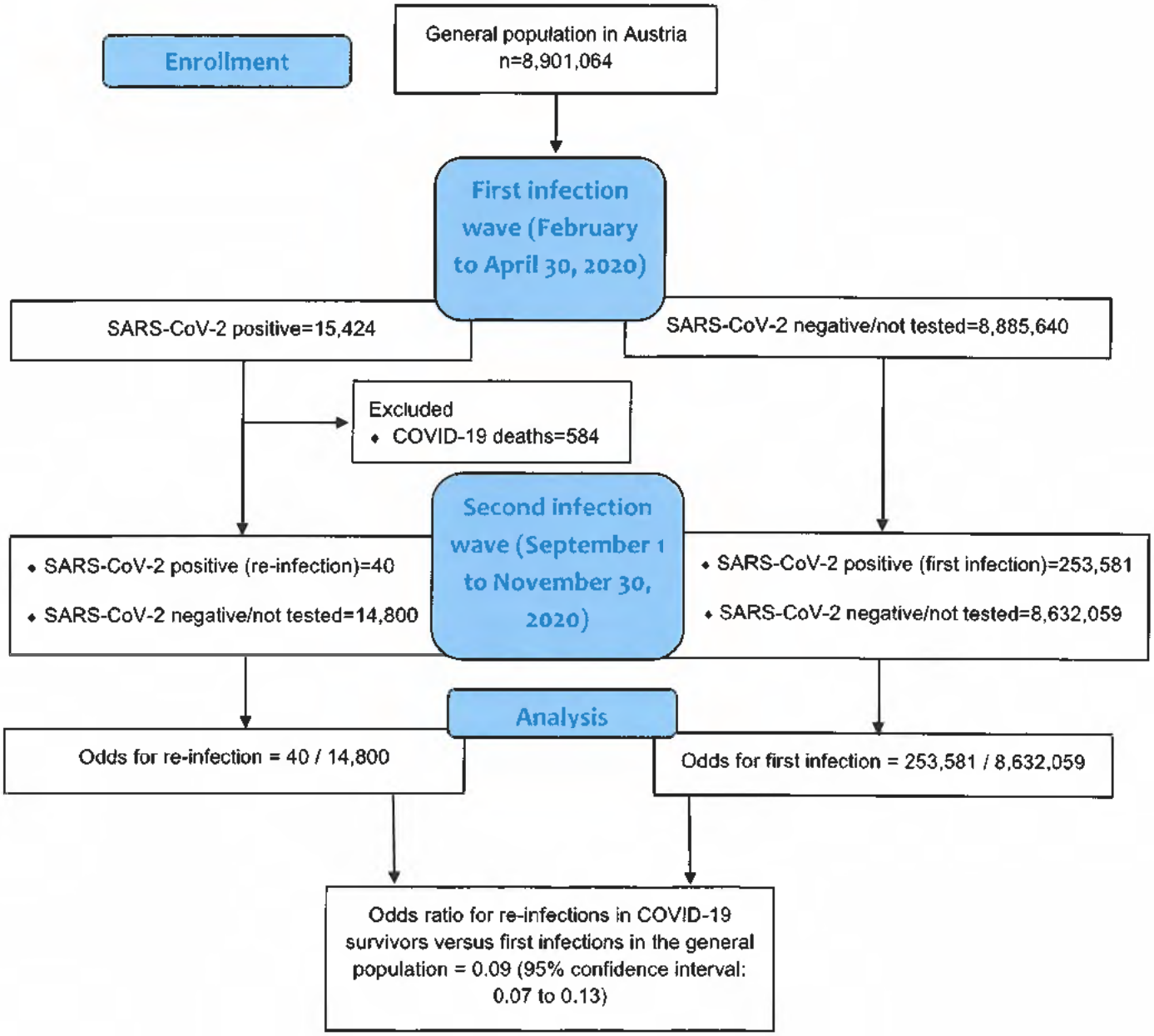

FIGURE 1 Analysis plan for calculating the odds ratio for re-infections versus first infections with SARS-CoV-2 in the general population in Austria

viral shedding for up to about 4 months.[5-7] This 4 month interval was also the main consideration to separate the time frame for the two waves. Of note, there were only relatively few documented SARS-CoV-2 cases (<0.15% of the Austrian population) from May to August.[15]

Regardless of the main reason for hospitalization, any hospitalized patient who was tested SARS-CoV-2 positive was classified as hospitalized in the ERS. All persons who were tested SARS-CoV-2 positive and died for whatever reanalyses were performed by using SPSS Version 25.0 (IBM SPSS Inc, Chicago, IL, USA).

3 | RESULTS

From 15 424 patients with SARS-CoV-2 positive tests in the first wave, 584 were recorded as COVID-19 deaths, so that our COVID-19 survivor group consists of 14 840 pa-

TABLE 1 Characteristics of 40 patients with re-infection

Gender	Age at first infection(years)	Time between infections (days)	Hospitalization	
			First wave	Second wave
Female	84	148	Yes	No
Female	53	223	No	No
Female	54	183	No	No
Male	34	215	Yes	No
Female	31	200	Unknown	Unknown
Female	25	206	No	No
Male	89	196	No	No
Female	39	175	No	No
Male	52	222	No	Unknown
Male	22	251	No	Unknown
Female	84	148	Yes	Yes
Male	79	238	Yes	Unknown
Female	23	236	No	Unknown
Female	55	214	No	No
Female	37	203	No	No
Female	23	222	No	No
Male	15	235	No	No
Female	76	219	Yes	Yes
Male	52	206	No	No
Female	72	172	No	No
Male	24	207	No	No
Female	51	221	No	No
Male	19	210	No	No
Female	43	246	No	No
Male	61	246	No	Unknown
Male	25	221	Yes	Yes
Male	47	232	No	No
Female	34	222	No	Yes
Female	31	231	No	No
Female	30	213	No	No
Female	54	173	Yes	Yes
Male	27	203	No	No
Female	23	172	No	No
Female	40	214	No	Unknown
Male	25	221	No	No
Female	93	237	Yes	Unknown
Female	26	227	No	No
Female	41	226	No	No
Female	48	216	No	No

percentile; minimum–maximum) at the first infection was 39.8 (25.9 to 54.5; 15.4 - 93.8) years. The mean (± standard deviation) time from the first to the tentative re-infection was 212 ± 25 days. Of the 40 tentative re-infections, 4, 12 and 24 were documented in September, October and November, respectively (among 18 106, 61 384 and 174 131 total infections, respectively).

Hospitalization status in numbers of patients coded as yes, no and unknown was 8, 31 and 1 for the first infection and 5, 27 and 8 for the tentative re-infection, respectively. Four patients were hospitalized during both infection waves. Unknown hospitalization data during the second wave are probably mainly due to a delay in hospitalization data entry into the ERS.

With follow-up on mortality available until December 23, only one 72-year-old woman died two days after her tentative re-infection diagnosis. She was not hospitalized and according to her medical records her cause of death ('acute vascular occlusion of an extremity with rhabdomyolysis') was not causally attributed to COVID-19.

4 | DISCUSSION

We documented a relatively low re-infection risk for SARS-CoV-2 in the general population of Austria by using data from the ERS. Patients with re-infections covered both genders, a wide age range and included also patients who were hospitalized during both infections.

Our study is, to the best of our knowledge, the first systematic investigation of tentative re-infection risk with SARS-CoV-2 in a large national population. Several case reports on SARS-CoV-2 re-infections in the general population indicate that there is at least some risk of re-infection, but they did not provide quantification of re-infection risk that requires a standardized comparison to the 'background' infection risk in the general population.[3-5] While data on immune responses to previous SARS-CoV-2 infections exist, they can only be regarded a proxy for a previous infection and the associated clinical protection against re-infections, thus requiring studies like ours to address the question to what extent patients who experienced PCR confirmed SARS-CoV-2 infections are protected against re-infections.[3-5] Importantly, the study by Lumley et al in 12 541 healthcare workers documented protection against re-infection for those who had anti-SARS-CoV-2 antibodies with a rate ratio (0.11) very similar to what we observed.[13] While the investigation by Lumley et al was SARS-CoV-2 PCR and antibody test data from 66 001 patients from a laboratory in south-west London documented 8 patients with evidence of re-infections, and calculated a relative risk of re-infections versus first infections of 0.0578 (95% CI: 0.0288 to 0.1160)[18] which is also compatible with our estimate.

Our data do not include detailed clinical characteristics of the patients with tentative re-infections but it is noteworthy that these patients covered both genders with a wide age range and included also several hospitalized patients. These data are of interest since previous studies indicate a high correlation between neutralizing antibodies against SARS-CoV-2 and COVID-19 severity. This in turn suggests that those patients with more severe infections may develop a stronger protective humoral immune response against SARS-CoV-2 compared to those with less severe infections. This hypothesis is, however, not strongly supported by our findings as several patients with tentative re-infections were already hospitalized during their first infection.[8] Regarding duration of acquired immunity against SARS-CoV-2 re-infections, we provide data with a median follow-up time of about 7 months. Importantly, there was no clear sign of decreasing protection against re-infections in descriptive analyses of monthly stratified re-infection cases.

In view of ongoing discussions on vaccination approaches regarding SARS-CoV-2, our data suggest that the protection against SARS-CoV-2 after natural infection is roughly similar to the highest estimates of SARS-CoV-2 vaccine efficacies among vaccines that have been authorized to-date, although a direct comparison cannot be made due to differences in study designs and study populations.[19,20] Nevertheless, we believe that based on our findings, waving urgent recommendations to undergo SARS-Cov-2-vaccination for persons with PCR-documented previous COVID-19 infection seems prudent as long as any shortage of vaccines is present.

Our findings on a significant protection against SARS-CoV-2 re-infections, provide also evidence for the rapid evolution of the pandemic towards 'herd immunity', in particular because of a huge underreporting of SARS-CoV-2 cases.[21,22] Therefore, the relatively high prevalence of individuals who were already infected with SARS-CoV-2 along with the currently rapidly increasing number of vaccinated individuals may work in concert towards an ensuring 'herd immunity' that will hopefully bend this pandemic within the near future.[2,23,24] This may already be the case in some countries such as India, where seroprevalence rose rapidly from 0.7% in May to 7% in August and 60%

suggested that about 76% of the population had been infected with SARS-CoV-2 by October 2020.[28] It is unknown whether there was an error with over-estimation of the first wave seroprevalence, or the resurgence can be explained by the advent of a new strain (P1) that has a high propensity for re-infection. Careful monitoring for new strains and for their ability to evade existing natural immune responses and vaccine-induced immunity is needed.

Our findings are limited due to lack of detailed clinical characteristics, the observational nature of our study design, and the strong dependence on the data quality of the ERS. The 40 tentative re-infections have quite similar demographics to the totality of COVID-19 documented cases in Austria, but data are limited for meaningful formal comparisons.[9] Data on hospitalizations are very sparse and hospitalization data during the second wave are missing for some participants, probably, due to a delay in reporting such data. Infections in the first wave are likely to have been far more common than the documented ones, so some of the general population controls may actually represent people already infected in the first wave. Moreover, the relative risk of re-infection may be over-estimated, if re-infection cases are artefacts of PCR false positives in either wave; and underestimated if people who were infected in the first wave were less likely to be tested in the second wave compared with other people having the same symptoms. In this context, Lumley et al reported that seropositive healthcare workers attended asymptomatic screening less often than seronegative healthcare workers with a rate ratio of 0.76 (95% CI: 0.73 to 0.80), a finding that is similar compared to another study from the UK.[10,13] Another limitation of our work is that we did not have access to viral sequencing data to compare first and re-infections, and it is not known how well our findings generalize to the re-infection risk concerning different genetic variants of SARS-CoV-2. Finally, we have to stress that our main findings are only a rough estimate of SARS-CoV-2 re-infection risk, requiring urgent confirmation in other populations and study settings.

In conclusion, we observed a relatively low tentative re-infection rate of SARS-CoV-2 in Austria that suggests a similar protection against SARS-CoV-2 infection compared to vaccine efficacies.[5,19,20] These data may be useful for decisions on public health measures and vaccination strategies to fight the COVID-19 pandemic.[2,19,20,23,24] Further studies are urgently needed to improve our knowledge on SARS-CoV-2 re-infection risk and its predisposing factors and clinical significance.

AUTHOR CONTRIBUTIONS

All authors have substantially contributed to the design, performance, analysis and reporting of the work. AC, LR and FA contributed to data collection. SP and JPAI analysed the data and wrote the manuscript.

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Exhibit "L"

Continued Effectiveness of COVID-19 Vaccination among Urban Healthcare Workers during Delta Variant Predominance

Running title: Continued COVID-19 VE during Delta Variant Predominance

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Abstract

Background: Data on COVID-19 vaccine effectiveness (VE) among healthcare workers (HCWs) during periods of delta variant predominance are limited.

Methods: We followed a population of urban Massachusetts HCWs (45% non-White) subject to epidemiologic surveillance. We accounted for covariates such as demographics and community background infection incidence, as well as information bias regarding COVID-19 diagnosis and vaccination status.

Results and Discussion: During the study period (December 16, 2020 to September 30, 2021), 4615 HCWs contributed to a total of 1,152,486 person-days at risk (excluding 309 HCWs with prior infection) and had a COVID-19 incidence rate of 5.2/10,000 (114 infections out of 219,842 person-days) for unvaccinated person-days and 0.6/10,000 (49 infections out of 830,084 person-days) for fully vaccinated person-days, resulting in an adjusted VE of 82.3% (95% CI: 75.1–87.4%). For the secondary analysis limited to the period of delta variant predominance in Massachusetts (i.e., July 1 to September 30, 2021), we observed an adjusted VE of 76.5% (95% CI: 40.9–90.6%). Independently, we found no re-infection among those with prior COVID-19, contributing to 74,557 re-infection-free person-days, adding to the evidence base for the robustness of naturally acquired immunity.

Background

Data on COVID-19 vaccine effectiveness (VE) among healthcare workers (HCWs) during periods of delta variant predominance are limited. Literature accounting for other potential determinants of infection rates is even more scarce.

Objective

To investigate the continued effectiveness of COVID-19 vaccination during the Delta variant predominance in a diverse and urban healthcare setting.

Methods and Findings

A community-based healthcare system in Massachusetts runs a COVID-19 vaccination program for employees (described previously (1)), with the Pfizer vaccine starting on December 16, Moderna on December 23, 2020, and J&J/Janssen in February 2021. Vaccination was available to all workers regardless of their in-person/remote working status from December 29, 2020. In addition, the system announced a vaccine mandate on August 16, 2021, which requires employees to receive their final dose by October 18, 2021 barring an approved religious or medical

We followed all actively serving HCWs in the system from December 16, 2020 to September 30, 2021, excluding those with prior COVID-19 infection from the main analyses. The outcome was having a positive PCR assay during the study period documented by the healthcare system's Occupational Health department (2). The established master database, comprised of workers' demographics, prior infection, *de novo* PCR positivity, vaccination (validated by the Massachusetts Immunization Information System and/or the healthcare system's medical records), and human resource administrative data, has been previously described (1, 2). For each HCW, we calculated the person-days at risk and categorized them according to vaccination status. A HCW's follow-up person-days were censored at the end of the study period, his/her termination date, the date tested positive for COVID, or the date he/she received a 3rd vaccine dose , whichever came first.

The Andersen-Gill extension of the Cox proportional hazards models were built to account for correlated data. We further adjusted for age, sex, race, and the Massachusetts statewide 7-day average of tested COVID cases (3) on the date the first dose was given to control for background rates. We estimated VE by calculating

analyses.

A total of 4,615 HCWs (average age of 45.0±13.3 years and female predominance (76.0%)) contributed to 1,152,486 person-days at risk during the study period. Forty-five percent of the study population was non-White (including 20% African American, 13.5% Hispanic, and 9.0% Asian). Of all HCWs, 4,418 (95.7%) had received at least one dose by the end of the study. Among them, 58.3% got Moderna, 39.4% Pfizer, 2.3% J&J/Janssen, and one (0.02%) got mixed doses of J&J/Janssen and Moderna. The results showed that throughout the study period, for fully vaccinated HCWs the VE is 82.3% (95% CI: 75.1–87.4%) after multivariable adjustment (Table, Figure).

We further conducted a secondary analysis limiting the study period from July 1, 2021 to September 30, 2021, corresponding to delta variant predominance in Massachusetts (4). We observed an incidence rate of 5.8/10,000 (15 events out of 25,910 person-days) for unvaccinated person-days and 1.3/10,000 (39 events out of 308,267 person-days) for 14 days after fully vaccinated, resulting in an adjusted VE of 76.5% (95% CI: 40.9–90.6%).

When we examined HCWs (n=423) with infections occurring before vaccination, no re-infection was observed, accumulating 74,557 re-infection-free person-days (starting 10 days after initial infection and censoring at the date of receiving their first vaccine dose). Further, after vaccination, previously infected HCWs did not contribute any breakthrough infection events among the vaccinated HCWs.

Discussion

To our knowledge, this study is one of the first in healthcare settings regarding continued VE during delta variant predominance. Our work also provides further evidence of naturally acquired immunity. We found similar VE against the delta variant, 76%, compared to another study's findings, 66% (5). Strengths included accounting for covariates and information bias such as demographics and background incidence, a multiethnic study population, consistent COVID-19 screening criteria, and well-validated vaccination records. Nonetheless, we did not examine individual manufacturers' VE due to a limited power.

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MMWR Morb Mortal Wkly Rep. 2021;70(34):1167-1169.

doi:10.15585/mmwr.mm7034e4

Table. Rate of infection during the study period (Dec 16, 2020 – Sep 30, 2021) across the four vaccination categories (excluding 309 people infected before Dec 15, 2020)

Status	Person-days	No. of infections	Rate per 10,000 person-days	Unadjusted vaccine effectiveness % (95% CI)	Adjusted vaccine effectiveness % (95% CI)*
Unvaccinated	219842	114	5.19	Reference	Reference
First dose (<14 days)	51329	17	3.31	44.8 (0.13–69.5)	38.8 (-10.8–66.2)
First dose (14+ days)†	51231	7	1.37	78.9 (51.6–90.8)	75.5 (43.9–89.3)
Fully vaccinated‡	830084	49	0.59	87.5 (83.0–90.8)	82.3 (75.1–87.4)

Vaccine effectiveness (95% CI) derived from the Andersen-Gill extension of the Cox proportional hazards model

* Adjust for age, sex, race, and the Massachusetts statewide 7-day average of new tested COVID-19 cases at the date for the first vaccine dose. Those with the race of "American Indian or Alaska Native", "Hawaiian or Pacific Islander", or "Two or More" were pooled into one level "other race".

† Not eligible for those receiving J&J/Janssen

‡ Equal or more than 14 days after single dose of J&J/Janssen or having received the second shot of Pfizer or Moderna

Figure. The Kaplan-Meier curve for the survival (i.e. infection-free) person-days across the four categories based on vaccination status

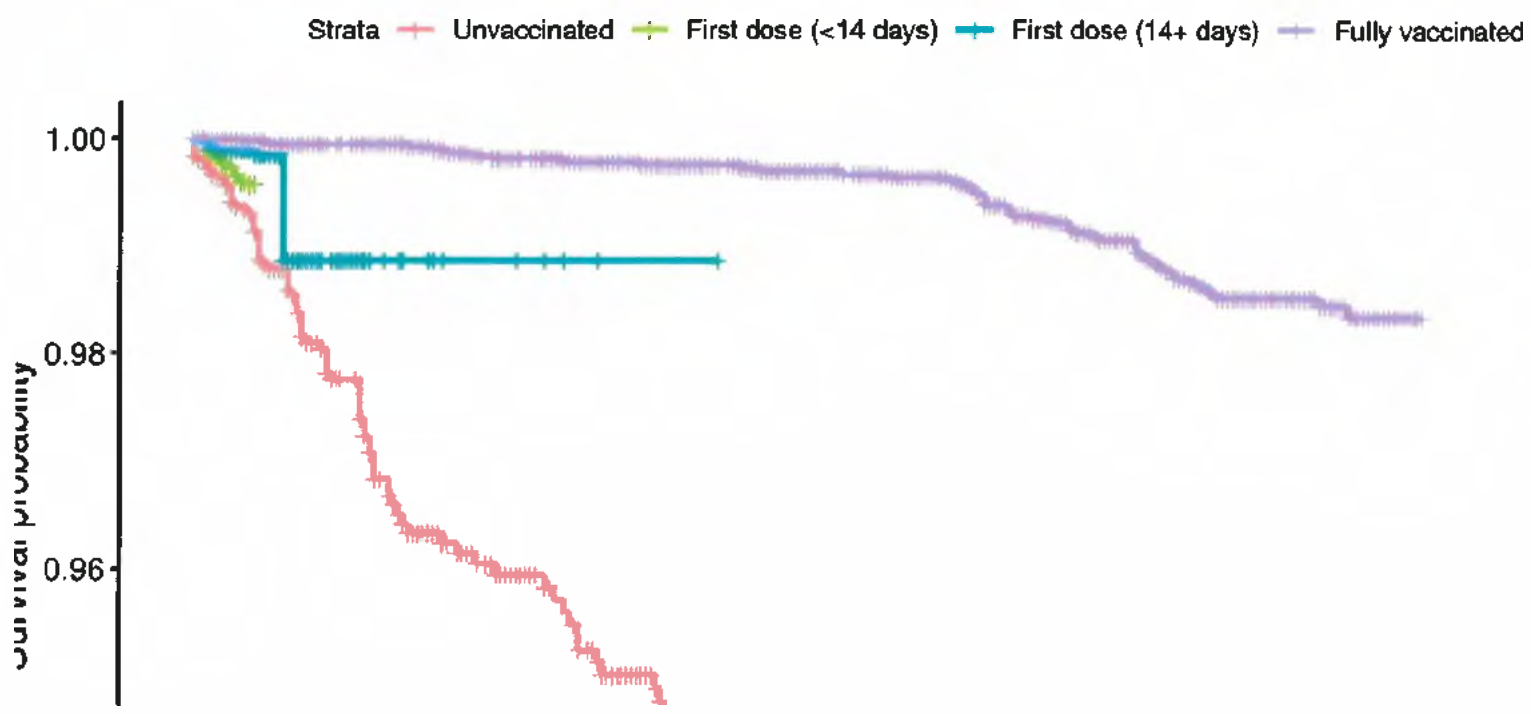
Strata
Unvaccinated
First dose (<14 days)
First dose (14+ days)
Fully vaccinated
1.00
0.98
0.96
Survival probability

Exhibit "M"

This is **Exhibit "M"** referred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at

135 Research Studies Affirm Naturally Acquired Immunity to Covid-19: Documented, Linked, and Quoted

BY PAUL ELIAS ALEXANDER OCTOBER 17, 2021 PUBLIC HEALTH 55 MINUTE READ

We should not force COVID vaccines on anyone when the evidence shows that naturally acquired immunity is equal to or more robust and superior to existing vaccines. Instead, we should respect the right of the bodily integrity of individuals to decide for themselves.

Public health officials and the medical establishment with the help of the politicized media are misleading the public with assertions that the COVID-19 shots provide greater protection than natural immunity. CDC Director Rochelle Walensky, for example, was deceptive in her October 2020 published *LANCET* statement that "there is no evidence for lasting protective immunity to SARS-CoV-2 following natural infection" and that "the consequence of waning immunity would present a risk to vulnerable populations for the indefinite future."

Immunology and virology 101 have taught us over a century that natural immunity confers protection against a respiratory virus's outer coat proteins, and not just one, e.g. the SARS-CoV-2 spike glycoprotein. There is even strong evidence for the persistence of antibodies. Even the CDC recognizes natural immunity for chicken-pox and measles, mumps, and rubella, but not for COVID-19.

This troubling situation of the vaccinated being infectious and transmitting the virus emerged in seminal nosocomial outbreak papers by Chau et al. (HCWs in Vietnam), the Finland hospital outbreak (spread among HCWs and patients), and the Israel hospital outbreak (spread among HCWs and patients). These studies also revealed that the PPE and masks were essentially ineffective in the healthcare setting. Again, the Marek's disease in chickens and the vaccination situation explains what we are potentially facing with these leaky vaccines (increased transmission, faster transmission, and more 'hotter' variants).

Moreover, existing immunity should be assessed before any vaccination, via an accurate, dependable, and reliable antibody test (or T cell immunity test) or be based on documentation of prior infection (a previous positive PCR or antigen test). Such would be evidence of immunity that is equal to that of vaccination and the immunity should be provided the same societal status as any vaccine-induced immunity. This will function to mitigate the societal anxiety with these forced vaccine mandates and societal upheaval due to job loss, denial of societal privileges etc. Tearing apart the vaccinated and the unvaccinated in a society, separating them, is not medically or scientifically supportable.

The Brownstone Institute previously documented 30 studies on natural immunity as it relates to Covid-19.

This follow-up chart is the most updated and comprehensive library list of 135 of the highest-quality, complete, most robust scientific studies and evidence reports/position statements on natural immunity as compared to

- Dr. Howard Tenenbaum, PhD (Faculty of Medicine, University of Toronto)
- Dr. Ramin Oskoui, MD (Foxhall Cardiology, Washington)
- Dr. Peter McCullough, MD (Truth for Health Foundation (TFH)), Texas
- Dr. Parvez Dara, MD (consultant, Medical Hematologist and Oncologist)

Evidence on natural immunity versus COVID-19 vaccine induced immunity:

Study / report title, author, and year published	**Predominant finding on natural immunity**
1) Necessity of COVID-19 vaccination in previously infected individuals, Shrestha, 2021	"Cumulative incidence of COVID-19 was examined among 52,238 employees in an American healthcare system. The cumulative incidence of SARS-CoV-2 infection remained almost zero among previously infected unvaccinated subjects, previously infected subjects who were vaccinated, and previously uninfected subjects who were vaccinated, compared with a steady increase in cumulative incidence among previously uninfected subjects who remained unvaccinated. Not one of the 1359 previously infected subjects who remained unvaccinated had a SARS-CoV-2 infection over the duration of the study. Individuals who have had SARS-CoV-2 infection are unlikely to benefit from COVID-19 vaccination…"
2) SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls, Le Bert, 2020	"Studied T cell responses against the structural (nucleocapsid (N) protein) and non-structural (NSP7 and NSP13 of *ORF1*) regions of SARS-CoV-2 in individuals convalescing from coronavirus disease 2019 (COVID-19) (n = 36). In all of these individuals, we found CD4 and CD8 T cells that recognized multiple regions of the N protein…showed that patients

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3) Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections,Gazit, 2021	"A retrospective observational study comparing three groups: (1) SARS-CoV-2-naïve individuals who received a two-dose regimen of the BioNTech/Pfizer mRNA BNT162b2 vaccine, (2) previously infected individuals who have not been vaccinated, and (3) previously infected *and* single dose vaccinated individuals found para a 13 fold increased risk of breakthrough Delta infections in double vaccinated persons, and a 27 fold increased risk for symptomatic breakthrough infection in the double vaccinated relative to the natural immunity recovered persons…the risk of hospitalization was 8 times higher in the double vaccinated (para)…this analysis demonstrated that natural immunity affords longer lasting and stronger protection against infection, symptomatic disease and hospitalization due to the Delta variant of SARS-CoV-2, compared to the BNT162b2 two-dose vaccine-induced immunity."
4) Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection, Le Bert, 2021	"Studied SARS-CoV-2–specific T cells in a cohort of asymptomatic (n = 85) and symptomatic (n = 75) COVID-19 patients after seroconversion…thus, asymptomatic SARS-CoV-2–infected individuals are not characterized by weak antiviral immunity; on the contrary, they mount a highly functional virus-specific cellular immune response."
5) Large-scale study of antibody titer decay following BNT162b2 mRNA vaccine or SARS-CoV-2 infection, Israel, 2021	"A total of 2,653 individuals fully vaccinated by two doses of vaccine during the study period and 4,361 convalescent patients were included. Higher SARS-CoV-2 IgG antibody titers were observed in vaccinated individuals (median 1581 AU/mL IQR [533.8-5644.6]) after the second vaccination, than in convalescent individuals (median 355.3 AU/mL IQR [141.2-998.7]; p<0.001). In vaccinated subjects, antibody titers decreased by up to 40% each subsequent month while in convalescents they decreased by less than 5% per month…this study demonstrates individuals who received the Pfizer-BioNTech mRNA vaccine have different kinetics of antibody levels compared to patients who had been

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6) SARS-CoV-2 re-infection risk in Austria, Pilz, 2021	Researchers recorded "40 tentative re-infections in 14, 840 COVID-19 survivors of the first wave (0.27%) and 253 581 infections in 8, 885, 640 individuals of the remaining general population (2.85%) translating into an odds ratio (95% confidence interval) of 0.09 (0.07 to 0.13)…relatively low re-infection rate of SARS-CoV-2 in Austria. Protection against SARS-CoV-2 after natural infection is comparable with the highest available estimates on vaccine efficacies." Additionally, hospitalization in only five out of 14,840 (0.03%) people and death in one out of 14,840 (0.01%) (tentative re-infection).
7) mRNA vaccine-induced SARS-CoV-2-specific T cells recognize B.1.1.7 and B.1.351 variants but differ in longevity and homing properties depending on prior infection status, Neidleman, 2021	"Spike-specific T cells from convalescent vaccinees differed strikingly from those of infection-naïve vaccinees, with phenotypic features suggesting superior long-term persistence and ability to home to the respiratory tract including the nasopharynx. These results provide reassurance that vaccine-elicited T cells respond robustly to the B.1.1.7 and B.1.351 variants, confirm that convalescents may not need a second vaccine dose."
8) Good news: Mild COVID-19 induces lasting antibody protection, Bhandari, 2021	"Months after recovering from mild cases of COVID-19, people still have immune cells in their body pumping out antibodies against the virus that causes COVID-19, according to a study from researchers at Washington University School of Medicine in St. Louis. Such cells could persist for a lifetime, churning out antibodies all the while. The findings, published May 24 in the journal Nature, suggest that mild cases of COVID-19 leave those infected with lasting antibody protection and that repeated bouts of illness are likely to be uncommon."
9) Robust neutralizing antibodies to SARS-CoV-2 infection persist for	"Neutralizing antibody titers against the SARS-CoV-2 spike protein persisted for at least 5 months after infection. Although continued monitoring of this cohort will be needed to confirm the longevity and

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10) Evolution of Antibody Immunity to SARS-CoV-2, Gaebler, 2020	"Concurrently, neutralizing activity in plasma decreases by five-fold in pseudo-type virus assays. In contrast, the number of RBD-specific memory B cells is unchanged. Memory B cells display clonal turnover after 6.2 months, and the antibodies they express have greater somatic hypermutation, increased potency and resistance to RBD mutations, indicative of continued evolution of the humoral response…we conclude that the memory B cell response to SARS-CoV-2 evolves between 1.3 and 6.2 months after infection in a manner that is consistent with antigen persistence."
11) Persistence of neutralizing antibodies a year after SARS-CoV-2 infection in humans, Haveri, 2021	"Assessed the persistence of serum antibodies following WT SARS-CoV-2 infection at 8 and 13 months after diagnosis in 367 individuals…found that NAb against the WT virus persisted in 89% and S-IgG in 97% of subjects for at least 13 months after infection."
12) Quantifying the risk of SARS-CoV-2 reinfection over time, Murchu, 2021	"Eleven large cohort studies were identified that estimated the risk of SARS-CoV-2 reinfection over time, including three that enrolled healthcare workers and two that enrolled residents and staff of elderly care homes. Across studies, the total number of PCR-positive or antibody-positive participants at baseline was 615,777, and the maximum duration of follow-up was more than 10 months in three studies. Reinfection was an uncommon event (absolute rate 0%–1.1%), with no study reporting an increase in the risk of reinfection over time."

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13) Natural immunity to covid is powerful. Policymakers seem afraid to say so, Makary, 2021	Makary writes "it's okay to have an incorrect scientific hypothesis. But when new data proves it wrong, you have to adapt. Unfortunately, many elected leaders and public health officials have held on far too long to the hypothesis that natural immunity offers unreliable protection against covid-19 — a contention that is being rapidly debunked by science. More than 15 studies have demonstrated the power of immunity acquired by previously having the virus. A 700,000-person study from Israel two weeks ago found that those who had experienced prior infections were 27 times less likely to get a second symptomatic covid infection than those who were vaccinated. This affirmed a June Cleveland Clinic study of health-care workers (who are often exposed to the virus), in which none who had previously tested positive for the coronavirus got reinfected. The study authors concluded that "individuals who have had SARS-CoV-2 infection are unlikely to benefit from covid-19 vaccination." And in May, a Washington University study found that even a mild covid infection resulted in long-lasting immunity."
14) SARS-CoV-2 elicits robust adaptive immune responses regardless of disease severity, Nielsen, 2021	"203 recovered SARS-CoV-2 infected patients in Denmark between April 3rd and July 9th 2020, at least 14 days after COVID-19 symptom recovery… report broad serological profiles within the cohort, detecting antibody binding to other human coronaviruses… the viral surface spike protein was identified as the dominant target for both neutralizing antibodies and $CD8^+$ T-cell responses. Overall, the majority of patients had robust adaptive immune responses, regardless of their disease severity."
15) Protection of previous SARS-CoV-2 infection is similar to that of BNT162b2 vaccine protection: A three-month nationwide experience	"Analyze an updated individual-level database of the entire population of Israel to assess the protection efficacy of both prior infection and vaccination in preventing subsequent SARS-CoV-2 infection, hospitalization with COVID-19, severe disease, and death due to COVID-19… vaccination was highly effective with overall estimated efficacy for documented infection of 92·8% (CI:[92·6, 93·0]); hospitalization 94·2%

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16) Incidence of Severe Acute Respiratory Syndrome Coronavirus-2 infection among previously infected or vaccinated employees, Kojima, 2021	"Employees were divided into three groups: (1) SARS-CoV-2 naïve and unvaccinated, (2) previous SARS-CoV-2 infection, and (3) vaccinated. Person-days were measured from the date of the employee first test and truncated at the end of the observation period. SARS-CoV-2 infection was defined as two positive SARS-CoV-2 PCR tests in a 30-day period… 4313, 254 and 739 employee records for groups 1, 2, and 3…previous SARS-CoV-2 infection and vaccination for SARS-CoV-2 were associated with decreased risk for infection or re-infection with SARS-CoV-2 in a routinely screened workforce. The was no difference in the infection incidence between vaccinated individuals and individuals with previous infection."
17) Having SARS-CoV-2 once confers much greater immunity than a vaccine—but vaccination remains vital, Wadman, 2021	"Israelis who had an infection were more protected against the Delta coronavirus variant than those who had an already highly effective COVID-19 vaccine…the newly released data show people who once had a SARS-CoV-2 infection were much less likely than never-infected, vaccinated people to get Delta, develop symptoms from it, or become hospitalized with serious COVID-19."
18) One-year sustained cellular and humoral immunities of COVID-19 convalescents, Zhang, 2021	"A systematic antigen-specific immune evaluation in 101 COVID-19 convalescents; SARS-CoV-2-specific IgG antibodies, and also NAb can persist among over 95% COVID-19 convalescents from 6 months to 12 months after disease onset. At least 19/71 (26%) of COVID-19 convalescents (double positive in ELISA and MCLIA) had detectable circulating IgM antibody against SARS-CoV-2 at 12m post-disease onset. Notably, the percentages of convalescents with positive SARS-CoV-2-specific T-cell responses (at least one of the SARS-CoV-2 antigen S1, S2, M and N protein) were 71/76 (93%) and 67/73 (92%) at 6m and 12m, respectively."

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19) Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19, Rodda, 2021	"Recovered individuals developed SARS-CoV-2-specific immunoglobulin (IgG) antibodies, neutralizing plasma, and memory B and memory T cells that persisted for at least 3 months. Our data further reveal that SARS-CoV-2-specific IgG memory B cells increased over time. Additionally, SARS-CoV-2-specific memory lymphocytes exhibited characteristics associated with potent antiviral function: memory T cells secreted cytokines and expanded upon antigen re-encounter, whereas memory B cells expressed receptors capable of neutralizing virus when expressed as monoclonal antibodies. Therefore, mild COVID-19 elicits memory lymphocytes that persist and display functional hallmarks of antiviral immunity."
20) Discrete Immune Response Signature to SARS-CoV-2 mRNA Vaccination Versus Infection, Ivanova, 2021	"Performed multimodal single-cell sequencing on peripheral blood of patients with acute COVID-19 and healthy volunteers before and after receiving the SARS-CoV-2 BNT162b2 mRNA vaccine to compare the immune responses elicited by the virus and by this vaccine…both infection and vaccination induced robust innate and adaptive immune responses, our analysis revealed significant qualitative differences between the two types of immune challenges. In COVID-19 patients, immune responses were characterized by a highly augmented interferon response which was largely absent in vaccine recipients. Increased interferon signaling likely contributed to the observed dramatic upregulation of cytotoxic genes in the peripheral T cells and innate-like lymphocytes in patients but not in immunized subjects. Analysis of B and T cell receptor repertoires revealed that while the majority of clonal B and T cells in COVID-19 patients were effector cells, in vaccine recipients clonally expanded cells were primarily circulating memory cells…we observed the presence of cytotoxic CD4 T cells in COVID-19 patients that were largely absent in healthy volunteers following immunization. While hyper-activation of inflammatory responses and cytotoxic cells may

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21) SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans, Turner, 2021	"Bone marrow plasma cells (BMPCs) are a persistent and essential source of protective antibodies… durable serum antibody titres are maintained by long-lived plasma cells—non-replicating, antigen-specific plasma cells that are detected in the bone marrow long after the clearance of the antigen … S-binding BMPCs are quiescent, which suggests that they are part of a stable compartment. Consistently, circulating resting memory B cells directed against SARS-CoV-2 S were detected in the convalescent individuals. Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans…overall, our data provide strong evidence that SARS-CoV-2 infection in humans robustly establishes the two arms of humoral immune memory: long-lived bone marrow plasma cells (BMPCs) and memory B-cells."
22) SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN), Jane Hall, 2021	"The SARS-CoV-2 Immunity and Reinfection Evaluation study… 30625 participants were enrolled into the study… a previous history of SARS-CoV-2 infection was associated with an 84% lower risk of infection, with median protective effect observed 7 months following primary infection. This time period is the minimum probable effect because seroconversions were not included. This study shows that previous infection with SARS-CoV-2 induces effective immunity to future infections in most individuals."
23) Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers, Houlihan, 2020	"Enrolled 200 patient-facing HCWs between March 26 and April 8, 2020… represents a 13% infection rate (i.e. 14 of 112 HCWs) within the 1 month of follow-up in those with no evidence of antibodies or viral shedding at enrolment. By contrast, of 33 HCWs who tested positive by serology but tested negative by RT-PCR at enrolment, 32 remained negative by RT-PCR through follow-up, and one tested positive by RT-PCR on days 8 and 13 after enrolment."

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25) Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells, Cohen, 2021	"Evaluate 254 COVID-19 patients longitudinally up to 8 months and find durable broad-based immune responses. SARS-CoV-2 spike binding and neutralizing antibodies exhibit a bi-phasic decay with an extended half-life of >200 days suggesting the generation of longer-lived plasma cells… most recovered COVID-19 patients mount broad, durable immunity after infection, spike IgG+ memory B cells increase and persist post-infection, durable polyfunctional CD4 and CD8 T cells recognize distinct viral epitope regions."
26) Single cell profiling of T and B cell repertoires following SARS-CoV-2 mRNA vaccine, Sureshchandra, 2021	"Used single-cell RNA sequencing and functional assays to compare humoral and cellular responses to two doses of mRNA vaccine with responses observed in convalescent individuals with asymptomatic disease… natural infection induced expansion of larger CD8 T cell clones occupied distinct clusters, likely due to the recognition of a broader set of viral epitopes presented by the virus not seen in the mRNA vaccine."
27) SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy, Abu-Raddad, 2021	"SARS-CoV-2 antibody-positive persons from April 16 to December 31, 2020 with a PCR-positive swab ≥14 days after the first-positive antibody test were investigated for evidence of reinfection, 43,044 antibody-positive persons who were followed for a median of 16.3 weeks… reinfection is rare in the young and international population of Qatar. Natural infection appears to elicit strong protection against reinfection with an efficacy ~95% for at least seven months."
28) Orthogonal SARS-CoV-2 Serological Assays Enable Surveillance of Low-Prevalence Communities and Reveal Durable Humoral Immunity, Ripperger, 2020	"Conducted a serological study to define correlates of immunity against SARS-CoV-2. Compared to those with mild coronavirus disease 2019 (COVID-19) cases, individuals with severe disease exhibited elevated virus-neutralizing titers and antibodies against the nucleocapsid (N) and the receptor binding domain (RBD) of the spike protein…neutralizing and spike-specific antibody production persists for at least 5–7 months… nucleocapsid antibodies frequently become undetectable by

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30) Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers, Lumley, 2021	"12,541 health care workers participated and had anti-spike IgG measured; 11,364 were followed up after negative antibody results and 1265 after positive results, including 88 in whom seroconversion occurred during follow-up…a total of 223 anti-spike–seronegative health care workers had a positive PCR test (1.09 per 10,000 days at risk), 100 during screening while they were asymptomatic and 123 while symptomatic, whereas 2 anti-spike–seropositive health care workers had a positive PCR test… the presence of anti-spike or anti-nucleocapsid IgG antibodies was associated with a substantially reduced risk of SARS-CoV-2 reinfection in the ensuing 6 months."
31) Researchers find long-lived immunity to 1918 pandemic virus, CIDRAP, 2008 and the actual 2008 NATURE journal publication by Yu	"A study of the blood of older people who survived the 1918 influenza pandemic reveals that antibodies to the strain have lasted a lifetime and can perhaps be engineered to protect future generations against similar strains…the group collected blood samples from 32 pandemic survivors aged 91 to 101..the people recruited for the study were 2 to 12 years old in 1918 and many recalled sick family members in their households, which suggests they were directly exposed to the virus, the authors report. The group found that 100% of the subjects had serum-neutralizing activity against the 1918 virus and 94% showed serologic reactivity to the 1918 hemagglutinin. The investigators generated B lymphoblastic cell lines from the peripheral blood mononuclear cells of eight subjects. Transformed cells from the blood of 7 of the 8 donors yielded secreting antibodies that bound the 1918 hemagglutinin." Yu: "here we show that of the 32 individuals tested that were born in or before 1915, each showed sero-reactivity with the 1918 virus, nearly 90 years after the pandemic. Seven of the eight donor samples tested had circulating B cells that secreted antibodies that bound the 1918 HA. We isolated B cells from subjects and generated five monoclonal antibodies that showed potent neutralizing activity against 1918 virus from three

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32) Live virus neutralisation testing in convalescent patients and subjects vaccinated against 19A, 20B, 20I/501Y.V1 and 20H/501Y.V2 isolates of SARS-CoV-2, Gonzalez, 2021	"No significant difference was observed between the 20B and 19A isolates for HCWs with mild COVID-19 and critical patients. However, a significant decrease in neutralisation ability was found for 20I/501Y.V1 in comparison with 19A isolate for critical patients and HCWs 6-months post infection. Concerning 20H/501Y.V2, all populations had a significant reduction in neutralising antibody titres in comparison with the 19A isolate. Interestingly, a significant difference in neutralisation capacity was observed for vaccinated HCWs between the two variants whereas it was not significant for the convalescent groups…the reduced neutralising response observed towards the 20H/501Y.V2 in comparison with the 19A and 20I/501Y.V1 isolates in fully immunized subjects with the BNT162b2 vaccine is a striking finding of the study."
33) Differential effects of the second SARS-CoV-2 mRNA vaccine dose on T cell immunity in naïve and COVID-19 recovered individuals, Camara, 2021	"Characterized SARS-CoV-2 spike-specific humoral and cellular immunity in naïve and previously infected individuals during full BNT162b2 vaccination…results demonstrate that the second dose increases both the humoral and cellular immunity in naïve individuals. On the contrary, the second BNT162b2 vaccine dose results in a reduction of cellular immunity in COVID-19 recovered individuals."
34) Op-Ed: Quit Ignoring Natural COVID Immunity, Klausner, 2021	"Epidemiologists estimate over 160 million people worldwide have recovered from COVID-19. Those who have recovered have an astonishingly low frequency of repeat infection, disease, or death."
35) Association of SARS-CoV-2 Seropositive Antibody Test With Risk of Future Infection, Harvey, 2021	"To evaluate evidence of SARS-CoV-2 infection based on diagnostic nucleic acid amplification test (NAAT) among patients with positive vs negative test results for antibodies in an observational descriptive cohort study of clinical laboratory and linked claims data…the cohort included 3 257 478 unique patients with an index antibody test…patients with positive antibody test results were initially more likely to have positive

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36) SARS-CoV-2 seropositivity and subsequent infection risk in healthy young adults: a prospective cohort study, Letizia, 2021	"Investigated the risk of subsequent SARS-CoV-2 infection among young adults (CHARM marine study) seropositive for a previous infection… enrolled 3249 participants, of whom 3168 (98%) continued into the 2-week quarantine period. 3076 (95%) participants…Among 189 seropositive participants, 19 (10%) had at least one positive PCR test for SARS-CoV-2 during the 6-week follow-up (1·1 cases per person-year). In contrast, 1079 (48%) of 2247 seronegative participants tested positive (6·2 cases per person-year). The incidence rate ratio was 0·18 (95% CI 0·11–0·28; p<0·001)…infected seropositive participants had viral loads that were about 10-times lower than those of infected seronegative participants (ORF1ab gene cycle threshold difference 3·95 [95% CI 1·23–6·67]; p=0·004)."
37) Associations of Vaccination and of Prior Infection With Positive PCR Test Results for SARS-CoV-2 in Airline Passengers Arriving in Qatar, Bertollini, 2021	"Of 9,180 individuals with no record of vaccination but with a record of prior infection at least 90 days before the PCR test (group 3), 7694 could be matched to individuals with no record of vaccination or prior infection (group 2), among whom PCR positivity was 1.01% (95% CI, 0.80%-1.26%) and 3.81% (95% CI, 3.39%-4.26%), respectively. The relative risk for PCR positivity was 0.22 (95% CI, 0.17-0.28) for vaccinated individuals and 0.26 (95% CI, 0.21-0.34) for individuals with prior infection compared with no record of vaccination or prior infection."
38) Natural immunity against COVID-19 significantly reduces the risk of reinfection: findings from a cohort of sero-survey participants, Mishra, 2021	"Followed up with a subsample of our previous sero-survey participants to assess whether natural immunity against SARS-CoV-2 was associated with a reduced risk of re-infection (India)… out of the 2238 participants, 1170 were sero-positive and 1068 were sero-negative for antibody against COVID-19. Our survey found that only 3 individuals in the sero-positive group got infected with COVID-19 whereas 127 individuals reported contracting the infection the sero-negative group…from the 3 sero-positives re-infected with COVID-19, one had hospitalization, but did not require oxygen support or critical care…development of antibody

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39) Lasting immunity found after recovery from COVID-19, NIH, 2021	"The researchers found durable immune responses in the majority of people studied. Antibodies against the spike protein of SARS-CoV-2, which the virus uses to get inside cells, were found in 98% of participants one month after symptom onset. As seen in previous studies, the number of antibodies ranged widely between individuals. But, promisingly, their levels remained fairly stable over time, declining only modestly at 6 to 8 months after infection… virus-specific B cells increased over time. People had more memory B cells six months after symptom onset than at one month afterwards… levels of T cells for the virus also remained high after infection. Six months after symptom onset, 92% of participants had CD4+ T cells that recognized the virus… 95% of the people had at least 3 out of 5 immune-system components that could recognize SARS-CoV-2 up to 8 months after infection."
40) SARS-CoV-2 Natural Antibody Response Persists for at Least 12 Months in a Nationwide Study From the Faroe Islands, Petersen, 2021	"The seropositive rate in the convalescent individuals was above 95% at all sampling time points for both assays and remained stable over time; that is, almost all convalescent individuals developed antibodies… results show that SARS-CoV-2 antibodies persisted at least 12 months after symptom onset and maybe even longer, indicating that COVID-19-convalescent individuals may be protected from reinfection."
41) SARS-CoV-2-specific T cell memory is sustained in COVID-19 convalescent patients for 10 months with successful development of stem cell-like memory T cells, Jung, 2021	"ex vivo assays to evaluate SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell responses in COVID-19 convalescent patients up to 317 days post-symptom onset (DPSO), and find that memory T cell responses are maintained during the study period regardless of the severity of COVID-19. In particular, we observe sustained polyfunctionality and proliferation capacity of SARS-CoV-2-specific T cells. Among SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells detected by activation-induced markers, the proportion of stem cell-like memory T (T_{SCM}) cells is increased, peaking at approximately 120 DPSO."

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42) Immune Memory in Mild COVID-19 Patients and Unexposed Donors Reveals Persistent T Cell Responses After SARS-CoV-2 Infection, Ansari, 2021	"Analyzed 42 unexposed healthy donors and 28 mild COVID-19 subjects up to 5 months from the recovery for SARS-CoV-2 specific immunological memory. Using HLA class II predicted peptide megapools, we identified SARS-CoV-2 cross-reactive $CD4^+$ T cells in around 66% of the unexposed individuals. Moreover, we found detectable immune memory in mild COVID-19 patients several months after recovery in the crucial arms of protective adaptive immunity; $CD4^+$ T cells and B cells, with a minimal contribution from $CD8^+$ T cells. Interestingly, the persistent immune memory in COVID-19 patients is predominantly targeted towards the Spike glycoprotein of the SARS-CoV-2. This study provides the evidence of both high magnitude pre-existing and persistent immune memory in Indian population."
43) COVID-19 natural immunity, WHO, 2021	"Current evidence points to most individuals developing strong protective immune responses following natural infection with SARSCoV-2. Within 4 weeks following infection, 90-99% of individuals infected with the SARS-CoV-2 virus develop detectable neutralizing antibodies. The strength and duration of the immune responses to SARS-CoV-2 are not completely understood and currently available data suggests that it varies by age and the severity of symptoms. Available scientific data suggests that in most people immune responses remain robust and protective against reinfection for at least 6-8 months after infection (the longest follow up with strong scientific evidence is currently approximately 8 months)."
44) Antibody Evolution after SARS-CoV-2 mRNA Vaccination, Cho, 2021	"We conclude that memory antibodies selected over time by natural infection have greater potency and breadth than antibodies elicited by vaccination…boosting vaccinated individuals with currently available mRNA vaccines would produce a quantitative increase in plasma neutralizing activity but not the qualitative advantage against variants obtained by vaccinating convalescent individuals."

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46) Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection, Dan, 2021	"Analyzed multiple compartments of circulating immune memory to SARS-CoV-2 in 254 samples from 188 COVID-19 cases, including 43 samples at ≥ 6 months post-infection…IgG to the Spike protein was relatively stable over 6+ months. Spike-specific memory B cells were more abundant at 6 months than at 1 month post symptom onset."
47) The prevalence of adaptive immunity to COVID-19 and reinfection after recovery – a comprehensive systematic review and meta-analysis of 12 011 447 individuals, Chivese, 2021	"Fifty-four studies, from 18 countries, with a total of 12 011 447 individuals, followed up to 8 months after recovery, were included. At 6-8 months after recovery, the prevalence of detectable SARS-CoV-2 specific immunological memory remained high; IgG – 90.4%… pooled prevalence of reinfection was 0.2% (95%CI 0.0 – 0.7, I^2 = 98.8, 9 studies). Individuals who recovered from COVID-19 had an 81% reduction in odds of a reinfection (OR 0.19, 95% CI 0.1 – 0.3, I^2 = 90.5%, 5 studies)."
48) Reinfection Rates among Patients who Previously Tested Positive for COVID-19: a Retrospective Cohort Study, Sheehan, 2021	"Retrospective cohort study of one multi-hospital health system included 150,325 patients tested for COVID-19 infection…prior infection in patients with COVID-19 was highly protective against reinfection and symptomatic disease. This protection increased over time, suggesting that viral shedding or ongoing immune response may persist beyond 90 days and may not represent true reinfection."
49) Assessment of SARS-CoV-2 Reinfection 1 Year After Primary Infection in a Population in Lombardy, Italy, Vitale, 2020	"The study results suggest that reinfections are rare events and patients who have recovered from COVID-19 have a lower risk of reinfection. Natural immunity to SARS-CoV-2 appears to confer a protective effect for at least a year, which is similar to the protection reported in recent vaccine studies."
50) Prior SARS-CoV-2 infection is associated with protection against	"We observed no symptomatic reinfections in a cohort of healthcare workers…this apparent immunity to re-infection was maintained for at least 6 months…test positivity rates were 0% (0/128 [95% CI: 0–2.9]) in

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51) mRNA vaccine-induced T cells respond identically to SARS-CoV-2 variants of concern but differ in longevity and homing properties depending on prior infection status, Neidleman, 2021	"In infection-naïve individuals, the second dose boosted the quantity and altered the phenotypic properties of SARS-CoV-2-specific T cells, while in convalescents the second dose changed neither. Spike-specific T cells from convalescent vaccinees differed strikingly from those of infection-naïve vaccinees, with phenotypic features suggesting superior long-term persistence and ability to home to the respiratory tract including the nasopharynx."
52) Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals, Grifoni, 2020	"Using HLA class I and II predicted peptide "megapools," circulating SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cells were identified in ~70% and 100% of COVID-19 convalescent patients, respectively. $CD4^+$ T cell responses to spike, the main target of most vaccine efforts, were robust and correlated with the magnitude of the anti-SARS-CoV-2 IgG and IgA titers. The M, spike, and N proteins each accounted for 11%–27% of the total $CD4^+$ response, with additional responses commonly targeting nsp3, nsp4, ORF3a, and ORF8, among others. For $CD8^+$ T cells, spike and M were recognized, with at least eight SARS-CoV-2 ORFs targeted."
53) NIH Director's Blog: Immune T Cells May Offer Lasting Protection Against COVID-19, Collins, 2021	"Much of the study on the immune response to SARS-CoV-2, the novel coronavirus that causes COVID-19, has focused on the production of antibodies. But, in fact, immune cells known as memory T cells also play an important role in the ability of our immune systems to protect us against many viral infections, including—it now appears—COVID-19.An intriguing new study of these memory T cells suggests they might protect some people newly infected with SARS-CoV-2 by remembering past encounters with other human coronaviruses. This might potentially explain why some people seem to fend off the virus and may be less susceptible to becoming severely ill with COVID-19."
54) Ultrapotent antibodies	"Our study demonstrates that convalescent subjects previously infected

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55) Why COVID-19 Vaccines Should Not Be Required for All Americans, Makary, 2021	"Requiring the vaccine in people who are already immune with natural immunity has no scientific support. While vaccinating those people may be beneficial – and it's a reasonable hypothesis that vaccination may bolster the longevity of their immunity – to argue dogmatically that they *must* get vaccinated has zero clinical outcome data to back it. As a matter of fact, we have data to the contrary: A Cleveland Clinic study found that vaccinating people with natural immunity did not add to their level of protection."
56) Protracted yet coordinated differentiation of long-lived SARS-CoV-2-specific CD8+ T cells during COVID-19 convalescence, Ma, 2021	"Screened 21 well-characterized, longitudinally-sampled convalescent donors that recovered from mild COVID-19…following a typical case of mild COVID-19, SARS-CoV-2-specific CD8+ T cells not only persist but continuously differentiate in a coordinated fashion well into convalescence, into a state characteristic of long-lived, self-renewing memory."
57) Decrease in Measles Virus-Specific CD4 T Cell Memory in Vaccinated Subjects, Naniche, 2004	"Characterized the profiles of measles vaccine (MV) vaccine-induced antigen-specific T cells over time since vaccination. In a cross-sectional study of healthy subjects with a history of MV vaccination, we found that MV-specific CD4 and CD8 T cells could be detected up to 34 years after vaccination. The levels of MV-specific CD8 T cells and MV-specific IgG remained stable, whereas the level of MV-specific CD4 T cells decreased significantly in subjects who had been vaccinated >21 years earlier."
58) Remembrance of Things Past: Long-Term B Cell Memory After Infection and Vaccination, Palm, 2019	"The success of vaccines is dependent on the generation and maintenance of immunological memory. The immune system can remember previously encountered pathogens, and memory B and T cells are critical in secondary responses to infection. Studies in mice have helped to understand how different memory B cell populations are generated following antigen exposure and how affinity for the antigen is

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59) SARS-CoV-2 specific memory B-cells from individuals with diverse disease severities recognize SARS-CoV-2 variants of concern, Lyski, 2021	"Examined the magnitude, breadth, and durability of SARS-CoV-2 specific antibodies in two distinct B-cell compartments: long-lived plasma cell-derived antibodies in the plasma, and peripheral memory B-cells along with their associated antibody profiles elicited after *in vitro* stimulation. We found that magnitude varied amongst individuals, but was the highest in hospitalized subjects. Variants of concern (VoC) -RBD-reactive antibodies were found in the plasma of 72% of samples in this investigation, and VoC-RBD-reactive memory B-cells were found in all but 1 subject at a single time-point. This finding, that VoC-RBD-reactive MBCs are present in the peripheral blood of all subjects including those that experienced asymptomatic or mild disease, provides a reason for optimism regarding the capacity of vaccination, prior infection, and/or both, to limit disease severity and transmission of variants of concern as they continue to arise and circulate."
60) Exposure to SARS-CoV-2 generates T-cell memory in the absence of a detectable viral infection, Wang, 2021	"T-cell immunity is important for recovery from COVID-19 and provides heightened immunity for re-infection. However, little is known about the SARS-CoV-2-specific T-cell immunity in virus-exposed individuals…report virus-specific $CD4^+$ and $CD8^+$ T-cell memory in recovered COVID-19 patients and close contacts…close contacts are able to gain T-cell immunity against SARS-CoV-2 despite lacking a detectable infection."
61) CD8+ T-Cell Responses in COVID-19 Convalescent Individuals Target Conserved Epitopes From Multiple Prominent SARS-CoV-2 Circulating Variants, Redd, 2021and Lee, 2021	"The CD4 and CD8 responses generated after natural infection are equally robust, showing activity against multiple "epitopes" (little segments) of the spike protein of the virus. For instance, CD8 cells responds to 52 epitopes and CD4 cells respond to 57 epitopes across the spike protein, so that a few mutations in the variants cannot knock out such a robust and in-breadth T cell response…only 1 mutation found in Beta variant-spike overlapped with a previously identified epitope (1/52), suggesting that virtually all anti-SARS-CoV-2 CD8+ T-cell responses should recognize these newly described variants."

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63) Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans, Mateus, 2020	"Found that the pre-existing reactivity against SARS-CoV-2 comes from memory T cells and that cross-reactive T cells can specifically recognize a SARS-CoV-2 epitope as well as the homologous epitope from a common cold coronavirus. These findings underline the importance of determining the impacts of pre-existing immune memory in COVID-19 disease severity."
64) Longitudinal observation of antibody responses for 14 months after SARS-CoV-2 infection, Dehgani-Mobaraki, 2021	"Better understanding of antibody responses against SARS-CoV-2 after natural infection might provide valuable insights into the future implementation of vaccination policies. Longitudinal analysis of IgG antibody titers was carried out in 32 recovered COVID-19 patients based in the Umbria region of Italy for 14 months after Mild and Moderately-Severe infection…study findings are consistent with recent studies reporting antibody persistency suggesting that induced SARS-CoV-2 immunity through natural infection, might be very efficacious against re-infection (>90%) and could persist for more than six months. Our study followed up patients up to 14 months demonstrating the presence of anti-S-RBD IgG in 96.8% of recovered COVID-19 subjects."
65) Humoral and circulating follicular helper T cell responses in recovered patients with COVID-19, Juno, 2020	"Characterized humoral and circulating follicular helper T cell (cTFH) immunity against spike in recovered patients with coronavirus disease 2019 (COVID-19). We found that S-specific antibodies, memory B cells and cTFH are consistently elicited after SARS-CoV-2 infection, demarking robust humoral immunity and positively associated with plasma neutralizing activity."
66) Convergent antibody responses to SARS-CoV-2 in convalescent individuals, Robbiani, 2020	"149 COVID-19-convalescent individuals…antibody sequencing revealed the expansion of clones of RBD-specific memory B cells that expressed closely related antibodies in different individuals. Despite low plasma titres, antibodies to three distinct epitopes on the RBD neutralized the virus with half-maximal inhibitory concentrations (IC_{50} values) as low as

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68) Had COVID? You'll probably make antibodies for a lifetime, Callaway, 2021	"People who recover from mild COVID-19 have bone-marrow cells that can churn out antibodies for decades…the study provides evidence that immunity triggered by SARS-CoV-2 infection will be extraordinarily long-lasting."
69) A majority of uninfected adults show preexisting antibody reactivity against SARS-CoV-2, Majdoubi, 2021	In greater Vancouver Canada, "using a highly sensitive multiplex assay and positive/negative thresholds established in infants in whom maternal antibodies have waned, we determined that more than 90% of uninfected adults showed antibody reactivity against the spike protein, receptor-binding domain (RBD), N-terminal domain (NTD), or the nucleocapsid (N) protein from SARS-CoV-2."
70) SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19, Braun, 2020	"The results indicate that spike-protein cross-reactive T cells are present, which were probably generated during previous encounters with endemic coronaviruses."
71) Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection, Wang, 2021	"A cohort of 63 individuals who have recovered from COVID-19 assessed at 1.3, 6.2 and 12 months after SARS-CoV-2 infection…the data suggest that immunity in convalescent individuals will be very long lasting."
72) One Year after Mild COVID-19: The Majority of Patients Maintain Specific Immunity, But One in Four Still Suffer from Long-Term Symptoms, Rank, 2021	"Long-lasting immunological memory against SARS-CoV-2 after mild COVID-19."
73) IDSA, 2021	"Immune responses to SARS-CoV-2 following natural infection can persist

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74) Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study, Holm Hansen, 2021	Denmark, "during the first surge (ie, before June, 2020), 533 381 people were tested, of whom 11 727 (2·20%) were PCR positive, and 525 339 were eligible for follow-up in the second surge, of whom 11 068 (2·11%) had tested positive during the first surge. Among eligible PCR-positive individuals from the first surge of the epidemic, 72 (0·65% [95% CI 0·51–0·82]) tested positive again during the second surge compared with 16 819 (3·27% [3·22–3·32]) of 514 271 who tested negative during the first surge (adjusted RR 0·195 [95% CI 0·155–0·246])."
75) Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity, Moderbacher, 2020	"Adaptive immune responses limit COVID-19 disease severity…multiple coordinated arms of adaptive immunity control better than partial responses…completed a combined examination of all three branches of adaptive immunity at the level of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell and neutralizing antibody responses in acute and convalescent subjects. SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells were each associated with milder disease. Coordinated SARS-CoV-2-specific adaptive immune responses were associated with milder disease, suggesting roles for both $CD4^+$ and $CD8^+$ T cells in protective immunity in COVID-19."
76) Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals, Ni, 2020	"Collected blood from COVID-19 patients who have recently become virus-free, and therefore were discharged, and detected SARS-CoV-2-specific humoral and cellular immunity in eight newly discharged patients. Follow-up analysis on another cohort of six patients 2 weeks post discharge also revealed high titers of immunoglobulin G (IgG) antibodies. In all 14 patients tested, 13 displayed serum-neutralizing activities in a pseudotype entry assay. Notably, there was a strong correlation between neutralization antibody titers and the numbers of virus-specific T cells."

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78) Negligible impact of SARS-CoV-2 variants on $CD4^+$ and $CD8^+$ T cell reactivity in COVID-19 exposed donors and vaccinees, Tarke, 2021	"Performed a comprehensive analysis of SARS-CoV-2-specific CD4+ and CD8+ T cell responses from COVID-19 convalescent subjects recognizing the ancestral strain, compared to variant lineages B.1.1.7, B.1.351, P.1, and CAL.20C as well as recipients of the Moderna (mRNA-1273) or Pfizer/BioNTech (BNT162b2) COVID-19 vaccines… the sequences of the vast majority of SARS-CoV-2 T cell epitopes are not affected by the mutations found in the variants analyzed. Overall, the results demonstrate that CD4+ and CD8+ T cell responses in convalescent COVID-19 subjects or COVID-19 mRNA vaccinees are not substantially affected by mutations."
79) A 1 to 1000 SARS-CoV-2 reinfection proportion in members of a large healthcare provider in Israel: a preliminary report, Perez, 2021	Israel, "out of 149,735 individuals with a documented positive PCR test between March 2020 and January 2021, 154 had two positive PCR tests at least 100 days apart, reflecting a reinfection proportion of 1 per 1000."
80) Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients, Iyer, 2020	"Measured plasma and/or serum antibody responses to the receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2 in 343 North American patients infected with SARS-CoV-2 (of which 93% required hospitalization) up to 122 days after symptom onset and compared them to responses in 1548 individuals whose blood samples were obtained prior to the pandemic…IgG antibodies persisted at detectable levels in patients beyond 90 days after symptom onset, and seroreversion was only observed in a small percentage of individuals. The concentration of these anti-RBD IgG antibodies was also highly correlated with pseudovirus NAb titers, which also demonstrated minimal decay. The observation that IgG and neutralizing antibody responses persist is encouraging, and suggests the development of robust systemic immune memory in individuals with severe infection."

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81) A population-based analysis of the longevity of SARS-CoV-2 antibody seropositivity in the United States, Alfego, 2021	"To track population-based SARS-CoV-2 antibody seropositivity duration across the United States using observational data from a national clinical laboratory registry of patients tested by nucleic acid amplification (NAAT) and serologic assays… specimens from 39,086 individuals with confirmed positive COVID-19…both S and N SARS-CoV-2 antibody results offer an encouraging view of how long humans may have protective antibodies against COVID-19, with curve smoothing showing population seropositivity reaching 90% within three weeks, regardless of whether the assay detects N or S-antibodies. Most importantly, this level of seropositivity was sustained with little decay through ten months after initial positive PCR."
82) What are the roles of antibodies versus a durable, high- quality T-cell response in protective immunity against SARS-CoV-2? Hellerstein, 2020	"Progress in laboratory markers for SARS-CoV2 has been made with identification of epitopes on CD4 and CD8 T-cells in convalescent blood. These are much less dominated by spike protein than in previous coronavirus infections. Although most vaccine candidates are focusing on spike protein as antigen, natural infection by SARS-CoV-2 induces broad epitope coverage, cross-reactive with other betacoronviruses."
83) Broad and strong memory $CD4^+$ and $CD8^+$ T cells induced by SARS-CoV-2 in UK convalescent COVID-19 patients, Peng, 2020	"Study of 42 patients following recovery from COVID-19, including 28 mild and 14 severe cases, comparing their T cell responses to those of 16 control donors…found the breadth, magnitude and frequency of memory T cell responses from COVID-19 were significantly higher in severe compared to mild COVID-19 cases, and this effect was most marked in response to spike, membrane, and ORF3a proteins…total and spike-specific T cell responses correlated with the anti-Spike, anti-Receptor Binding Domain (RBD) as well as anti-Nucleoprotein (NP) endpoint antibody titre…furthermore showed a higher ratio of SARS-CoV-2-specific $CD8^+$ to $CD4^+$ T cell responses…immunodominant epitope clusters and peptides containing T cell epitopes identified in this study will provide

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84) Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19, Sekine, 2020	"SARS-CoV-2-specific memory T cells will likely prove critical for long-term immune protection against COVID-19…mapped the functional and phenotypic landscape of SARS-CoV-2-specific T cell responses in unexposed individuals, exposed family members, and individuals with acute or convalescent COVID-19…collective dataset shows that SARS-CoV-2 elicits broadly directed and functionally replete memory T cell responses, suggesting that natural exposure or infection may prevent recurrent episodes of severe COVID-19."
85) Potent SARS-CoV-2-Specific T Cell Immunity and Low Anaphylatoxin Levels Correlate With Mild Disease Progression in COVID-19 Patients, Lafron, 2021	"Provide a full picture of cellular and humoral immune responses of COVID-19 patients and prove that robust polyfunctional $CD8^+$ T cell responses concomitant with low anaphylatoxin levels correlate with mild infections."
86) SARS-CoV-2 T-cell epitopes define heterologous and COVID-19 induced T-cell recognition, Nelde, 2020	"The first work identifying and characterizing SARS-CoV-2-specific and cross-reactive HLA class I and HLA-DR T-cell epitopes in SARS-CoV-2 convalescents (n = 180) as well as unexposed individuals (n = 185) and confirming their relevance for immunity and COVID-19 disease course… cross-reactive SARS-CoV-2 T-cell epitopes revealed pre-existing T-cell responses in 81% of unexposed individuals, and validation of similarity to common cold human coronaviruses provided a functional basis for postulated heterologous immunity in SARS-CoV-2 infection…intensity of T-cell responses and recognition rate of T-cell epitopes was significantly higher in the convalescent donors compared to unexposed individuals, suggesting that not only expansion, but also diversity spread of SARS-CoV-2 T-cell responses occur upon active infection."
87) Karl Friston: up to 80%	"Results have just been published of a study suggesting that 40%-60% of

Study / report title, author, and year published	Predominant finding on natural immunity
88) CD8⁺ T cells specific for an immunodominant SARS-CoV-2 nucleocapsid epitope cross-react with selective seasonal coronaviruses, Lineburg, 2021	"Screening of SARS-CoV-2 peptide pools revealed that the nucleocapsid (N) protein induced an immunodominant response in HLA-B7⁺ COVID-19-recovered individuals that was also detectable in unexposed donors… the basis of selective T cell cross-reactivity for an immunodominant SARS-CoV-2 epitope and its homologs from seasonal coronaviruses, suggesting long-lasting protective immunity."
89) SARS-CoV-2 genome-wide mapping of CD8 T cell recognition reveals strong immunodominance and substantial CD8 T cell activation in COVID-19 patients, Saini, 2020	"COVID-19 patients showed strong T cell responses, with up to 25% of all CD8⁺ lymphocytes specific to SARS-CoV-2-derived immunodominant epitopes, derived from ORF1 (open reading frame 1), ORF3, and Nucleocapsid (N) protein. A strong signature of T cell activation was observed in COVID-19 patients, while no T cell activation was seen in the 'non-exposed' and 'high exposure risk' healthy donors."
90) Equivalency of Protection from Natural Immunity in COVID-19 Recovered Versus Fully Vaccinated Persons: A Systematic Review and Pooled Analysis, Shenai, 2021	"Systematic review and pooled analysis of clinical studies to date, that (1) specifically compare the protection of natural immunity in the COVID-recovered versus the efficacy of full vaccination in the COVID-naive, and (2) the added benefit of vaccination in the COVID-recovered, for prevention of subsequent SARS-CoV-2 infection…review demonstrates that natural immunity in COVID-recovered individuals is, at least, equivalent to the protection afforded by full vaccination of COVID-naïve populations. There is a modest and incremental relative benefit to vaccination in COVID-recovered individuals; however, the net benefit is marginal on an absolute basis."
91) ChAdOx1nCoV-19 effectiveness during an unprecedented surge in SARS CoV-2 infections,	"The third key finding is that previous infections with SARS-CoV-2 were significantly protective against all studied outcomes, with an effectiveness of 93% (87 to 96%) seen against symptomatic infections, 89% (57 to 97%) against moderate to severe disease and 85% (-9 to 98%)

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92) SARS-CoV-2 specific T cells and antibodies in COVID-19 protection: a prospective study, Molodtsov, 2021	"Explore the impact of T cells and to quantify the protective levels of the immune responses…5,340 Moscow residents were evaluated for the antibody and cellular immune responses to SARS-CoV-2 and monitored for COVID-19 up to 300 days. The antibody and cellular responses were tightly interconnected, their magnitude inversely correlated with infection probability. Similar maximal level of protection was reached by individuals positive for both types of responses and by individuals with antibodies alone…T cells in the absence of antibodies provided an intermediate level of protection."
93) Negligible impact of SARS-CoV-2 variants on $CD4^+$ and $CD8^+$ T cell reactivity in COVID-19 exposed donors and vaccinees, Tarke, 2021	"Demonstrate that the sequences of the vast majority of SARS-CoV-2 T cell epitopes are not affected by the mutations found in the variants analyzed. Overall, the results demonstrate that CD4+ and CD8+ T cell responses in convalescent COVID-19 subjects or COVID-19 mRNA vaccinees are not substantially affected by mutations found in the SARS-CoV-2 variants."
94) Anti- SARS-CoV-2 Receptor Binding Domain Antibody Evolution after mRNA Vaccination, Cho, 2021	"SARS-CoV-2 infection produces B-cell responses that continue to evolve for at least one year. During that time, memory B cells express increasingly broad and potent antibodies that are resistant to mutations found in variants of concern."
95) Seven-month kinetics of SARS-CoV-2 antibodies and role of pre-existing antibodies to human coronaviruses, Ortega, 2021	"Impact of pre-existing antibodies to human coronaviruses causing common cold (HCoVs), is essential to understand protective immunity to COVID-19 and devise effective surveillance strategies…after the peak response, anti-spike antibody levels increase from ~150 days post-symptom onset in all individuals (73% for IgG), in the absence of any

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96) Immunodominant T-cell epitopes from the SARS-CoV-2 spike antigen reveal robust pre-existing T-cell immunity in unexposed individuals, Mahajan, 2021	"Findings suggest that SARS-CoV-2 reactive T-cells are likely to be present in many individuals because of prior exposure to flu and CMV viruses."
97) Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals, Ni, 2020	"Collected blood from COVID-19 patients who have recently become virus-free, and therefore were discharged, and detected SARS-CoV-2-specific humoral and cellular immunity in eight newly discharged patients… In all 14 patients tested, 13 displayed serum-neutralizing activities in a pseudotype entry assay. Notably, there was a strong correlation between neutralization antibody titers and the numbers of virus-specific T cells."
98) Neutralizing Antibody Responses to Severe Acute Respiratory Syndrome Coronavirus 2 in Coronavirus Disease 2019 Inpatients and Convalescent Patients, Wang, 2020	"117 blood samples were collected from 70 COVID-19 inpatients and convalescent patients…the neutralizing antibodies were detected even at the early stage of disease, and a significant response was shown in convalescent patients."
99) Not just antibodies: B cells and T cells mediate immunity to COVID-19, Cox, 2020	"Reports that antibodies to SARS-CoV-2 are not maintained in the serum following recovery from the virus have caused alarm…the absence of specific antibodies in the serum does not necessarily mean an absence of immune memory."
100) T-cell immunity to	"Although T-cell responses to SARS-CoV-2

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101) Durable SARS-CoV-2 B cell immunity after mild or severe disease, Ogega, 2021	"Multiple studies have shown loss of severe acute respiratory syndrome coronavirus 2-specific (SARS-CoV-2-specific) antibodies over time after infection, raising concern that humoral immunity against the virus is not durable. If immunity wanes quickly, millions of people may be at risk for reinfection after recovery from coronavirus disease 2019 (COVID-19). However, memory B cells (MBCs) could provide durable humoral immunity even if serum neutralizing antibody titers decline… data indicate that most SARS-CoV-2-infected individuals develop S-RBD-specific, class-switched rMBCs that resemble germinal center-derived B cells induced by effective vaccination against other pathogens, providing evidence for durable B cell-mediated immunity against SARS-CoV-2 after mild or severe disease."
102) Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection., Ng, 2016	"All memory T cell responses detected target the SARS-Co-V structural proteins… these responses were found to persist up to 11 years post-infection… knowledge of the persistence of SARS-specific celullar immunity targeting the viral structural proteins in SARS-recovered individuals is important."
103) Adaptive immunity to SARS-CoV-2 and COVID-19, Sette, 2021	"The adaptive immune system is important for control of most viral infections. The three fundamental components of the adaptive immune system are B cells (the source of antibodies), CD4+ T cells, and CD8+ T cells…a picture has begun to emerge that reveals that CD4+ T cells, CD8+ T cells, and neutralizing antibodies all contribute to control of SARS-CoV-2 in both non-hospitalized and hospitalized cases of COVID-19."
104) Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance	"These findings provide support for the prognostic value of early functional SARS-CoV-2-specific T cells with important implications in vaccine design and immune monitoring."

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105) SARS-CoV-2–specific $CD8^+$ T cell responses in convalescent COVID-19 individuals, Kared, 2021	"A multiplexed peptide-MHC tetramer approach was used to screen 408 SARS-CoV-2 candidate epitopes for $CD8^+$ T cell recognition in a cross-sectional sample of 30 coronavirus disease 2019 convalescent individuals…Modelling demonstrated a coordinated and dynamic immune response characterized by a decrease in inflammation, increase in neutralizing antibody titer, and differentiation of a specific $CD8^+$ T cell response. Overall, T cells exhibited distinct differentiation into stem cell and transitional memory states (subsets), which may be key to developing durable protection."
106) S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit, Nguyen-Contant, 2021	"Most importantly, we demonstrate that infection generates both IgG and IgG MBCs against the novel receptor binding domain and the conserved S2 subunit of the SARS-CoV-2 spike protein. Thus, even if antibody levels wane, long-lived MBCs remain to mediate rapid antibody production. Our study results also suggest that SARS-CoV-2 infection strengthens pre-existing broad coronavirus protection through S2-reactive antibody and MBC formation."
107) Persistence of Antibody and Cellular Immune Responses in Coronavirus Disease 2019 Patients Over Nine Months After Infection, Yao, 2021	A cross-sectional study to assess the virus-specific antibody and memory T and B cell responses in coronavirus disease 2019 (COVID-19) patients up to 343 days after infection…found that approximately 90% of patients still have detectable immunoglobulin (Ig)G antibodies against spike and nucleocapsid proteins and neutralizing antibodies against pseudovirus, whereas ~60% of patients had detectable IgG antibodies against receptor-binding domain and surrogate virus-neutralizing antibodies… SARS-CoV-2-specific IgG+ memory B cell and interferon-γ-secreting T cell responses were detectable in more than 70% of patients…coronavirus 2-specific immune memory response persists in most patients approximately 1 year after infection, which provides a promising sign for prevention from reinfection and vaccination strategy."

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109) Decreasing Seroprevalence of Measles Antibodies after Vaccination – Possible Gap in Measles Protection in Adults in the Czech Republic, Smetana, 2017	"A long-term high rate of seropositivity persists after natural measles infection. By contrast, it decreases over time after vaccination. Similarly, the concentrations of antibodies in persons with measles history persist for a longer time at a higher level than in vaccinated persons."
110) Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection, Wrammert, 2011	"The expansion of these rare types of memory B cells may explain why most people did not become severely ill, even in the absence of pre-existing protective antibody titers"…found "extraordinarily" powerful antibodies in the blood of nine people who caught the swine flu naturally and recovered from it."…unlike antibodies elicited by annual influenza vaccinations, most neutralizing antibodies induced by pandemic H1N1 infection were broadly cross-reactive against epitopes in the hemagglutinin (HA) stalk and head domain of multiple influenza strains. The antibodies were from cells that had undergone extensive affinity maturation."
111) Reinfection With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Patients Undergoing Serial Laboratory Testing, Qureshi, 2021	"Reinfection was identified in 0.7% (n = 63, 95% confidence interval [CI]: .5%–.9%) during follow-up of 9119 patients with SARS-CoV-2 infection."
112) Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals	"Interrogated antibody and antigen-specific memory B cells over time in 33 SARS-CoV-2 naïve and 11 SARS-CoV-2 recovered subjects… In SARS-CoV-2 recovered individuals, antibody and memory B cell responses were significantly boosted after the first vaccine dose; however, there was no

Study / report title, author, and year published	Predominant finding on natural immunity
113) Covid-19: Do many people have pre-existing immunity?Doshi, 2021	"Six studies have reported T cell reactivity against SARS-CoV-2 in 20% to 50% of people with no known exposure to the virus… in a study of donor blood specimens obtained in the US between 2015 and 2018, 50% displayed various forms of T cell reactivity to SARS-CoV-2… Researchers are also confident that they have made solid inroads into ascertaining the origins of the immune responses. "Our hypothesis, of course, was that it's so called 'common cold' coronaviruses, because they're closely related…we have really shown that this is a true immune memory and it is derived in part from common cold viruses."
114) Pre-existing and *de novo* humoral immunity to SARS-CoV-2 in humans, Ng, 2020	"We demonstrate the presence of pre-existing humoral immunity in uninfected and unexposed humans to the new coronavirus. SARS-CoV-2 S-reactive antibodies were readily detectable by a sensitive flow cytometry-based method in SARS-CoV-2-uninfected individuals and were particularly prevalent in children and adolescents."
115) Phenotype of SARS-CoV-2-specific T-cells in COVID-19 patients with acute respiratory distress syndrome, Weiskopf, 2020	"We detected SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in 100% and 80% of COVID-19 patients, respectively. We also detected low levels of SARS-CoV-2-reactive T-cells in 20% of the healthy controls, not previously exposed to SARS-CoV-2 and indicative of cross-reactivity due to infection with 'common cold' coronaviruses."
116) Pre-existing immunity to SARS-CoV-2: the knowns and unknowns, Sette, 2020	"T cell reactivity against SARS-CoV-2 was observed in unexposed people…it is speculated that this reflects T cell memory to circulating 'common cold' coronaviruses."
117) Pre-existing immunity against swine-origin H1N1 influenza viruses in the general	"Memory T-cell immunity against S-OIV is present in the adult population and that such memory is of similar magnitude as the pre-existing memory against seasonal H1N1 influenza…the conservation of a large fraction of T-cell epitopes suggests that the severity of an S-OIV infection,

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118) Cellular immune correlates of protection against symptomatic pandemic influenza, Sridhar, 2013	"The 2009 H1N1 pandemic (pH1N1) provided a unique natural experiment to determine whether cross-reactive cellular immunity limits symptomatic illness in antibody-naive individuals… Higher frequencies of pre-existing T cells to conserved CD8 epitopes were found in individuals who developed less severe illness, with total symptom score having the strongest inverse correlation with the frequency of interferon-γ (IFN-γ)(+) interleukin-2 (IL-2)(-) CD8(+) T cells ($r = -0.6$, $P = 0.004$)… CD8(+) T cells specific to conserved viral epitopes correlated with cross-protection against symptomatic influenza."
119) Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans, Wilkinson, 2012	"Precise role of T cells in human influenza immunity is uncertain. We conducted influenza infection studies in healthy volunteers with no detectable antibodies to the challenge viruses H3N2 or H1N1…mapped T cell responses to influenza before and during infection…found a large increase in influenza-specific T cell responses by day 7, when virus was completely cleared from nasal samples and serum antibodies were still undetectable. Pre-existing CD4+, but not CD8+, T cells responding to influenza internal proteins were associated with lower virus shedding and less severe illness. These CD4+ cells also responded to pandemic H1N1 (A/CA/07/2009) peptides and showed evidence of cytotoxic activity."
120) Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine, CDC, MMWR, 2009	"No increase in cross-reactive antibody response to the novel influenza A (H1N1) virus was observed among adults aged >60 years. These data suggest that receipt of recent (2005–2009) seasonal influenza vaccines is unlikely to elicit a protective antibody response to the novel influenza A (H1N1) virus."
121) No one is naive: the significance of	"Memory T cells that are specific for one virus can become activated during infection with an unrelated heterologous virus, and might have

Study / report title, author, and year published	Predominant finding on natural immunity
122) Intrafamilial Exposure to SARS-CoV-2 Induces Cellular Immune Response without Seroconversion, Gallais, 2020	"Individuals belonging to households with an index COVID-19 patient, reported symptoms of COVID-19 but discrepant serology results… All index patients recovered from a mild COVID-19. They all developed anti-SARS-CoV-2 antibodies and a significant T cell response detectable up to 69 days after symptom onset. Six of the eight contacts reported COVID-19 symptoms within 1 to 7 days after the index patients but all were SARS-CoV-2 seronegative… exposure to SARS-CoV-2 can induce virus-specific T cell responses without seroconversion. T cell responses may be more sensitive indicators of SARS-Co-V-2 exposure than antibodies…results indicate that epidemiological data relying only on the detection of SARS-CoV-2 antibodies may lead to a substantial underestimation of prior exposure to the virus."
123) Protective immunity after recovery from SARS-CoV-2 infection, Kojima, 2021	"It important to note that antibodies are incomplete predictors of protection. After vaccination or infection, many mechanisms of immunity exist within an individual not only at the antibody level, but also at the level of cellular immunity. It is known that SARS-CoV-2 infection induces specific and durable T-cell immunity, which has multiple SARS-CoV-2 spike protein targets (or epitopes) as well as other SARS-CoV-2 protein targets. The broad diversity of T-cell viral recognition serves to enhance protection to SARS-CoV-2 variants, with recognition of at least the alpha (B.1.1.7), beta (B.1.351), and gamma (P.1) variants of SARS-CoV-2. Researchers have also found that people who recovered from SARS-CoV infection in 2002–03 continue to have memory T cells that are reactive to SARS-CoV proteins 17 years after that outbreak. Additionally, a memory B-cell response to SARS-CoV-2 evolves between 1·3 and 6·2 months after infection, which is consistent with longer-term protection."
124) This 'super antibody' for COVID fights off multiple coronaviruses	"This 'super antibody' for COVID fights off multiple coronaviruses…12 antibodies…that was involved in the study, isolated from people who had been infected with either SARS-CoV-2 or its close relative SARS-CoV"

Study / report title, author, and year published	Predominant finding on natural immunity
125) SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19, Wu, 2020	"Taken together, our data indicate sustained humoral immunity in recovered patients who suffer from symptomatic COVID-19, suggesting prolonged immunity."
126) Evidence for sustained mucosal and systemic antibody responses to SARS-CoV-2 antigens in COVID-19 patients, Isho, 2020	"Whereas anti-CoV-2 IgA antibodies rapidly decayed, IgG antibodies remained relatively stable up to 115 days PSO in both biofluids. Importantly, IgG responses in saliva and serum were correlated, suggesting that antibodies in the saliva may serve as a surrogate measure of systemic immunity."
127) The T-cell response to SARS-CoV-2: kinetic and quantitative aspects and the case for their protective role, Bertoletti, 2021	"Early appearance, multi-specificity and functionality of SARS-CoV-2-specific T cells are associated with accelerated viral clearance and with protection from severe COVID-19."
128) The longitudinal kinetics of antibodies in COVID-19 recovered patients over 14 months, Eyran, 2020	"Found a significantly faster decay in naïve vaccinees compared to recovered patients suggesting that the serological memory following natural infection is more robust compared to vaccination. Our data highlights the differences between serological memory induced by natural infection vs. vaccination."
129) Continued Effectiveness of COVID-19	"Followed a population of urban Massachusetts HCWs…we found no re-infection among those with prior COVID-19, contributing to 74,557 re-

Study / report title, author, and year published	Predominant finding on natural immunity
130) Immunity to COVID-19 in India through vaccination and natural infection, Sarraf, 2021	"Compared the vaccination induced immune response profile with that of natural infection, evaluating thereby if individuals infected during the first wave retained virus specific immunity…the overall immune response resulting from natural infection in and around Kolkata is not only to a certain degree better than that generated by vaccination, especially in the case of the Delta variant, but cell mediated immunity to SARS-CoV-2 also lasts for at least ten months after the viral infection."
131) Asymptomatic or mild symptomatic SARS-CoV-2 infection elicits durable neutralizing antibody responses in children and adolescents, Garrido, 2021	"Evaluated humoral immune responses in 69 children and adolescents with asymptomatic or mild symptomatic SARS-CoV-2 infection. We detected robust IgM, IgG, and IgA antibody responses to a broad array of SARS-CoV-2 antigens at the time of acute infection and 2 and 4 months after acute infection in all participants. Notably, these antibody responses were associated with virus-neutralizing activity that was still detectable 4 months after acute infection in 94% of children. Moreover, antibody responses and neutralizing activity in sera from children and adolescents were comparable or superior to those observed in sera from 24 adults with mild symptomatic infection. Taken together, these findings indicate that children and adolescents with mild or asymptomatic SARS-CoV-2 infection generate robust and durable humoral immune responses that can likely contribute to protection from reinfection."
132) T cell response to SARS-CoV-2 infection in humans: A systematic review, Shrotri, 2021	"Symptomatic adult COVID-19 cases consistently show peripheral T cell lymphopenia, which positively correlates with increased disease severity, duration of RNA positivity, and non-survival; while asymptomatic and paediatric cases display preserved counts. People with severe or critical disease generally develop more robust, virus-specific T cell responses. T cell memory and effector function has been demonstrated against multiple viral epitopes, and, cross-reactive T cell responses have been demonstrated in unexposed and uninfected adults, but the significance

Study / report title, author, and year published	Predominant finding on natural immunity
133) Severity of SARS-CoV-2 Reinfections as Compared with Primary Infections, Abu-Raddad, 2021	"Reinfections had 90% lower odds of resulting in hospitalization or death than primary infections. Four reinfections were severe enough to lead to acute care hospitalization. None led to hospitalization in an ICU, and none ended in death. Reinfections were rare and were generally mild, perhaps because of the primed immune system after primary infection."
134) Assessment of the Risk of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reinfection in an Intense Re-exposure Setting, Abu-Raddad, 2021	"SARS-CoV-2 reinfection can occur but is a rare phenomenon suggestive of protective immunity against reinfection that lasts for at least a few months post primary infection."
135) Increased risk of infection with SARS-CoV-2 Beta, Gamma, and Delta variant compared to Alpha variant in vaccinated individuals, Andeweg, 2021	"Analyzed 28,578 sequenced SARS-CoV-2 samples from individuals with known immune status obtained through national community testing in the Netherlands from March to August 2021. They found evidence for an "increased risk of infection by the Beta (B.1.351), Gamma (P.1), or Delta (B.1.617.2) variants compared to the Alpha (B.1.1.7) variant after vaccination. No clear differences were found between vaccines. However, the effect was larger in the first 14-59 days after complete vaccination compared to 60 days and longer. In contrast to vaccine-induced immunity, no increased risk for reinfection with Beta, Gamma or Delta variants relative to Alpha variant was found in individuals with infection-induced immunity."

Author

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Research paper

An observational study of breakthrough SARS-CoV-2 Delta variant infections among vaccinated healthcare workers in Vietnam

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ABSTRACT

Background: Data on breakthrough SARS-CoV-2 Delta variant infections in vaccinated individuals are limited.
Methods: We studied breakthrough infections among Oxford-AstraZeneca vaccinated healthcare workers in an infectious diseases hospital in Vietnam. We collected demographic and clinical data alongside serial PCR testing, measurement of SARS-CoV-2 antibodies, and viral whole-genome sequencing.
Findings: Between 11th–25th June 2021 (7-8 weeks after the second dose), 69 staff tested positive for SARS-CoV-2. 62 participated in the study. Most were asymptomatic or mildly symptomatic and all recovered. Twenty-two complete-genome sequences were obtained; all were Delta variant and were phylogenetically distinct from contemporary viruses obtained from the community or from hospital patients admitted prior to the outbreak. Viral loads inferred from Ct values were 251 times higher than in cases infected with the original strain in March/April 2020. Median time from diagnosis to negative PCR was 21 days (range 8–33). Neutralizing antibodies (expressed as percentage of inhibition) measured after the second vaccine dose, or at diagnosis, were lower in cases than in uninfected, fully vaccinated controls (median (IQR): 69.4 (50.7-89.1) vs. 91.3 (79.6-94.9), p=0.005 and 59.4 (32.5-73.1) vs. 91.1 (77.3-94.2), p=0.043). There was no correlation between vaccine-induced neutralizing antibody levels and peak viral loads or the development of symptoms.
Interpretation: Breakthrough Delta variant infections following Oxford-AstraZeneca vaccination may cause asymptomatic or mild disease, but are associated with high viral loads, prolonged PCR positivity and low levels of vaccine-induced neutralizing antibodies. Epidemiological and sequence data suggested ongoing transmission had occurred between fully vaccinated individuals.
Funding: Wellcome and NIH/NIAID

This is **Exhibit "N"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at

[redacted]

1. Introduction

SARS-CoV-2 Delta variant is approximately 60% more transmissible than the Alpha (B.1.1.7) variant, and has rapidly spread worldwide [1], posing a significant threat to global COVID-19 control. The Delta variant possesses mutations in the spike protein (including

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Research in context

Evidence before this study

To date, existing data showed that breakthrough Delta variant infections among vaccinated people had comparable viral loads with those in unvaccinated individuals infected with the Delta variants, but there has been no study comparing viral loads of breakthrough infections with those in cases infected with the original SARS-CoV-2 strains detected in early 2020. Using PCR or viral culture, previous reports also showed that cases of breakthrough infections had a short duration of viral shedding of 7 days or less, but none has reported robust evidence demonstrating the transmission of SARS-CoV-2 between fully vaccinated people. Most recently, a study in Israel identified a correlation between neutralizing antibody titers after the second dose and at diagnosis and breakthrough infection with the Alpha variant.

Added value of this study

This study examined 62 (asymptomatic (n=13) and mildly symptomatic (n=49)) cases of breakthrough Delta variant infections among Oxford-AstraZeneca vaccinated healthcare workers in an infectious diseases hospital in Ho Chi Minh City, Vietnam, and demonstrated evidence of secondary transmission between vaccinated individuals through the analysis of epidemiological and viral whole-genome sequence data. Peak viral loads assessed by Ct value were 251 times higher than those in cases infected with the original SARS-CoV-2 strain detected in Vietnam between March and April 2020. Vaccine-induced neutralizing antibodies after the second dose and at diagnosis were lower than those in the matched uninfected, fully vaccinated controls, but they were not associated with peak viral loads (i.e. infectivity) or the development of symptoms during the course of infection.

Implications of all the available evidence

Breakthrough Delta variant infections are associated with asymptomatic or mild disease, but resulted in high viral loads and prolonged PCR positivity. In this study, high viral loads coupled with a poorly ventilated indoor setting without in-office mask wearing might have facilitated the transmission of the Delta variant between vaccinated individuals, emphasizing that social distancing measures remain critical to reduce the transmission of SARS-CoV-2 Delta variant, event in countries where vaccination coverage is high. The absence of correlation between neutralizing antibody levels and peak viral loads suggested that vaccine might not lower the transmission potential of breakthrough infection cases.

L452R and T478K) that makes the virus less susceptible to neutralizing antibodies generated by current vaccines or natural infection [2,3]. These features have raised concern about Delta variant vaccine escape potential and breakthrough infections.

Data on vaccine breakthrough infections, especially those caused by the Delta variant, are limited [4,5]. Likewise, little is known about the associated serological markers (i.e. vaccine induced neutralizing antibody levels) of breakthrough infections, especially those infected with the Delta variant [6]. And scarce epidemiological and molecular data exist regarding to the transmission of SARS-CoV-2 delta variant between fully vaccinated individuals. Such knowledge is critical to informing the development and deployment of COVID-19 vaccine, and the implementation of infection control measures.

Between 11th and 25th June 2021, an outbreak of breakthrough infections occurred among staff members of an infectious diseases hospital, the Hospital for Tropical Diseases (HTD), in Ho Chi Minh City, Vietnam. Nearly all of the staff had received a second dose of Oxford-AstraZeneca vaccine 7 weeks previously. The first case (patient 1), a 41-year old man, was identified by PCR on 11th June 2021 having experienced body pain and tiredness. Following the diagnosis of patient 1, HTD expanded the PCR screening for SARS-CoV-2 to all staff members. By the end of 12th June 2021, 52 additional members were found to be infected, including all 6 members who shared an office with patient 1.

Following Vietnamese Government regulations, HTD was locked down for two weeks (12th-26th June 2021), with no one allowed to enter or leave the hospital. Further PCR testing of all staff during this period identified 16 additional positive cases, totaling 69 infected members from 20/34 departments, corresponding to an overall PCR positive rate of 8% (69/866) at the hospital-wide level. Serological testing for SARS-CoV-2 N protein antibodies (indicating naturally acquired infection) was carried out on 683 staff (including those who stayed in HTD during the lockdown and the infected cases) between 13th and 16th June 2021, but none was positive.

Here, we report the results of in-depth investigations of the outbreak. We focused our analysis on the clinical features, viral evolution and dynamics of viral loads and antibody responses during the course of breakthrough infections.

2. Materials and methods

2.1. Setting

HTD is a 550-bed tertiary referral hospital for patients with infectious diseases in southern Vietnam [7]. HTD has around 900 members of staff and 34 departments. All offices except one are equipped with air conditioners that recirculate the air without mechanical ventilation (Supplementary Figure 1).

As an infectious hospital, HTD is responsible for COVID-19 patient management. At the time of the outbreak, three departments were dedicated for COVID-19 patients, and around 70 patients were being treated at HTD. Access to COVID-19 patient departments was restricted to medical staff providing COVID-19 patient care. During the lockdown, HTD temporarily stopped receiving new patients, including those with COVID-19.

2.2. The vaccine evaluation study: a brief description

HTD staff members were amongst the first people in Vietnam to be offered the Oxford-AstraZeneca COVID-19 vaccine, and 554 vaccinated staff have been participating in an ongoing vaccine evaluation study since March 2021. The detailed descriptions about the study was previously reported [8]. In brief, the first vaccine doses were given on 8th March 2021; the second doses were given in the last two weeks of April 2021. The window time between the two doses was 6 weeks (Figure 1). Blood sampling was scheduled for the time points before and approximately at 14 days after each dose, and at week 4, and month 3, 6, and 12 after the first dose. The outbreak coincided with the blood-sampling schedule at month 3 after dose 1 (i.e. between 8th–15th June 2021, Figure 1).

2.3. The breakthrough infection study

2.3.1. Data and sample collection

We collected demographic, vaccination history, and clinical data alongside the results of routine SARS-CoV-2 PCR testing from the study participants, conducted at least once every three days during hospitalization.

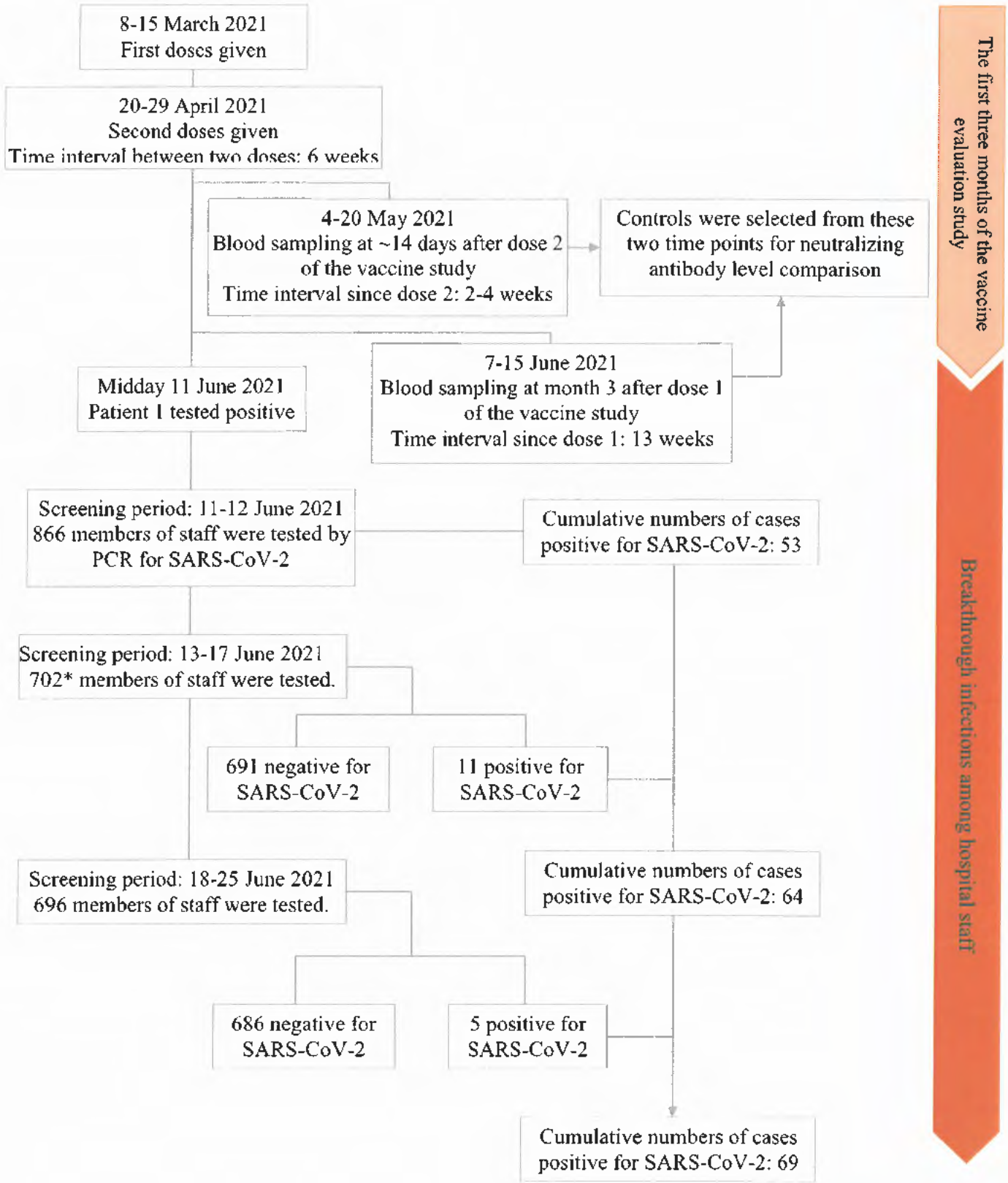

Figure 1. Flowchart showing timelines and results of SARS-CoV-2 RT-PCR screening before and during the lockdown (11-25 June 2021). **Notes to** Figure 1. *The remaining members of staff were working from home.

2.3.2. Nasopharyngeal-throat swab collection, PCR testing and viral load conversion

Nasopharyngeal swabs were collected and placed in 1mL of viral transport medium, and 200uL was used for viral RNA extraction using the MagNApure 96 platform (Roche Diagnostics, Germany), according to the manufacturer's instructions. For SARS-CoV-2 RNA detection, we used real-time RT-PCR assay with primers and probe targeted at the envelope protein-coding gene (TIB MOLBIOL) [9] with a cutoff of

40 cycles. PCR Ct values were converted to RNA loads using an in-house established formula ($y = -0.3092x + 12.553$, $R^2 = 0.9963$, where y is viral load and x is Ct value) based on 10-fold dilution series of in-vitro transcribed RNA [9,10].

2.3.3. Whole genome sequencing and sequence analysis

Whole-genome sequences of SARS-CoV-2 were directly obtained from leftover RNA after PCR testing using ARTIC protocol [11] and Illumina reagents on a MiSeq platform with the inclusion of a negative control in every sequencing run. The obtained reads from individual samples were mapped to a SARS-CoV-2 reference genome (GISIAD sequence ID: EPI_ISL_1942165) to generate the consensuses using Geneious software (Biomatter, New Zealand). SARS-CoV-2 variant assignment was carried out using Pangolin [12]. Detection of amino acid changes as compared to the original Wuhan strain was done using COV-GLUE [13]. Maximum likelihood phylogenetic tree was reconstructed using IQ-TREE [14].

2.3.4. Blood collection and SARS-CoV-2 antibody measurement

For SARS-CoV-2 antibody measurement, we obtained 2ml of EDTA plasma from each study participant at diagnosis and every week after diagnosis for a duration of up to three weeks. Any study participants, who were discharged before the time point scheduled for weekly sampling, were not subjected to additional blood collection. When convenient, we used leftover blood samples collected as part of the initial investigation of the outbreak.

We measured antibodies (IgM and IgG) against SARS-CoV-2 nucleocapsid (N) protein using Elecsys Anti-SARS-CoV-2 assay (Diagnostics, Germany) with a cutoff index of 1.0, and SARS-CoV-2 neutralizing antibodies using SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) (GenScript, USA) with a cutoff of 30% [15]. The experiments were carried according to the manufacturers' instructions.

2.3.5. Additional data for case-control analyses

To assess the potential association between vaccine breakthrough infections and the levels of vaccine-induced neutralizing antibodies, we compared available antibody levels measured after the second dose (approximately 14 days) and at month 3 after the first vaccine dose in fully vaccinated, uninfected staff (controls) with the cases [8]. To ensure the statistical power of the study, we applied a matching ratio of 1:3 (1 case and 3 controls). We matched cases with the uninfected, fully vaccinated controls for age and gender (Supplementary Tables 1).

For viral load comparison, we used previously reported data from SARS-CoV-2 infected cases from the early phase of the pandemic in Vietnam between March and April 2020 (Supplementary Table 2) [7]. Herein, we used the term "original strain" to refer to the SARS-CoV-2 strain detected in those earlier patients.

2.4. Data analysis

Data analysis was carried out using SPSS v27 (SPSS Inc, Chicago, US) or Graphpad Prism v9.0.2 (Graphpad Software, California, US). For comparison between groups of categorical data, we used the Fisher exact test for expected frequencies of <5, otherwise, we used the Chi-squared test. The Mann-Whitney U test was used for nonparametric comparisons of data. The repeated mesurement of viral loads and antibodies over the time were tested using ANOVA and the non-parametric Friedman test, respectively. For comparison between the viral loads of the controls and the case patients we used multivariate regression with type-II sum of square and age, gender, symptomatic status and comorbidity as variables in order to control for potential associated confounding effects. For comparison between neutralizing antibodies after vaccination and at diagnosis between cases and control, we applied the same statistical approach with age, gender and the number days since dose two as variables. We also applied the same multivariate regression analysis approach to assess the association between peak viral loads during the course of breakthrough infections and neutralizing antibodies measured at diagnosis.

2.5. Ethics

The study was approved by the Institutional Review Board of HTD and the Oxford Tropical Research Ethics Committee, University of Oxford, UK. Written informed consent was obtained from all the participants. Whole-genome sequencing of SARS-CoV-2 formed part of the national response to the COVID-19 outbreak in Vietnam. Accordingly, obtaining informed consent was deemed unnecessary.

2.6. Role of the funders

The funders of the study had no role in data collection, analysis, interpretation, writing of the manuscript and the decision to submit the manuscript.

3. Results

3.1. Demographics and clinical features

In total 69/866 (8%) staff members were found positive for SARS-CoV-2 by PCR testing during the lockdown period. As per the national COVID-19 management policy in Vietnam, all the 69 infected members of HTD staff were admitted to HTD for clinical follow-up. Their associated departments are listed in Supplementary Table 4. They all were invited to participate in an ongoing observational study [7]. And 62 (including 53/53 tested positive on 11th-12th June and 9/16 tested positive between 13th-25th June) consented to have their clinical features reported. The 7 staff members who did not enter the study were excluded by chance. Comparison between them and those included did not suggest bias was introduced, male/female ratio: 3/4 vs. 33/29, p=0.702 and median age in years (IQR): 36 (27-53) vs. 42 (32-50), p=0.582, respectively.

Of the 62 study participants, two had received one dose of Oxford-AstraZeneca vaccine, and 60 (including patient 1) had received two doses. Two presented with symptoms at diagnosis (11th and 15th June), and 47 developed symptoms between 1-15 days after diagnosis (Supplementary Figure 2). Chest x-ray examination was performed on 34 cases. Of these, three had evidence of pneumonia, including one requiring oxygen supplementation for three days because of shortness of breath. This staff member was fully vaccinated. Otherwise, they all were either mildly symptomatic or asymptomatic (i.e. no observed symptoms during the course of infection) (Table 1). All recovered uneventfully.

3.2. Viral loads

At diagnosis, median PCR Ct value of the 62 study participants was 31.9 (IQR: 23.3 - 34.9), equivalent to $\log_{10}$ copies per mL of 4.4 (IQR: 3.5–7.1); eleven (21%) of the first 53 cases from 5 different departments had high viral loads (i.e. a Ct value of between 14.0 and 22.6), equivalent to $\log_{10}$ copies per mL of 7.3–9.9, including patient 1 and 4/6 members sharing the office with him.

Of the 49 symptomatic cases, high viral loads were observed around 2-3 days before and after symptom onset (Figure 2A). Their peak viral loads measured at any time point during the course of infection were higher than that of asymptomatic cases (median (IQR): 16.5 (15.6–17.9) vs. 30.8 (16.4–33.9), equivalent to median $\log_{10}$ viral load of 9.2 copies per mL (IQR: 8.7–9.4) vs. 4.7 copies per mL (IQR: 3.8–9.2), p=0.004, respectively) (Figure 2B and Supplementary Figure 3). The median time from diagnosis to PCR negative was 21 days (range 8–33).

Table 1
Demographics and clinical characteristics of the study participants

Signs/Symptoms	All cases (n=62)	Male (n=33)	Female (n=29)
Age, y, median (IQR)	41.5 (32-50)	41 (34-50)	43 (29-50)
Occupation, n (%)			
Nurse	13 (21)	5 (15)	8 (28)
Pharmacist	10 (16)	3 (9)	7 (24)
IT	7 (11)	7 (21)	0
Clinician	7 (11)	5 (15)	2 (7)
Accountant	4 (6)	0	4 (14)
Technical staff	3 (5)	3 (9)	0
Cleaner	2 (3)	2 (6)	0
Others^	16 (26)	8 (24)	8 (28)
Symptomatic, n (%)	49 (79)	24 (73)	25 (86)
Acute respiratory infection^^	43 (69)	23 (70)	20 (69)
PCR diagnosis to illness onset, d, (median; IQR)*	4 (2-7)	3 (2-6)	5 (2-8)
Comorbidity[#], n (%)	17 (27)	9 (27)	8 (28)
COVID-19 vaccination[¥], n (%)	62 (100)	33 (100)	29 (100)
Two doses	60 (97)	33 (100)	27 (93)
One dose	2 (3)	0	2 (7)
Fever, n (%)	17 (27)	9 (27)	8 (27)
Cough, n (%)	23 (37)	19 (58)	14 (48)
Sore throat, n (%)	21 (34)	9 (27)	12 (41)
Runny nose, n (%)	22 (36)	9 (27)	13 (45)
Loss of smell, n %)	24 (39)	14 (42)	10 (35)
Loss of taste, n (%)	5 (8)	3 (9)	2 (7)
Muscle pain, n (%)	17 (27)	13 (39)	4 (14)
Headache, n (%)	12 (19)	6 (18)	6 (21)
Chest pain, n (%)	2 (3)	0	2 (7)
Nausea, n (%)	5 (8)	3 (9)	2 (7
Others, n (%)[$]	5 (8)	1 (3)	4 (14)
Shortness of breath	2 (4)	0	2 (6)
Pneumonia, n (%)**	3 (5)	0	3 (10)

Notes to Table 1
IQR: interquartile range
^including data entry (n=1), driver (n=4) and specialists with additional details not available (n=11)
^^Runny nose, cough, sore throat, muscle pain, chest pain, chills, or shortness of breath
*Symptomatic cases only
**One requiring oxygen supplementation via nasal cannula for 3 days.
[¥] All receiving Oxford-AstraZeneca vaccine; the second doses were given in last 2 weeks of April 2021.
[#] Overweight (n=6), obese (n=3), hypertension (n=3), hepatitis B (n=2), diabetes (n=1), pregnancy (n=1), diabetes and hepatitis B (n=1).
[$] Chills (n=2), sweating (n=1), giddiness (n=1), red eyes (n=1), and diarrhea (n=1)

Compared with peak viral loads in SARS-CoV-2 infected cases detected in Vietnam between March and April 2020, median peak viral loads of the breakthrough cases were equivalent to 251 times higher (median $\log_{10}$ viral load in copies per mL (IQR): 9.1 (8.7–9.4) vs. 6.7 (4.7–7.4), p=0.001). The differences between the groups were even greater among symptomatic cases (median $\log_{10}$ viral load in copies per mL (IQR): 9.2 (8.7–9.4) vs. 7.0 (6.3–7.6), p<0.001). In those without symptoms, however, viral loads were similar (median $\log_{10}$ viral load in copies per mL (IQR): 4.7 (3.8–9.2) vs. 4.9 (3.2-8.6) p=0.540) (Figure 2B).

3.3. Whole genome sequencing

A total of 22 whole genome sequences of SARS-CoV-2 were obtained from 22 fully vaccinated staff members (including patient 1 and 1/7 staff member not participating in the present study) from 9 different HTD departments (Supplementary Table 3). All were assigned to the SARS-CoV-2 Delta variant. They were either identical or different from each other by only 1 to 7 nucleotides and no novel amino acid changes were identified among them. Phylogenetically, the 22 sequences clustered tightly together and were separated from the contemporary Delta variant sequences obtained from cases of community transmission in HCMC and from the COVID-19 patients admitted HTD prior to the outbreak (Figure 3).

3.4. Antibody development

A total of 210 plasma samples were collected from the 62 study participants, including 61 at diagnosis, and 61 at week 1, 57 at week 2 and 31 at week 3 after diagnosis. The missing data was either attributed to lost to follow up (n=1 each at diagnosis and at week 1 after diagnosis, and n=3 at week 2) or early discharge (n=1 and n=31 at week 2 and 3, respectively).

The 61 at-diagnosis plasma samples were collected 1-3 days before PCR diagnosis (n=7 as part of the initial outbreak investigation between 13th and 16th June 2021), on the day of diagnosis (n=2), and 1 day (n=43) and 2 days (n=9) after diagnosis. Of these, all but three had detectable neutralizing antibodies, with comparable levels between symptomatic and asymptomatic cases (median % of inhibition (IQR): 69.0 (46.5-86.7) vs. 69.7 (61.1-81.8), p=0.918) (Supplementary Figure 4). There was no correlation between neutralizing antibodies at diagnosis and peak viral loads during the course of infection (R^2<0.001 and p=0.835) (Figure 4). At week 2 and 3 after diagnosis, neutralizing antibody levels of the cases significantly increased (p<0.001, Supplementary Figure 4).

The seroconversion rates for antibodies against N protein steadily increased from 0% at baseline to 65% (20/31) at week 3 after diagnosis. Asymptomatic patients had slightly lower seroconversion rates than symptomatic patients (week 1: 0% (0/13) vs. 4% (2/48), p=1.0, week 2: 0% (0/13) vs. 36% (16/44), p=0.012 and week 3: 40% (2/5) vs. 69% (18/26), p=0.317, respectively) (Supplementary Figure 5).

3.5. Case-control analyses of neutralizing antibodies after vaccination and at diagnosis

Ten of the 62 infected staff members participating in the study had neutralizing antibodies measured at 14 days after their second vaccine dose and at diagnosis (month 3 after the first dose). Neutralizing antibody levels measured at day 14 post vaccination and at diagnosis were similar (median % of inhibition (IQR): 69.4 (50.7-89.1) vs. 59.4 (32.5-73.2), p=0.353). However, compared with the 30 controls (matching ratio 1:3), these 10 cases had lower day-14 and at-diagnosis neutralizing antibody levels (median % of inhibition (IQR): 69.4 (50.7-89.1) vs. 91.3 (79.6-94.9), p=0.005 and 59.4 (32.5-73.1) vs. 91.1 (77.3-94.2), p=0.043, respectively) (Figure 5).

Case-control analysis for all 62 study participants was hampered by the unavailability of the controls. However, there was no difference in demographics and the levels of neutralizing antibodies measured at diagnosis between the 10 infected staff members included for case-control analysis and those who was not included (Supplementary Table 4).

4. Discussion

We studied Oxford-AstraZeneca vaccine breakthrough infections associated with SARS-CoV-2 Delta variant among staff in a 550-bed infectious diseases hospital in HCMC, Vietnam between 11th and 25th June 2021. The infections occurred 7-8 weeks after most staff received a second vaccine dose. 62/69 infected staff members participated in the clinical study. One required oxygen supplementation for three days but all other infections were either asymptomatic or mild and everyone made a full recovery, consistent with vaccine's effectiveness at protecting against severe disease [16–19]. Yet, we report for the first time the serological markers associated with breakthrough Delta variant infection, and the likely transmission of the Delta variant between fully vaccinated individuals.

All of the 23 whole-genome sequences of SARS-CoV-2 obtained from the infected, fully vaccinated staff members clustered tightly on

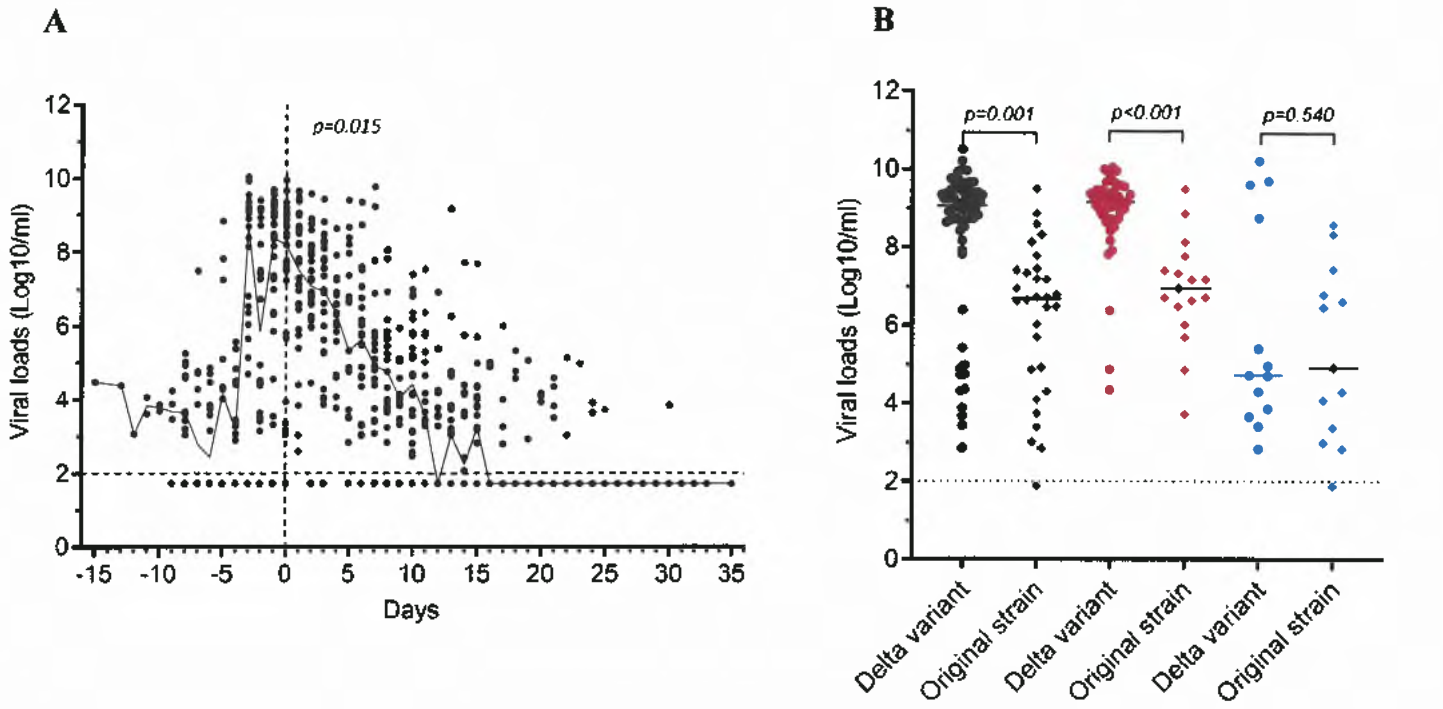

Figure 2. Viral load analyses. A) plot outlining kinetics of viral loads in relation to illness onset of the 49 symptomatic participants, B) comparison between peak viral loads of breakthrough infections (cases) and those (controls) infected with old SARS-CoV-2 strains detected between March and April 2020 in Vietnam. **Notes to Figure 2:** Vertical dashed line indicates the time point of illness onset. Horizontal dashed line indicates detection limit of PCR assay. A) Black lines indicates median viral loads, B) black dots represent for whole groups, red dots represent for symptomatic cases and blue dots represent for asymptomatic cases.

a phylogenetic tree and were distinct from contemporary Delta variant genomes obtained from the community and the hospital prior to the outbreak. Furthermore, the outbreak appeared to localize to a poorly ventilated administrative office, where all 7 members were fully vaccinated but became infected. These findings strongly suggest transmission from and between fully vaccinated individuals. We cannot definitively exclude a common external source, however, but other features indicate it was unlikely. At HTD, access to COVID-19 patient departments was restricted to medical staff and the majority of the infections occurred amongst non-medical staff. Additionally, because community transmission of SARS-CoV-2 had been escalating in HCMC since May 2021, public gatherings of more than 10 people had been banned. Thus it seems unlikely that the outbreak originated from hospital patients or was linked to a mass gathering, superspreader event outside the hospital.

At the beginning of the outbreak (11th June 2021), only patient 1 presented with symptoms. Therefore, secondary transmission may have occurred prior to the development or in the absence of symptoms, a well-recognised phenomenon in unvaccinated people [7]. In our study, transmission may be attributed to several factors. Firstly, high viral loads, >7 $\log_{10}$ copies per mL, which has been strongly correlated with positive culture (i.e. infectiousness) [10,20], was recorded at diagnosis in 11 of the first 53 positive cases. Second, HTD offices are typically equipped with air conditioners without mechanical ventilation systems, an indoor setting that could facilitate the transmission of SARS-CoV-2 [5,21]. Third, mask wearing in the office was not mandatory at the time.

At diagnosis neutralizing antibody levels in a subset of the infected staff were comparable with that measured at 14 days after the second vaccine dose. Additionally, prior to the outbreak, HTD staff members remained naïve to the infection, as evidenced by the absence of N protein antibodies in all staff members [22,23]. Therefore, neutralizing antibodies at diagnosis were likely derived from vaccination rather than natural infection.

Lower levels of neutralizing antibodies after vaccination and at diagnosis were associated with breakthrough infections in a recent report from Israel [6], consistent with our findings. However, in contrast with the Israeli study, we found no correlation between vaccine-induced neutralizing antibody levels at diagnosis and viral loads inferred from PCR Ct values (a surrogate of infectivity). We also found evidence of ongoing transmission between vaccinated staff members, while no secondary transmission was documented in the Israeli study. The difference in the responsible variants (Alpha in the Israeli study and Delta in present study) might explain the different findings. Additionally, our study also showed no correlation between neutralizing antibody levels at diagnosis and the development of symptoms. Collectively, while neutralizing antibodies might be a surrogate of protection, especially against severe diseases as a whole [24], they might not be good indicators of infection progression and infectiousness for breakthrough Delta variant infection.

At the beginning of the outbreak, none of the HTD members of staff (including the PCR confirmed cases) tested positive for N-protein antibodies, which only develop in response to whole-virus based vaccine and natural infection. Additionally, between 12th and 14th May 2021, all members of HTD staff were subjected to a periodic testing for SARS-CoV-2 by PCR, but none was positive. These features suggest that the infected cases were captured at an early phase of the infection. Therefore, by carefully following up the patients during hospitalization, we have also provided new insights into the natural history of breakthrough Delta variant infections. We found viral loads of breakthrough Delta variant infection cases were 251 times higher than those of the infected cases detected during the early phase of the pandemic in 2020[7], with high viral loads recorded around 2-3 days before and after the development of symptoms. Notably, a recent report from the US showed comparable viral loads between vaccinated and unvaccinated individuals infected with the Delta variant.[5] Additionally, a study from China showed viral loads in people infected with the Delta variant were 1000 times higher than those in individuals infected with SARS-CoV-2 19A/19B strains detected in

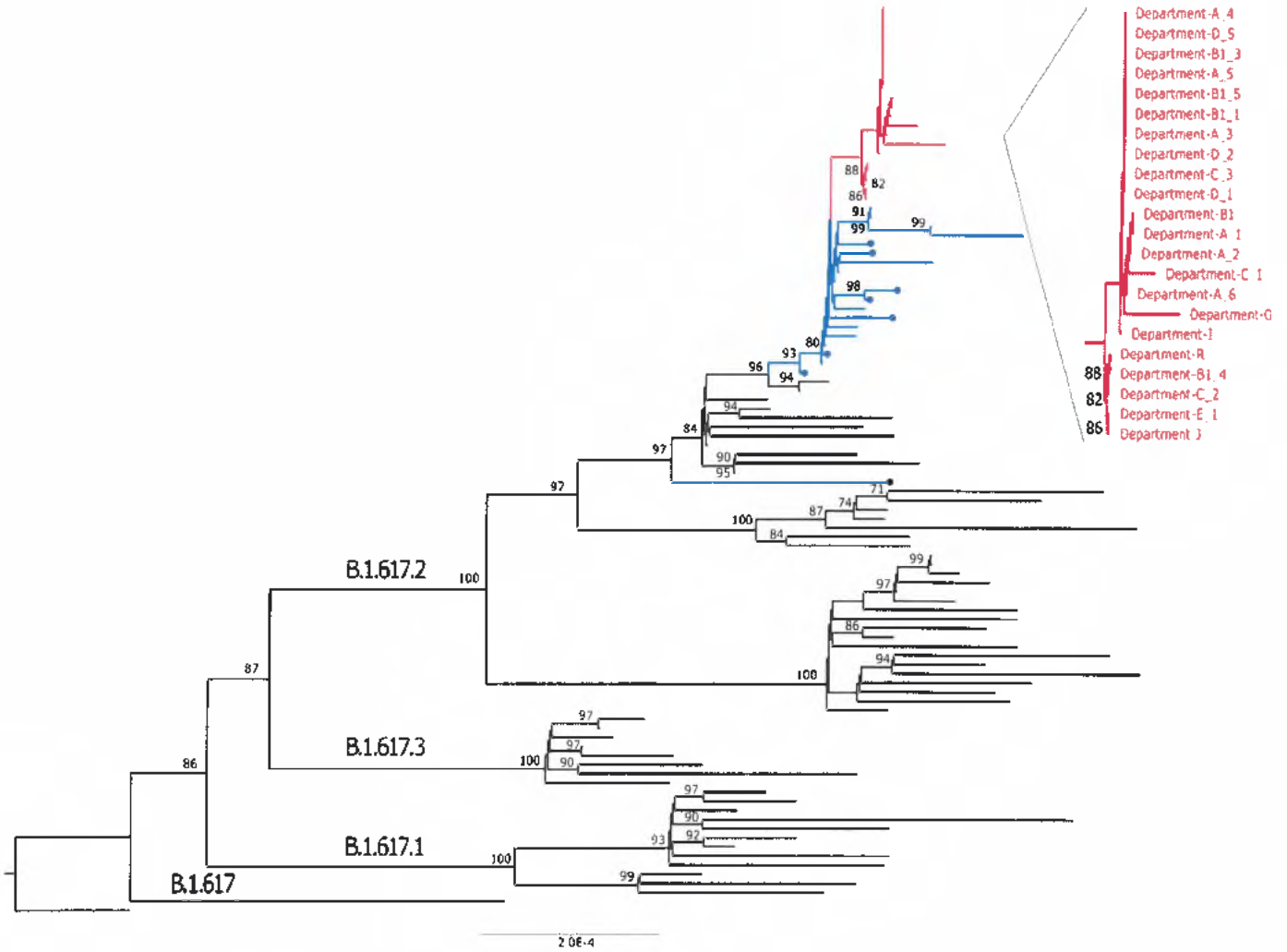

Figure 3. Maximum likelihood tree illustrating the relatedness between SARS-CoV-2 Delta variant strains obtained from cases of vaccine breakthrough infection (red) and contemporary Delta variant sequences obtained from cases of community transmission in Ho Chi Minh City (blue) and from COVID-19 patients admitted to HTD prior to the outbreaks (in blue marked with dots).

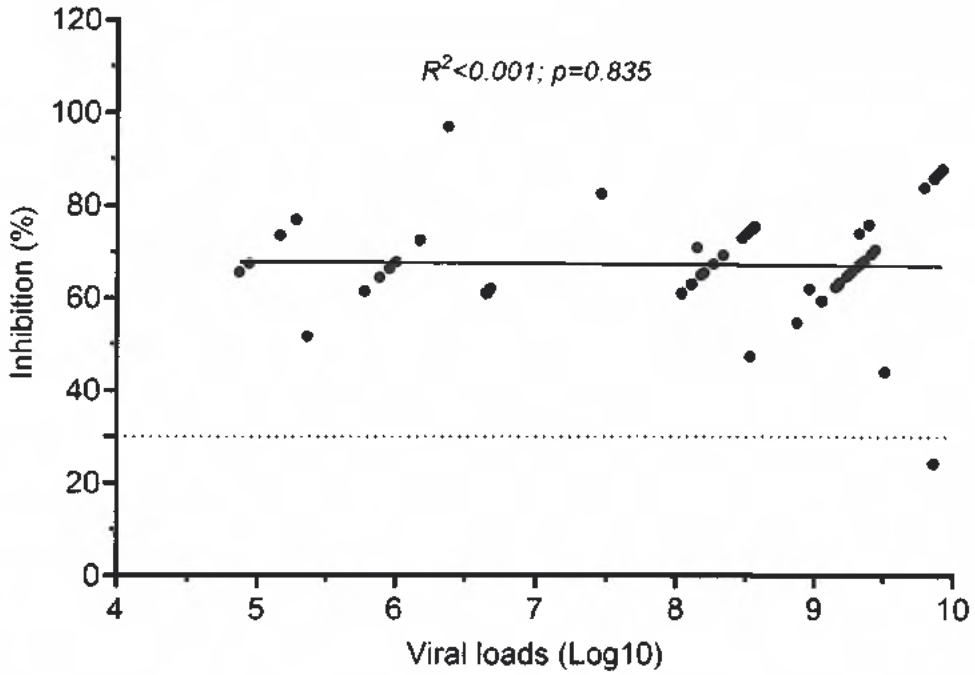

Figure 4. Correlation between neutralizing antibodies at diagnosis and peak viral loads during the course of infection

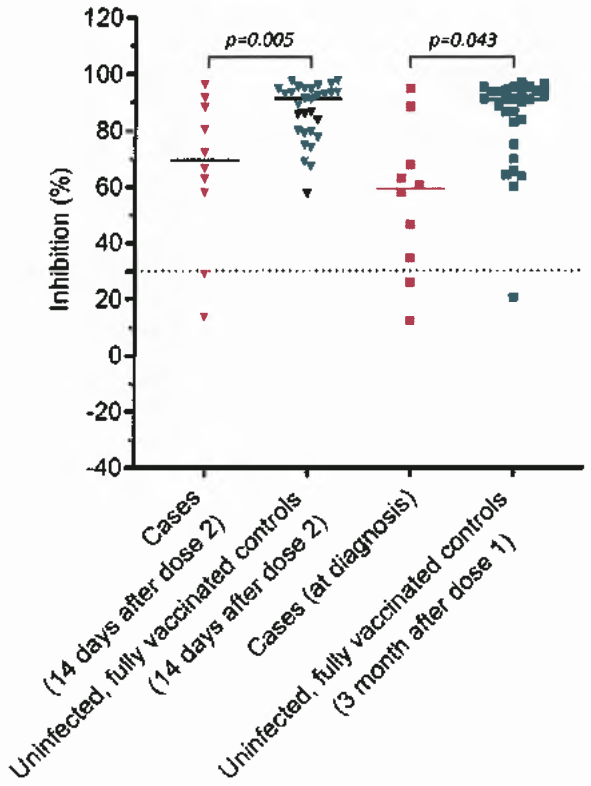

Figure 5. Comparison between neutralizing antibody levels of the 10 case patients (red) and 30 uninfected, fully vaccinated controls (grey green)

China in early 2020 [25]. Collectively, high viral loads might explain the current rapid expansion of the Delta variant, even in the countries with high vaccination coverage. Additionally, previous reports showed short duration of PCR positivity or culturable viruses (2–7 days after diagnosis) among breakthrough infections [26,27]. In contrast, we found prolonged PCR positivity was up to 33 days in our study participants.

Our study has several limitations. First, we only obtained SARS-CoV-2 genomes from 21/62 study participants, leaving the responsible variants in the remaining infected cases unknown. Second, we only focused our analysis on a hospital setting for a duration of two weeks. Therefore, our findings might not be generalizable for the general population, of which exposure to the virus might be different. Additionally, the short duration of the study coupled with the uncertain exposure to the virus prevented us from quantifying the risk of infection between vaccinated and unvaccinated individuals [28]. Third, we did not perform virus isolation to assess the duration of viral shedding, relying instead on PCR Ct values as a surrogate of infectivity. Fourth, information about other potential confounders (including BMI) was not available for inclusion in the case-control analysis. Last, none of the infected staff members was older than 60 years. Therefore, milder disease might be anticipated and breakthrough Delta variant infections in older people with comorbidities may be more severe.

In summary, we report SARS-CoV-2 Delta variant breakthrough infections among vaccinated health care workers with likely transmission between them. Most experienced mild symptoms and all recovered uneventfully. The infections were associated with high viral loads, prolonged PCR positivity, and low levels of neutralizing antibodies after vaccination and at diagnosis.

Contributors

Study design: NVVC, NTD, LMH, NTT, GT, LVT

Study implementation and patient enrolment: VMQ, DBK, NTP, HPT, NTNM, NCH, LMH, LMT, LMY

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Laboratory testing: LAN, BTTT, VVP, TNPT, VTV, TTTT, NTT, HTB, HTKN, LTTU

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All authors reviewed, approved, and agreed to submit the final version of the manuscript for publication.

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Data sharing

SARS-CoV-2 sequences have been submitted to GISIAD (gisaid.org). De-identified study data are available for access by accredited researchers in accordance with data sharing policies of OUCRU.

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Declaration of Competing Interest

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101143.

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Exhibit "O"

Shedding of Infectious SARS-CoV-2 Despite Vaccination

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This is **Exhibit** "O" referred to in the
Affidavit of Nadr Jomha

[redacted]

Abstract

The SARS-CoV-2 Delta variant is highly transmissible and contains mutations that confer partial immune escape. We compared RT-PCR cycle threshold (Ct) data from 699 test-positive anterior nasal swab specimens from fully vaccinated (n = 310) or unvaccinated (n=389) individuals. We observed low Ct values (<25) in 212 of 310 fully vaccinated (68%) and 246 of 389 (63%) unvaccinated individuals. Testing a subset of these low-Ct samples revealed infectious SARS-CoV-2 in 15 of 17 specimens (88%) from unvaccinated individuals and 37 of 39 (95%) from vaccinated people. To determine whether infectious virus titers differed in vaccinated and unvaccinated persons, we performed plaque assays on an additional set of 48 samples with Ct <25, finding no difference in infectious virus titer between groups.

Main text

Introduction

The SARS-CoV-2 Delta variant is highly transmissible and contains mutations that confer partial immune escape. Outbreak investigations suggest that vaccinated persons can spread Delta[1,2] but it is uncertain whether vaccine-induced immune responses reduce nasal viral RNA burden or the titer of infectious SARS-CoV-2 in people infected despite vaccination relative to unvaccinated persons.

Methods

To estimate nasal viral RNA burden, we compared RT-PCR cycle threshold (Ct) data from 699 test-positive anterior nasal swab specimens from fully vaccinated (n = 310) or unvaccinated (n=389) individuals. "Fully vaccinated" is defined as having received a second mRNA vaccine dose or single adenovirus vector vaccine dose ≥ 2 weeks prior to testing positive. Samples were collected in Wisconsin 29 June through 31 July 2021 and tested by a single contract laboratory. During the study period, estimated prevalence of Delta variants in Wisconsin increased from 69% to over 95%. Vaccination status was determined via self-reporting and validated with state immunization records (**Supplemental Figure 1**). Infectious virus was quantified using plaque assays on a subset of samples with Ct values <25.

Results

RT-PCR Ct values <25 had previously been associated with shedding of infectious SARS-CoV-2. We observed low Ct values (<25) in 212 of 310 fully vaccinated (68%; **Figure 1A**) and 246 of 389 (63%) unvaccinated individuals. Low Ct values were detected in vaccinated people regardless of symptoms at the time of testing (**Figure 1B**). Ct values <25 were detected in 7 of 24 unvaccinated (29%; CI: 13-51%) and 9 of 11 fully vaccinated asymptomatic individuals (82%; CI: 48-97%), and 158 of 232 unvaccinated (68%, CI: 62-74%) and 156 of 225 fully vaccinated (69%; CI: 63-75%) symptomatic individuals. Testing a subset of these low-Ct samples revealed infectious SARS-CoV-2 in 15 of 17 specimens (88%) from unvaccinated individuals and 37 of 39 (95%) from vaccinated people. Infectious virus was detected in the sole specimen tested from an asymptomatic fully vaccinated individual (**Supplemental Figure 2**). Although few asymptomatic individuals were sampled, these results indicate that even asymptomatic, fully vaccinated people might shed infectious SARS-CoV-2.

To determine whether infectious virus titers differed in vaccinated and unvaccinated persons, we performed plaque assays on an additional set of 48 samples with Ct <25, finding no difference in infectious virus titer between groups (**Figure 1C**). Notably time from symptom onset to testing did not vary by vaccination status, suggesting that our observations are not confounded by biases in test-seeking behavior between vaccinated and unvaccinated persons (p=0.40; **Supplemental Figure 3**).

Discussion

Combined with other studies[3,4] these data indicate that vaccinated as well as unvaccinated individuals infected with the Delta variant might transmit infection, though other studies suggest this may be relatively inefficient[5]. Importantly, we show that infectious SARS-CoV-2 is found at similar titers in vaccinated and unvaccinated persons when specimen Ct values are low. The inclusion of viruses from multiple counties without a linking outbreak (more than 80% of samples were not associated with an outbreak known to public health), indicate that Delta-lineage SARS-CoV-2 can achieve low Ct values consistent with transmissibility in fully vaccinated individuals across a range of settings.

Preventing infections with the Delta variant is therefore critical to stem transmission. Vaccinated and unvaccinated persons should get tested when symptomatic or after close contact with someone with suspected or confirmed COVID-19. Continued adherence to non-pharmaceutical interventions during periods of high community transmission to mitigate spread of COVID-19 remain important for both vaccinated and unvaccinated individuals. Moreover, the administration of an additional vaccine dose after the initial vaccine series dramatically reduces susceptibility to infection with the Delta variant[6], providing another valuable strategy for interrupting transmission.

Figure

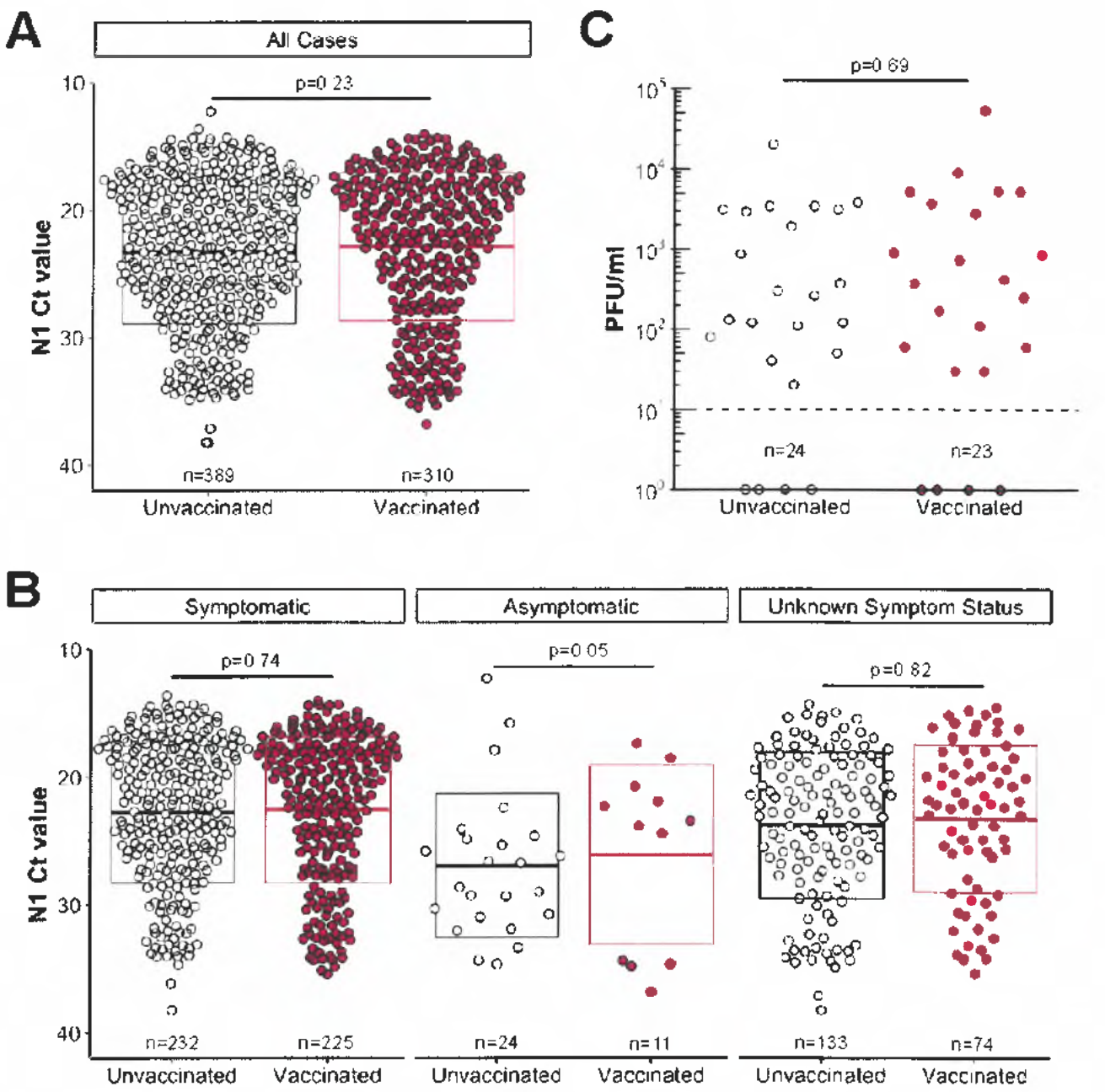
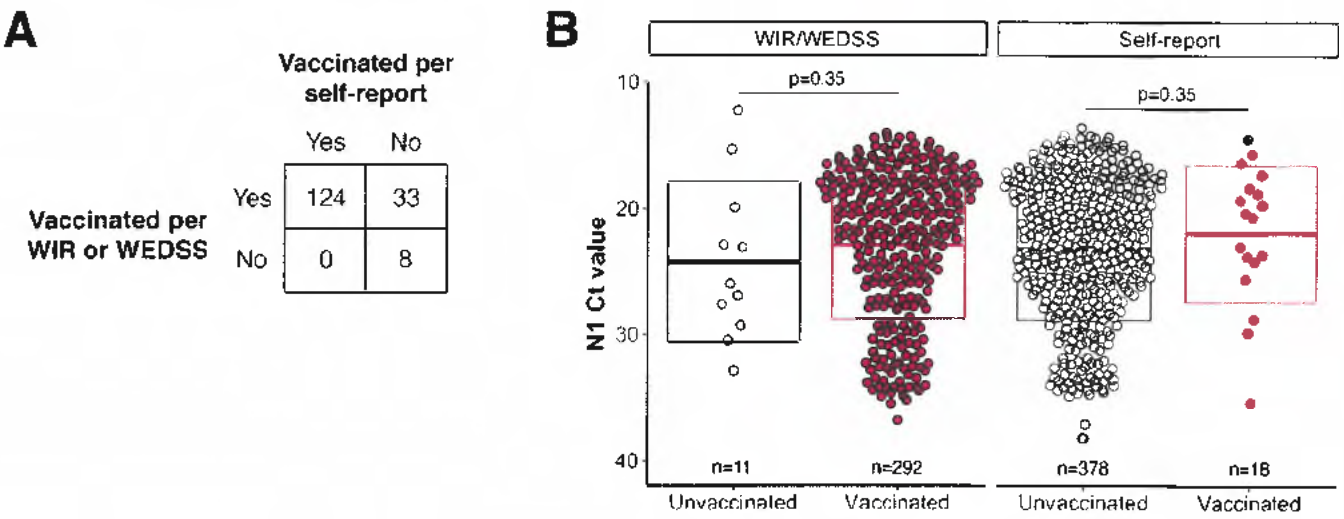


Figure 1. Individuals infected with SARS-CoV-2 despite full vaccination have low Ct values and shed similar amounts of infectious virus as uninfected individuals. A. Ct values for SARS-CoV-2-positive specimens grouped by vaccination status. RT-PCR was performed by Exact Sciences Corporation, responsible for over 10% of all PCR tests in Wisconsin during this period, using a qualitative diagnostic assay targeting the SARS-CoV-2 N gene (oligonucleotides identical to CDC's N1 primer and probe set) that has been authorized for emergency use by FDA

(https://www.fda.gov/media/138328/download). **B.** N1 Ct values for SARS-CoV-2-positive specimens grouped by vaccination status for individuals who were symptomatic or asymptomatic, or those whose symptom status was not determined, at the time of testing. **C.** We performed plaque assays on Vero E6 TMPRSS2 cells on a subset of specimens. Specimens were selected by N1 Ct-matching between fully vaccinated and unvaccinated persons, then specimens from persons with unknown vaccination status were excluded from the analysis. Infectious titers are expressed as plaque-forming units (pfu) per milliliter specimen. Specimens underwent a freeze-thaw cycle prior to virus titration. In **A** and **B**, boxplots represent mean N1 Ct values +/- one standard deviation. P-values were calculated by comparing mean Ct values by independent two-group Mann-Whitney U tests.

Supplemental materials

Supplemental figure 1



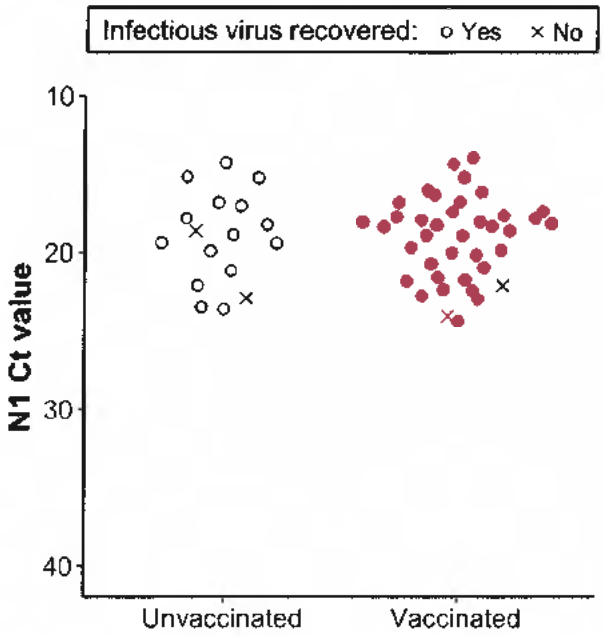
Supplemental figure 1. Concordance between self-reported vaccination status and the Wisconsin Immunization Registry (WIR) or Wisconsin Electronic Disease Surveillance System (WEDSS). For all individuals, vaccination status was determined using WIR/WEDSS electronic registries when data were available. Individuals were identified as unvaccinated at the time of testing if WIR/WEDSS data indicated receipt of a first SARS-CoV-2 vaccine dose after the test date.

Individuals were considered fully vaccinated based on WIR/WEDSS data if the registries indicated receipt of a final vaccine dose at least 14 days prior to testing. For individuals whose vaccination status could not be verified in WIR/WEDSS, self-reported data collected at the time of testing were used. Individuals were considered unvaccinated based on self-report only if there was an explicit declaration of unvaccinated status in the self-reported data. Individuals were considered fully vaccinated based on self-report if they fulfilled all of the following criteria: (1) indicated that they had received a COVID vaccine prior to testing; (2) indicated that they did not require another vaccine dose; and (3) reported a date of last vaccine dose that was at least 14 days prior to testing.

Specimens lacking data on vaccination status were excluded from the study. Specimens from partially vaccinated individuals (incomplete vaccine series, or <14 days post-final dose) were also excluded. Fully vaccinated status was determined by WIR/WEDSS for 292 specimens and by self-reported data for 18. Unvaccinated status was determined by WIR/WEDSS for 11 and by self-reported data by 378. **A.** Of the 699 specimens with vaccination status available from at least one source, 165 specimens had data available from both sources. For self-reporting, under-reporting of full vaccination status (33/157)

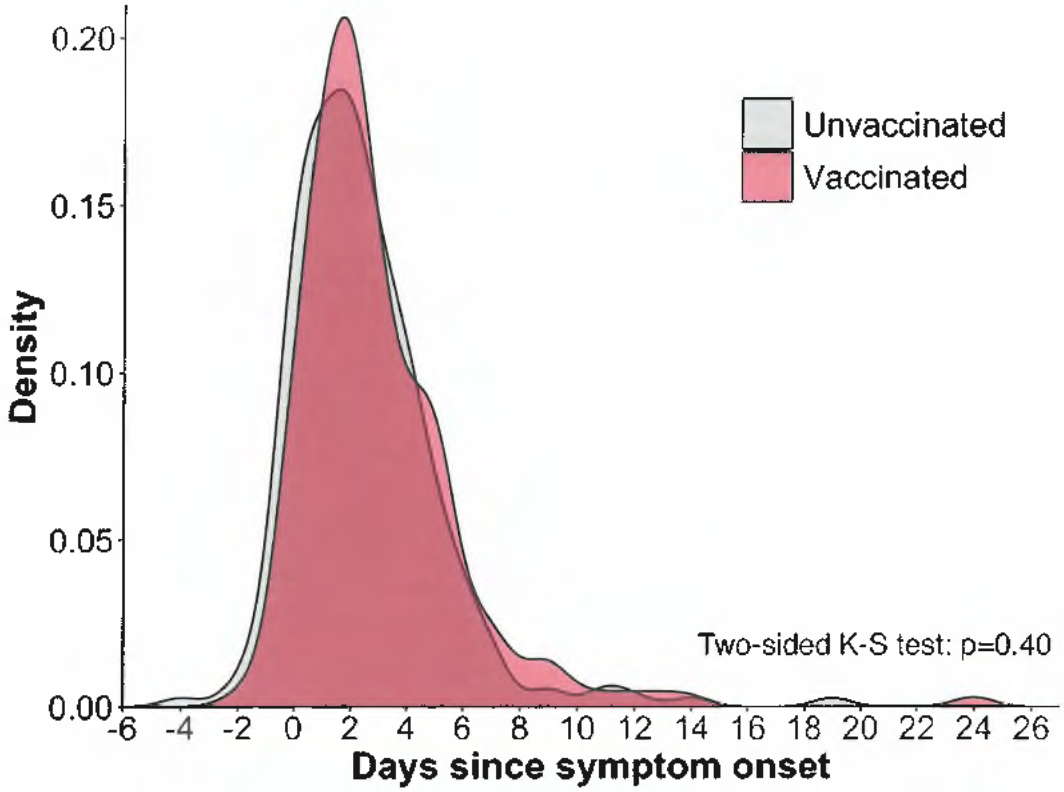
was more common than over-reporting (0/124). **B.** N1 Ct values for SARS-CoV-2-positive specimens grouped by vaccination status for individuals whose vaccination status was determined by WIR/WEDDS or by self-reported data. Boxplots represent mean N1 Ct values +/- one standard deviation. P-values were calculated by comparing mean Ct values by independent two-group Mann-Whitney U tests.

Supplemental figure 2



Supplemental figure 2. Infectious SARS-CoV-2 detected in the majority of fully vaccinated individuals with low Ct values. Infectiousness was determined for a subset of N1 Ct-matched specimens with Ct <25 by inoculation onto Vero E6 TMPRSS2 cells and determining presence of cytopathic effects (CPE) after 5 days in culture. Specimens with unknown vaccination status were excluded from the analysis. Circles indicate presence of CPE; 'X' indicates no CPE detected.

Supplemental figure 3



Supplemental figure 3. Density distributions of unvaccinated and vaccinated specimen collection dates by day since symptom onset. Day 0 on the x-axis denotes self-reported day of symptom onset. Negative values for days indicate specimen collection prior to symptom onset. Symptom onset data were available for n=263 unvaccinated cases and n=232 vaccinated cases.

Conflict of interest

The authors declare no conflicting interests.

Ethics statement

Per the University of Wisconsin-Madison IRB, this project qualifies as public health surveillance activities as defined in the Common Rule, 45 CFR 46.102(l)(2). As such, the project is not deemed to be research regulated under the Common Rule and therefore, does not require University of Wisconsin-Madison IRB review and oversight.

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Data availability

Data and processing workflows are available at https://go.wisc.edu/p22l16. To protect potentially personally identifiable information, the publicly available dataset contains only PCR Ct values, vaccine status, symptom status, virus culture status, and days from symptom onset to testing for each specimen.

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Exhibit "P"

This is **Exhibit "P"** referred to in the
Affidavit of Nadr Jomha

Rapid communication

Nosocomial outbreak caused by the SARS-CoV-2 Delta variant in a highly vaccinated population, Israel, July 2021

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A nosocomial outbreak of SARS-CoV-2 Delta variant infected 42 patients, staff and family members; 39 were fully vaccinated. The attack rate was 10.6% (16/151) among exposed staff and reached 23.7% (23/97) among exposed patients in a highly vaccinated population, 16–26 weeks after vaccination (median: 25 weeks). All cases were linked and traced to one patient. Several transmissions occurred between individuals wearing face masks. Fourteen of 23 patients became severely sick or died, raising a question about possible waning immunity.

Israel was one of the first countries to achieve a high level of full vaccination with the Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer, Mainz, Germany/New York, United States (US)) vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). From May through mid-June 2021, with more than 55% of the population fully vaccinated, new cases decreased to less than two cases per million, with no social restrictions, indicative of very high vaccine effectiveness [1,2]. Since mid-June, a sharp increase in cases has been observed, attributed to the SARS-CoV-2 Delta variant (Phylogenetic Assignment of Named Global Outbreak (Pango) lineage designation B.1.617.2 and AY.* sublineages), which by mid-July constituted more than 95% of sequenced virus isolates in Israel [3]. This variant was assessed to have higher transmissibility than the Alpha variant (B.1.1.7 and Q.* sublineages) [4].

We present an investigation of a coronavirus disease (COVID-19) outbreak that started from one unidentified COVID-19 patient, with extensive, rapid nosocomial spread among vaccinated, including individuals wearing surgical masks.

Setting

Meir Medical Center has 780 beds, most rooms accommodate three to four patients, 1 m apart with separation curtain partitions between beds. Starting in March 2020, patients have been encouraged to wear surgical masks. Although use was inconsistent, it was enforced during patient–staff encounters for both sides. On the dedicated COVID-19 ward, dedicated staff members worked with full personal protective equipment (PPE): N-95 mask, face shield, gown, gloves and hair cover.

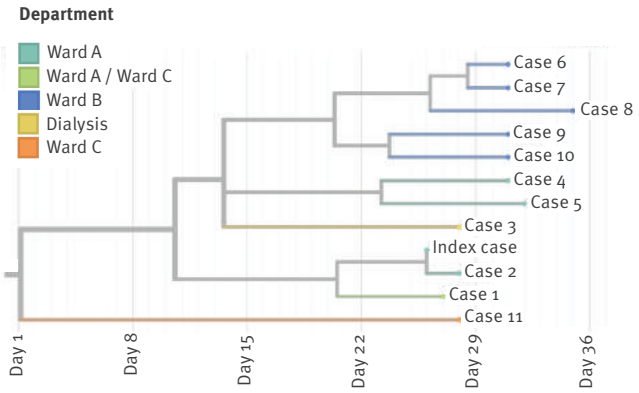
Outbreak investigation

Contact investigations were carried out by trained infection control personnel and were initiated after suspected nosocomial acquisition or COVID-19 diagnosis of a staff member confirmed by positive PCR for SARS-CoV-2. All exposed individuals were PCR-tested for SARS-CoV-2. All those testing positive were considered as a COVID-19 case. All data were collected in real time and included all patients and personnel exposed to a case, last negative SARS-CoV-2 test, presence of symptoms, date of symptom onset, any sick family member, and vaccination status and date. All exposed individuals were PCR-tested for SARS-CoV-2. Whenever more than one patient was identified as COVID-19 case, all staff and patients on the ward were screened regardless of a known encounter with the positive case. All exposed patients found negative in the first screening, were cohorted and rescreened 7 days post exposure. All identified cases were either transferred to a dedicated COVID-19 unit or discharged as per clinical status.

The index case was a fully vaccinated haemodialysis patient in their 70s. They were admitted to Ward A in

Figure

Whole genome-based phylogenetic tree of SARS-CoV-2 Delta isolates, nosocomial outbreak, Israel, July 2021 (n =12)



The tree was constructed using Nextstrain's Augur pipeline and visualised with Auspice [8]. The numbers represent the Patient numbering used in the manuscript text and Table.

mid-July with fever and cough and placed in a room with three other patients. On admission day, the index case was not tested for SARS-CoV-2, because their symptoms were mistaken for possible bloodstream infection exacerbating congestive heart failure. During their stay, the index case and one roommate were dialysed every other day in the dialysis unit. Four days after admission, the index case was diagnosed with COVID-19 by PCR for SARS-CoV-2 E gene with a quantitative cycle (Cq) value of 13.59; the case was therefore transferred to a COVID-19-dedicated unit of Ward B. On the same day, all three of this case's roommates on Ward A were screened for SARS-CoV-2 and tested positive and were transferred to the dedicated ward or discharged.

The contact investigation included Ward A, the dialysis unit (contacts of the index case) and Ward C following a 1-day stay of Case 1. This investigation revealed a total of 27 COVID-19 cases by SARS-CoV-2 PCR: 16 patients, including the index case, nine staff and two family members.

The COVID-19 diagnosed cases were transferred on the day of their diagnosis to a COVID-19 unit on Ward B, which operated as a mixed ward because of the small number of COVID-19 patients in our hospital at the time. Half the ward was dedicated to COVID-19 patients, with dedicated staff in full PPE, while half remained a regular ward. The index case was treated on transfer day by a healthcare worker (HCW) who had recovered from COVID-19 a year earlier, and was vaccinated once, as per Israeli guidelines [5]. Three days after transfer day, this HCW attended a room in the regular ward with three patients of whom two developed symptoms compatible with COVID-19 2 days later and tested positive for SARS-CoV-2. Contact investigation on Ward B identified a total of 19 COVID-19 cases by SARS-CoV-2 PCR: 10 staff, including the aforementioned HCW, eight patients, including the three above, and one family member.

The calculated attack rate among all exposed patients and staff was 10.6% (16/151) for staff and 23.7% (23/97) for patients, in a population with 96.2% vaccination rate (238 vaccinated/248 exposed individuals).

Sequencing and analysis

Sequence and patient data were obtained via the Israel National Consortium of SARS-CoV-2 sequencing. FASTQ files underwent processing, mapping to the reference genome (NC_045512.2) and construction of consensus FASTA sequences as previously described [6]. All sequence data were deposited and are available in GISAID [7]. Phylogenetic trees were constructed using NextStrain's Augur pipeline and visualised with auspice [8].

We conducted phylogenetic analysis on the whole-genome SARS-CoV-2 sequences that were available for 12 cases in this outbreak, including staff and patients from Wards A, B and C and dialysis departments (Figure). All were infected with the Delta variant and epidemiologically and phylogenetically connected to the same outbreak except for Case 11 from Ward C. Case 11 and three staff members identified on Ward C were not considered as part of this outbreak. The three staff members from Ward C were exposed to both Case 1 and Case 11 and therefore the source of their infection could not be verified.

Demographic and clinical information

Of the 42 cases diagnosed in this outbreak, 38 were fully vaccinated with two doses of the Comirnaty vaccine, one was recovered with one vaccination and three were unvaccinated. The median age was 55 years (interquartile range (IQR): 36–77.5) and 24 were female. Twenty-three were patients, 16 staff members and three family members. The median time from second vaccine dose to breakthrough infection was 177 days (range 111–194). On the day of diagnosis, only 24 individuals were symptomatic, but in the following days, 36 had become symptomatic. All staff (median age: 33 years; range: 22–48) remained asymptomatic or with mild disease. Among the patients (median age: 77 years; range: 42–93; median time from second vaccine dose to infection: 176 days; range: 143-188), eight became severely ill, six critically ill and five of the critically ill died (Table). The patient population was considerably older than staff and all patients had comorbidities: diabetes mellitus (n = 9), hypertension (n = 16), ischemic heart disease (n = 12), congestive heart failure (n = 7), dementia (n = 5), body mass index › 30 (n = 8), chronic renal failure (n = 11) of whom six were on dialysis. Eight patients were immunocompromised.

The median Cq values on diagnosis days were 19.9 (IQR: 17.8–25.1) and were lower for symptomatic individuals

Table

Case data, nosocomial COVID-19 outbreak, Israel, July 2021 (n = 23)

Case	Age group (years)	Gap (days) vaccine to diagnosis	Cq	COVID-19 maximal disease severity	Died
Index	70-79	169	13.6	Critical	Yes
1	80-89	172	15	Critical	Yes
2	50-59	175	18	Severe	No
3	60-69	176	17.6	Severe	No
4	80-89	181	20.5	Severe	No
6	40-49	143	15	Moderate	No
7	70-79	182	16	Critical	Yes
9	50-59	Not vaccinated	24	Mild	No
10	80-89	171	28	Severe	No
Na	60-69	168	18.5	Severe	No
Na	70-79	182	36	Mild	No
Na	80-89	177	31.8	Severe	No
Na	70-79	187	22	Critical	No
Na	70-79	184	14	Severe	No
Na	80-89	186	21	Asymptomatic	No
Na	90-99	173	18	Critical	Yes
Na	70-79	174	38	Severe	No
Na	70-79	176	NA	Mild	No
Na	90-99	176	NA	Critical	Yes
Na	80-89	188	NA	Mild	No
Na	60-69	183	27	Asymptomatic	No
Na	80-89	Not vaccinated	NA	Mild	No
Na	50-59	152	21.3	Asymptomatic	No

Na: not applicable; NA: not available.

(median: 18.2; IQR: 15.7–21.7) than for asymptomatic individuals (median: 22; IQR: 18–28), but the difference was not statistically significant.

Ethical statement

The clinical data of this work was from an outbreak investigation; thus ethical approval was waived by the Meir Medical Center Ethical committee. The bioinformatics work was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Sheba Medical Center institutional review board (7045–20-SMC). Patient consent was waived because the study used remains of clinical samples and the analysis used anonymous clinical data.

Discussion

We have investigated a nosocomial COVID-19 outbreak involving the SARS-CoV-2 Delta variant among a highly vaccinated population. The attack rate among exposed individuals reached 23.3% in patients and 10.3% in staff, with 96.2% vaccination rate among exposed individuals. Moreover, several transmissions probably occurred between two individuals both wearing surgical masks, and in one instance using full PPE, including N-95 mask, face shield, gown and gloves.

In a recent publication by Bernal et al., the effectiveness of full vaccination with the Comirnaty vaccine against the Delta variant was high, although lower than against the Alpha variant (88% vs 93.7%) [9]. This was not the experience in Israel, with a rapid increase in cases since June 2021 despite a high vaccination rate [1].

Although reports of breakthrough infections are increasing [10-12], this communication emphasises several points. It challenges the assumption that high universal vaccination rates will lead to herd immunity and prevent COVID-19 outbreaks. This was probably true for the wild-type SARS-CoV-2 virus, but in the outbreak described here, 96.2% of the exposed population was vaccinated. Infection advanced rapidly (many cases became symptomatic within 2 days of exposure), and viral load was high. Another accepted view is that, when facing a possible mismatch between the SARS-CoV-2 variant and vaccine or waning immunity, the combination of vaccine and face mask should provide the necessary protection. Although some transmission between staff members could have occurred without masks, all transmissions between patients and staff occurred between masked and vaccinated individuals, as experienced in an outbreak from Finland [12]. We cannot rule out that protection measures were not optimally implemented, however, transmissibility in summer 2021 differs from our experiences in the previous 18 months. Whether this can be attributed to the low Cq and high transmissibility of the Delta variant is not

clear. Of note, in our cases, in particular case patients, the time from vaccination was considerable. The shortest interval was 142 days (5 months), and many of our case patients advanced to severe disease. Data from Israel imply that the main reason for the increase in COVID-19 cases in summer is indeed waning immunity, and a third vaccine dose, 5 months after the second dose will possibly result in trend reversal [13,14].

Conclusion

This nosocomial outbreak exemplifies the high transmissibility of the SARS-CoV-2 Delta variant among twice vaccinated and masked individuals. This suggests some waning of immunity, albeit still providing protection for individuals without comorbidities. However, a third vaccine dose may be needed, particularly in individuals with risk factors for severe COVID-19. Appropriate use of masks, especially in high-risk settings is advised.

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Conflict of interest

None declared.

Authors' contributions

Pnina Shitrit was responsible for data acquisition and interpretation, revising the manuscript and final approval of the version to be published. Neta S. Zuckerman was responsible for sequencing and bioinformatics, revising the manuscript and final approval of the version to be published. Orna Mor was responsible for sample collection, revising the manuscript and final approval of the version to be published. Bat-Sheva Gottesman was responsible for interpretation of data, revising the manuscript and final approval of the version to be published. Michal Chowers was responsible for analysis of the data, drafting the work and final approval of the version to be published.

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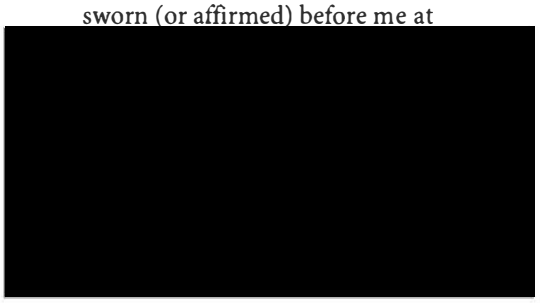
Exhibit "Q"

This is **Exhibit "Q"** referred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at



Rapid communication

An outbreak caused by the SARS-CoV-2 Delta variant (B.1.617.2) in a secondary care hospital in Finland, May 2021

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An outbreak caused by the SARS-CoV-2 Delta variant (B.1.617.2) spread from one inpatient in a secondary care hospital to three primary care facilities, resulting in 58 infections including 18 deaths in patients and 45 infections in healthcare workers (HCW). Only one of the deceased cases was fully vaccinated. Transmission occurred despite the use of personal protective equipment by the HCW, as advised in national guidelines, and a high two-dose COVID-19 vaccination coverage among permanent staff members in the COVID-19 cohort ward.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant of concern (Phylogenetic Assignment of Named Global Outbreak (Pango) lineage designation B.1.617.2) has been suggested to be more transmissible than the Alpha (B.1.1.7) variant [1], which is more transmissible than the wild-type SARS-CoV-2 virus [2-4]. We describe here an outbreak caused by the Delta variant that originated from one inpatient in a secondary care hospital and spread within the hospital and to three primary care facilities; we describe our experiences in controlling it. Cases were detected among patients, healthcare workers (HCW) and in the community. Both symptomatic and asymptomatic infections were found among vaccinated HCW, and secondary transmission occurred from those with symptomatic infections despite use of personal protective equipment (PPE).

Setting and outbreak onset

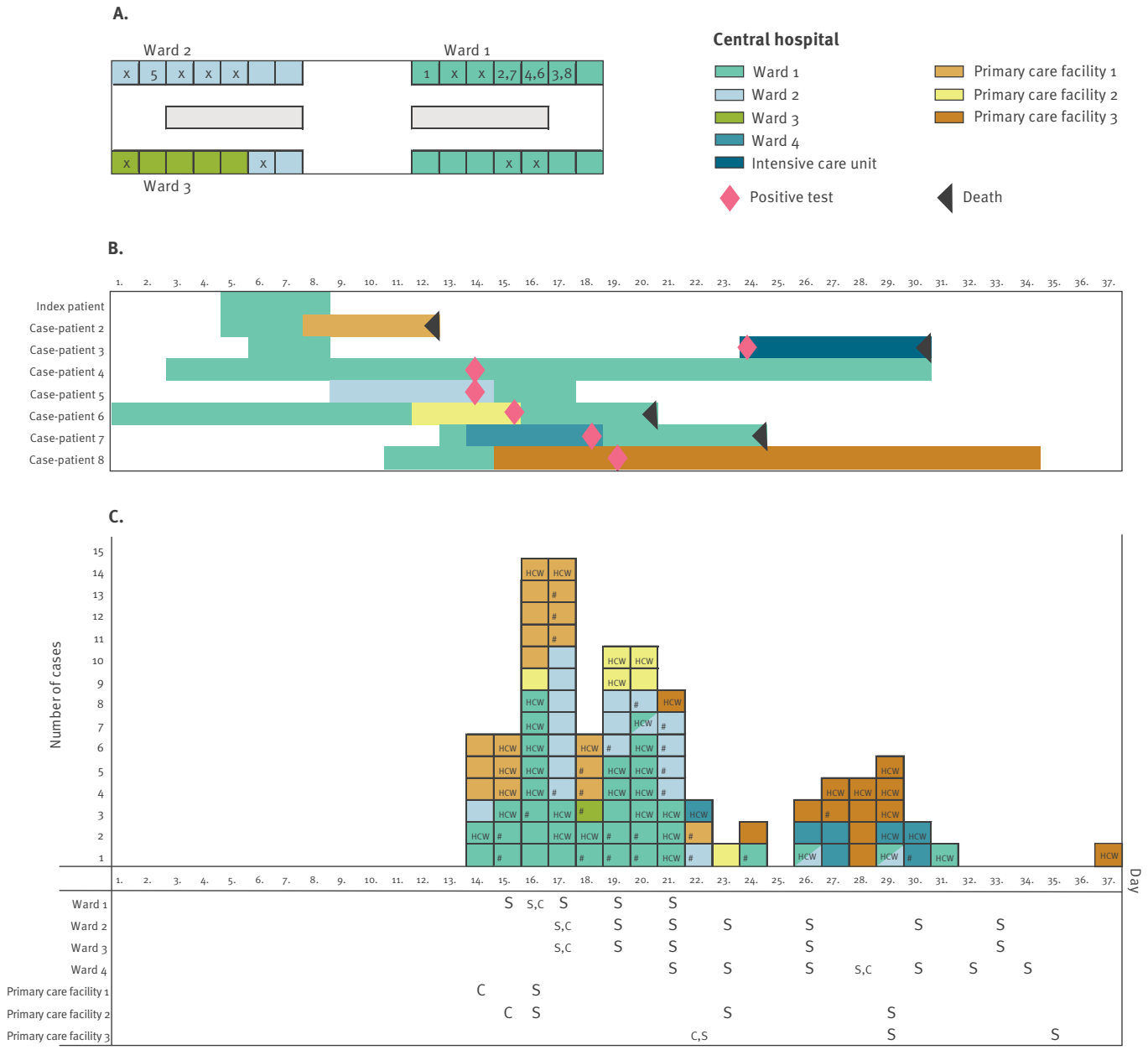
Tavastia Proper healthcare district (HD), with a population of 171,000, is one of the 20 geographically and administratively defined HD in Finland. This HD has one central hospital providing secondary care and six healthcare facilities providing primary care, one of which is a district hospital while the other five are healthcare centre wards. The central hospital has 184 beds in eight wards and an intensive care unit (ICU). In the central hospital, patients who have symptoms compatible with coronavirus disease (COVID-19) are tested upon admission with a nasopharyngeal SARS-CoV-2 real time reverse transcriptase (RT-PCR) test (Supplementary data). Patients with a high clinical suspicion of COVID-19 are treated in isolation until there are at least two negative tests 24 hours apart.

In early May 2021, a patient with COVID-19-associated pneumonia and travel history in Asia was hospitalised for 4 days in the COVID-19 cohort in Ward 1 of the central hospital. This index patient had a positive test for SARS-CoV-2 9 days before hospitalisation and was admitted to an isolation room. Six days after the discharge of the index patient, two secondary case-patients tested positive for SARS-CoV-2 in Wards 1 and 2 (Figure). Exposed roommates (n = 8) and unvaccinated healthcare workers (n = 11, 10 of whom were HCW students) were quarantined. As additional cases were detected, Wards 1, 2 and 3 were closed from new admissions. All patients were treated with contact and droplet precautions three days following the identification of the first secondary cases.

The outbreak spread to four wards in the central hospital. According to the place of exposure, most cases (31 case-patients and 21 HCW-cases) were in Wards 1 and 2, one case-patient was in Ward 3 and seven cases (four case-patients and three HCW-cases) were in another ward located on a different floor (Ward 4).

Figure.

Outbreak caused by the SARS-CoV-2 Delta variant from one infected index patient in a central hospital, Tavastia Proper healthcare district, Finland, May 2021 (n = 103)



C: ward closure; COVID-19: coronavirus disease; HCW: healthcare worker; S: ward screening; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

A) Start of the outbreak in Ward 1 (COVID-19 cohort), 2 and 3 in the central hospital. The rooms of the index case (1), case-patients 2-8 and other case-patients (›8, marked with x) are indicated.

B) Hospitalisations in the central hospital and transfers to primary healthcare facilities for the index patient and case-patients 2–8 during the outbreak. A pink diamond indicates the date of SARS-CoV-2 laboratory confirmation and the black triangle indicates the date of death. The index patient had a SARS-CoV-2-positive test several days before hospital admission. Case-patient 2 was not laboratory-confirmed for SARS-CoV-2 before death, but had symptoms and transmitted the disease to a family member, healthcare workers and a roommate, who were all laboratory-confirmed for SARS-CoV-2.

C) Epidemic curve showing case-patients and HCW-cases by date when a positive SARS-CoV-2 sample was taken and place of exposure. Case-patients whose diagnosis was made after discharge from the exposure ward are marked with #. The date of diagnosis was missing for three HCW-cases in Primary care facility 1. Three HCW-cases who had worked both in Wards 1 and 2 are marked accordingly.

Table 1.

Characteristics of COVID-19 case-patients and healthcare worker-cases, Tavastia Proper health district, Finland, May 2021 (n = 103)

Characteristics	Case-patients (n = 58)	HCW-cases (n = 45)	Total (n = 103)
Age in years (range)	73 (30–97)	38 (19–62)	-
Sex: men (%)	28 (48)	0 (0)	
Deaths (%)	18 (31)	0 (0)	
Vaccinated against SARS-CoV-2[a]			
Two doses	2	18	20
One dose	42	6	48
Unvaccinated	14	21	35
Place of exposure to SARS-CoV-2			
Central hospital[b]	36	26	62
Primary care facility 1	13	9	22
Primary care facility 2	3	3	6
Primary care facility 3	6	7	13

COVID-19: coronavirus disease; HCW: healthcare workers; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

[a] The vast majority of HCW (>90%) were vaccinated with Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer, Mainz, Germany/New York, United States).

[b] Central hospital includes Wards 1-4.

Exposure to SARS-CoV-2 occurred in almost every unit of the central hospital. Some exposed patients had been transferred to four of the six primary care facilities. In three of these four facilities, the outbreak spread through transfers that took place before the outbreak was detected. In the fourth facility, the transfer happened after outbreak detection and the exposed patient was quarantined and there was no further spread.

Sequencing detection of the Delta variant

The sequencing results of the index patient as well as results of Case-patients 4 and 5, a staff member exposed to Case-patient 2, and several other (n = 32) outbreak-related samples showed that the outbreak was caused by the Delta variant (GISAID Accession IDs: EPI_ISL_2557176-EPI_ISL_2557210) [5].

In total, 317 laboratory findings positive for SARS-CoV-2 were notified to the National Infectious Diseases Register (NIDR) in Tavastia Proper HD in May 2021; 44% (141/317) were sequenced (NIDR, data taken 28 June 2021) and 41% (58/141) were the Delta variant, all but one with known transmission chains.

Ethical statement

As the data displayed in this article is a result of an outbreak investigation (legal task of Finnish Institute for Health and Welfare and HD according to the Communicable Disease Act), ethical approval was not needed.

Extent and spread of the outbreak

We defined a case-patient or HCW-case as a person with a positive SARS-CoV-2 RT-PCR test with a known exposure to a SARS-CoV-2 outbreak, either when admitted (patient) or when working in one of the four healthcare facilities (HCW).

In total, 58 case-patients were detected (Table 1), 18 of whom died. For the deceased case-patients, the median age was 80 years (range: 62–96), 11 were men, one was vaccinated with two doses, 11 with one dose and six were unvaccinated. For the majority of the deceased case-patients, COVID-19 likely contributed to their death. All had an underlying condition requiring hospital treatment prior to COVID-19 infection. There were 45 HCW-cases in four healthcare facilities (the central hospital and three primary care facilities; Table 1). There were no hospitalisations or deaths among the HCW-cases. Secondary infections (n = 62) occurred also in community in close contacts of case-patients and HCW-cases.

At the time of the outbreak, 24 of 29 permanent HCW in Ward 1 were fully vaccinated with two doses of Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer, Mainz, Germany/New York, United States), two of 29 had received one dose of Comirnaty when preceded by laboratory-confirmed SARS-CoV-2 within 6 months, two of 29 had received one dose of Comirnaty, and one of 29 was unvaccinated. In Ward 2, all (17/17) permanent HCW were fully vaccinated with Comirnaty. Vaccinations were given at a 3-week interval until mid-February

Table 2.

Healthcare worker-cases and vaccination status by occupation, Tavastia Proper healthcare district, Finland, May 2021 (n = 45)

HCW occupation	Vaccinated[a]		Unvaccinated	Total
	2 doses	1 dose		
HCW with direct patient contact[b]	17	4	7	28
HCW students	0	0	8	8
Other staff[c]	1	2	6	9
Total	18	6	21	45

HCW: healthcare worker; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

[a] The vast majority of HCW (> 90%) were vaccinated with Comirnaty (BNT162b2 mRNA, BioNTech-Pfizer, Mainz, Germany/New York, United States).

[b] Includes 2 HCW with one dose of vaccine and previous laboratory-confirmed SARS-CoV-2 infection.

[c] Includes hospital cleaners and secretaries.

2021, and thereafter the interval was extended to 12 weeks.

There were a few patients who contracted COVID-19 who had stayed solely in their single or two-person room and were cared for by fully vaccinated HCW (with universal masking), suggesting transmission from a vaccinated HCW-case. All staff members in Wards 1, 2 and 3 were screened for SARS-CoV-2 by RT-PCR the week after outbreak detection; screening in Ward 4 on the other floor was done the following week after the detection of secondary cases. Five asymptomatic infections were identified among fully vaccinated staff members; one developed symptoms only after the positive RT-PCR screening test (CT value: 17) and four remained asymptomatic (CT values: 28, 32, 33 and 34, suggesting low infectivity [6]). There was no secondary transmission from the four identified asymptomatic fully vaccinated HCW.

There was high vaccine coverage among permanent staff in the central hospital, but lower for HCW in primary healthcare facilities and among students in training and staff members who had no direct patient contact (Table 2). This is in line with the strict national COVID-19 vaccination strategy during a period of limited vaccine supply, which emphasised the prioritisation of HCW based on their statute and job description in order to have more vaccines available for elderly people and medical risk groups.

Among the fully vaccinated, symptomatic HCW-cases in the central hospital (n = 8), there were five cases for whom we had follow-up data unconfounded by other exposures to evaluate secondary transmission. Two HCW-cases with symptoms transmitted the infection to their household contacts and patients and one who also infected a HCW colleague within 4 days from the symptom onset. One HCW-case with symptoms transmitted the infection only to a household contact nearly 2 weeks after the disease onset of the HCW-case. Two HCW-cases did not infect anyone. We were not able to obtain complete data for the secondary transmission from all case-patients.

Of all the identified HCW-cases, five remained completely asymptomatic. The remaining HCW (n = 36, information missing for n = 4) had at least mild symptoms that, for a few, developed after screening. At the very beginning of the outbreak in Ward 1 and 2 and later in Ward 4, there was transmission that we could not trace; this could only be explained by infected HCW, suggesting that we most likely were not able to identify all HCW cases with mild or no symptoms.

Infection control measures

In the central hospital, COVID-19 patients are cohorted in Ward 1, which has 28 beds in 14 rooms. Four isolation rooms are equipped with negative pressure, while for the other 10 rooms, incoming air enters from a common supply line through a room-specific pipe and outgoing air exits through a room-specific pipe to a common exit line. The air supply and exit lines for Ward 1 are separated from those of Ward 2 and Ward 3 located at the same floor. Some staff members, medical doctors, physiotherapists and cleaning staff are shared by Ward 1 and 2.

HCW used PPE (visor, long sleeved apron, gloves and surgical mask) in COVID-19 patients' care. FFP2/3 respirators were used in aerosol-generating procedures and intensive care. Surgical masks were used by HCW in all contexts with patient contact (i.e. universal masking). Visitors were only allowed if they were asymptomatic and they were advised in hand hygiene and surgical mask wearing. Patients were encouraged to use surgical masks.

Discussion

Since the start of the COVID-19 pandemic and as of 7 July 2021 in Finland, the cumulative number of laboratory-confirmed COVID-19 cases was 97,049 and that of COVID-19-related deaths was 976 (population: 5.5 million inhabitants). Before the outbreak described here, the 14-day incidence for laboratory-confirmed SARS-CoV-2 infections was 57 per 100,000 inhabitants for Tavastia Proper HD, and 52 cases per 100,000 inhabitants in the total Finnish population (source: NIDR). During the two weeks preceding the outbreak, 30% (856/2,848) of SARS-CoV-2-positive samples in Finland were sequenced (Supplementary data); of these, 57 were consistent with the Delta variant. Up to this point, there had only been one case of SARS-CoV-2 Delta variant in Tavastia Proper HD with a known transmission chain and no secondary transmission. Of note, at the start of the outbreak, vaccination coverage was the same in Tavastia Proper HD as in all of Finland i.e. 35% for the first dose and 4% for the second dose. The majority of those vaccinated were in the older age and medical risk groups.

This outbreak, which was caused by the SARS-CoV-2 Delta variant and led to 18 deaths in elderly people, occurred in four healthcare facilities despite the use of PPE, increasing vaccine coverage and universal masking by HCW. The technical department of the hospital did not deem possible that ventilation could explain the outbreak. Direct and indirect contact transmission are not considered to have an important role in SARS-CoV-2 transmission, compared to droplet transmission [7]. Pre-symptomatic yet infectious COVID-19 patients with varying incubation periods made it difficult to contain transmission both in the community and healthcare settings. It is possible that transmission occurred from asymptomatic/pre-symptomatic HCW to patients and other HCW. Although the first case-patients were diagnosed within 24 hours from symptom onset, the delay was enough for the outbreak to spread via patient transfer.

Breakthrough infections with the Delta variant and further transmission from fully vaccinated, symptomatic HCW occurred. Secondary transmission followed similar asymmetry as described with SARS-CoV-2 in unvaccinated individuals [8-10]. A recent study suggests reduced vaccine effectiveness of 36% against symptomatic disease caused by the Delta variant after one dose of Comirnaty vaccine [11], but excellent protection after full course; depending on disease severity, Comirnaty vaccine provides 88–92% protection against the Delta variant [11,12], and we saw similar rates among the fully vaccinated HCW in Ward 1.

To control the outbreak, surgical masks were replaced by FFP2 respirators when HCW are in close contact with a laboratory-confirmed COVID-19 patient, as supported by literature [10,13,14]. Previously FFP2/3 respirators were only used in aerosol-generating procedures and intensive care units. The Finnish national guidelines regarding use of PPE when treating patients with COVID-19 were changed accordingly based on ongoing discussion [15], even though we could not prove the airborne transmission during the outbreak. This was supported by the current good availability of FFP2 respirators.

In conclusion, this outbreak demonstrated that, despite full vaccination and universal masking of HCW, breakthrough infections by the Delta variant via symptomatic and asymptomatic HCW occurred, causing nosocomical infections. As the Delta variant continues to spread in Europe, we suggest that utilization of FFP2/3 respirators while treating COVID-19 patients should be included in national guidelines.

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Conflict of interest

None declared.

Authors' contributions

Iivo Hetemäki was responsible for collecting the data, writing the first draft of the manuscript and editing the manuscript during the writing process. Sohvi Kääriäinen was responsible for editing the manuscript during the whole writing process, communicating between the authors and collecting data from different sources. Pirjo Alho was responsible for coordinating the outbreak investigation, collecting data and commenting the manuscript. Janne Mikkola was responsible for coordinating the outbreak investigation, collecting data and commenting the manuscript. Niina Ikonen was responsible for laboratory methodology and results and commenting the manuscript. Carita Savolainen-Kopra was responsible for laboratory methodology and results and commenting the manuscript. Hanna Nohynek was responsible for commenting and discussing the vaccinology related parts. Outi Lyytikäinen was supervising the guiding the writing process as well as revising the manuscript.

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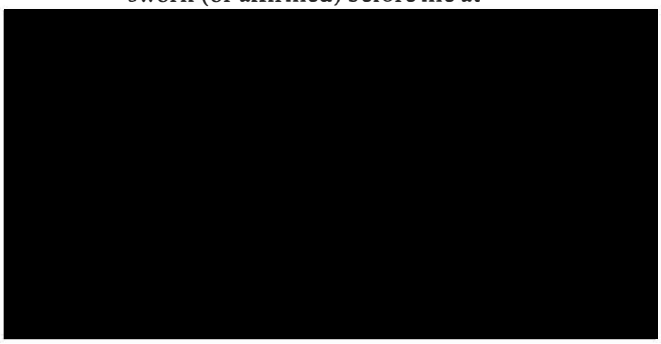
Exhibit "R"

This is **Exhibit "R" r**eferred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at



Outbreak of SARS-CoV-2 Infections, Including COVID-19 Vaccine Breakthrough Infections, Associated with Large Public Gatherings — Barnstable County, Massachusetts, July 2021

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On July 30, 2021, this report was posted as an MMWR *Early Release on the* MMWR *website (https://www.cdc.gov/mmwr).*

During July 2021, 469 cases of COVID-19 associated with multiple summer events and large public gatherings in a town in Barnstable County, Massachusetts, were identified among Massachusetts residents; vaccination coverage among eligible Massachusetts residents was 69%. Approximately three quarters (346; 74%) of cases occurred in fully vaccinated persons (those who had completed a 2-dose course of mRNA vaccine [Pfizer-BioNTech or Moderna] or had received a single dose of Janssen [Johnson & Johnson] vaccine ≥14 days before exposure). Genomic sequencing of specimens from 133 patients identified the B.1.617.2 (Delta) variant of SARS-CoV-2, the virus that causes COVID-19, in 119 (89%) and the Delta AY.3 sublineage in one (1%). Overall, 274 (79%) vaccinated patients with breakthrough infection were symptomatic. Among five COVID-19 patients who were hospitalized, four were fully vaccinated; no deaths were reported. Real-time reverse transcription–polymerase chain reaction (RT-PCR) cycle threshold (Ct) values in specimens from 127 vaccinated persons with breakthrough cases were similar to those from 84 persons who were unvaccinated, not fully vaccinated, or whose vaccination status was unknown (median = 22.77 and 21.54, respectively). The Delta variant of SARS-CoV-2 is highly transmissible (*1*); vaccination is the most important strategy to prevent severe illness and death. On July 27, CDC recommended that all persons, including those who are fully vaccinated, should wear masks in indoor public settings in areas where COVID-19 transmission is high or substantial.* Findings from this investigation suggest that even jurisdictions without substantial or high COVID-19 transmission might consider expanding prevention strategies, including masking in indoor public settings regardless of vaccination status, given the potential risk of infection during attendance at large public gatherings that include travelers from many areas with differing levels of transmission.

During July 3–17, 2021, multiple summer events and large public gatherings were held in a town in Barnstable County, Massachusetts, that attracted thousands of tourists from across the United States. Beginning July 10, the Massachusetts Department of Public Health (MA DPH) received reports of an increase in COVID-19 cases among persons who reside in or recently visited Barnstable County, including in fully vaccinated persons. Persons with COVID-19 reported attending densely packed indoor and outdoor events at venues that included bars, restaurants, guest houses, and rental homes. On July 3, MA DPH had reported a 14-day average COVID-19 incidence of zero cases per 100,000 persons per day in residents of the town in Barnstable County; by July 17, the 14-day average incidence increased to 177 cases per 100,000 persons per day in residents of the town (*2*).

During July 10–26, using travel history data from the state COVID-19 surveillance system, MA DPH identified a cluster of cases among Massachusetts residents. Additional cases were identified by local health jurisdictions through case investigation. COVID-19 cases were matched with the state immunization registry. A cluster-associated case was defined as receipt of a positive SARS-CoV-2 test (nucleic acid amplification or antigen) result ≤14 days after travel to or residence in the town in Barnstable County since July 3. COVID-19 vaccine breakthrough cases were those in fully vaccinated Massachusetts residents (those with documentation from the state immunization registry of completion of COVID-19 vaccination as recommended by the Advisory Committee on Immunization Practices,† ≥14 days before exposure). Specimens were submitted for whole genome sequencing§ to either the Massachusetts State Public Health Laboratory or the Broad Institute of the Massachusetts Institute of

* https://www.cdc.gov/coronavirus/2019-ncov/vaccines/fully-vaccinated.html

† As of May 2021, ACIP recommended that all adults aged ≥18 years receive any of the three COVID-19 vaccines available in the United States via Emergency Use Authorization from the Food and Drug Administration, including Pfizer-BioNTech, Moderna, and Janssen; persons aged ≥12 years are eligible to receive the Pfizer-BioNTech COVID-19 vaccine. Full vaccination is defined as receipt of 2 doses of the Pfizer-BioNTech or Moderna COVID-19 vaccines or 1 dose of Janssen COVID-19 vaccine ≥14 days before exposure.

§ Genomic sequencing was performed using Illumina NovaSeq using the NEB LunaScript RT ARTIC SARS-CoV-2 Kit. Novel mutations were not identified in the spike protein of the cluster-associated genomes compared with genomes collected during the same period from ongoing genomic surveillance efforts at Broad Institute. Raw and assembled genomic data are publicly available under NCBI BioProject PRJNA715749.

Summary

What is already known about this topic?

Variants of SARS-CoV-2 continue to emerge. The B.1.617.2 (Delta) variant is highly transmissible.

What is added by this report?

In July 2021, following multiple large public events in a Barnstable County, Massachusetts, town, 469 COVID-19 cases were identified among Massachusetts residents who had traveled to the town during July 3–17; 346 (74%) occurred in fully vaccinated persons. Testing identified the Delta variant in 90% of specimens from 133 patients. Cycle threshold values were similar among specimens from patients who were fully vaccinated and those who were not.

What are the implications for public health practice?

Jurisdictions might consider expanded prevention strategies, including universal masking in indoor public settings, particularly for large public gatherings that include travelers from many areas with differing levels of SARS-CoV-2 transmission.

Technology and Harvard University. Ct values were obtained for 211 specimens tested using a noncommercial real-time RT-PCR panel for SARS-CoV-2 performed under Emergency Use Authorization at the Broad Institute Clinical Research Sequencing Platform. On July 15, MA DPH issued the first of two Epidemic Information Exchange notifications to identify additional cases among residents of U.S. jurisdictions outside Massachusetts associated with recent travel to the town in Barnstable County during July 2021. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC policy.[¶]

By July 26, a total of 469 COVID-19 cases were identified among Massachusetts residents; dates of positive specimen collection ranged from July 6 through July 25 (Figure 1). Most cases occurred in males (85%); median age was 40 years (range = <1–76 years). Nearly one half (199; 42%) reported residence in the town in Barnstable County. Overall, 346 (74%) persons with COVID-19 reported symptoms consistent with COVID-19.[**] Five were hospitalized; as of July 27, no deaths were reported. One hospitalized patient (age range = 50–59 years) was not vaccinated and had multiple underlying medical conditions.[††] Four additional, fully vaccinated patients[§§] aged 20–70 years were also hospitalized, two of whom had underlying medical conditions. Initial genomic sequencing of specimens from 133 patients identified the Delta variant in 119 (89%) cases and the Delta AY.3 sublineage in one (1%) case; genomic sequencing was not successful for 13 (10%) specimens.

Among the 469 cases in Massachusetts residents, 346 (74%) occurred in persons who were fully vaccinated; of these, 301 (87%) were male, with a median age of 42 years. Vaccine products received by persons experiencing breakthrough infections were Pfizer-BioNTech (159; 46%), Moderna (131; 38%), and Janssen (56; 16%); among fully vaccinated persons in the Massachusetts general population, 56% had received Pfizer-BioNTech, 38% had received Moderna, and 7% had received Janssen vaccine products. Among persons with breakthrough infection, 274 (79%) reported signs or symptoms, with the most common being cough, headache, sore throat, myalgia, and fever. Among fully vaccinated symptomatic persons, the median interval from completion of ≥14 days after the final vaccine dose to symptom onset was 86 days (range = 6–178 days). Among persons with breakthrough infection, four (1.2%) were hospitalized, and no deaths were reported. Real-time RT-PCR Ct values in specimens from 127 fully vaccinated patients (median = 22.77) were similar to those among 84 patients who were unvaccinated, not fully vaccinated, or whose vaccination status was unknown (median = 21.54) (Figure 2).

Transmission mitigation measures included broadening testing recommendations for persons with travel or close contact with a cluster-associated case, irrespective of vaccination status; local recommendations for mask use in indoor settings, irrespective of vaccination status; deployment of state-funded mobile testing and vaccination units in the town in Barnstable County; and informational outreach to visitors and residents. In this tourism-focused community, the Community Tracing Collaborative[¶¶] conducted outreach to hospitality workers, an international workforce requiring messaging in multiple languages.

The call from MA DPH for cases resulted in additional reports of cases among residents of 22 other states who had traveled to the town in Barnstable County during July 3–17, as well as reports of secondary transmission; further analyses are ongoing. As of July 3, estimated COVID-19 vaccination coverage among the eligible population in Massachusetts was 69% (*3*). Further investigations and characterization of breakthrough infections and vaccine effectiveness among this highly vaccinated population are ongoing.

[¶] 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. Sect.241(d); 5 U.S.C. Sect.552a; 44 U.S.C. Sect.3501 et seq.

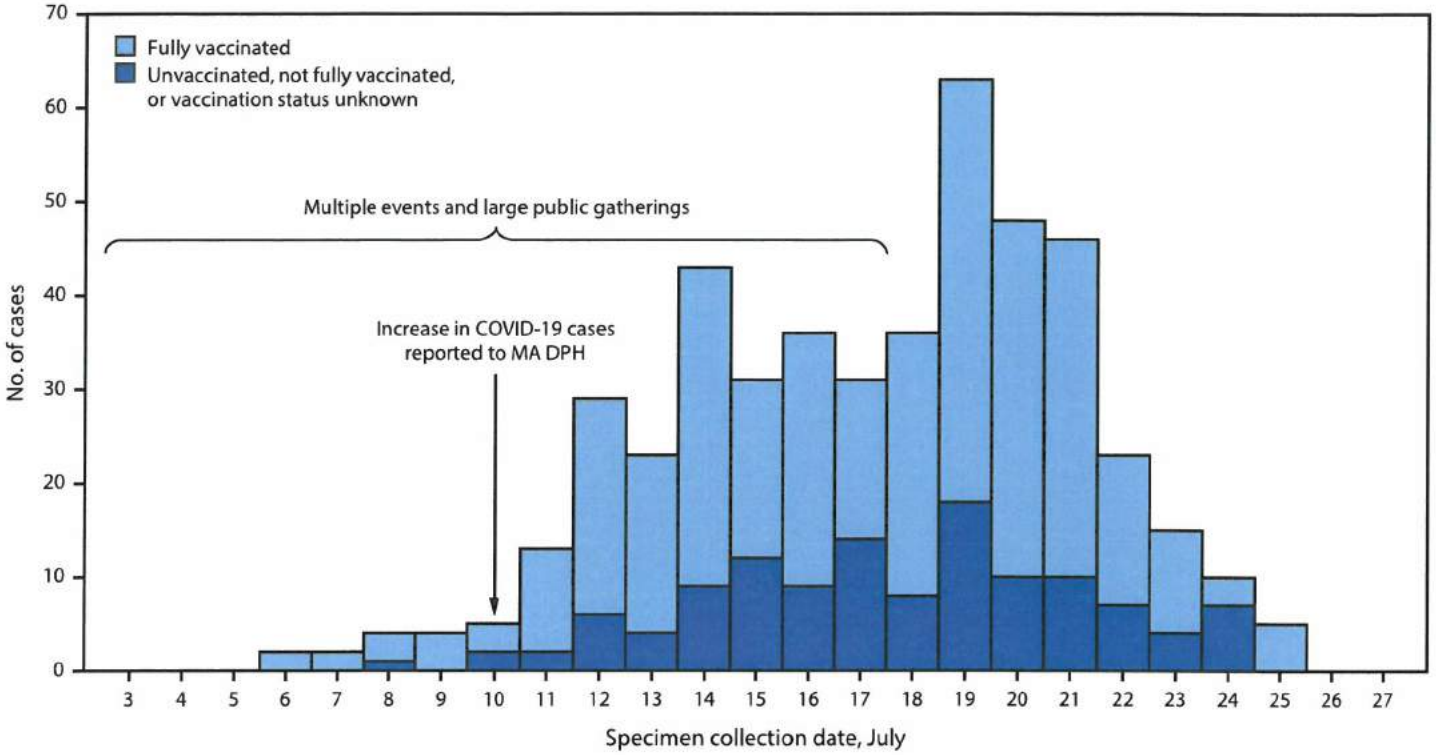
[**] COVID-like symptoms were based on the Council of State and Territorial Epidemiologists surveillance case definition for COVID-19. https://ndc.services.cdc.gov/case-definitions/coronavirus-disease-2019-2020-08-05/

[††] https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html

[§§] One vaccinated, hospitalized COVID-19 patient had received the Pfizer-BioNTech vaccine and three had received the Janssen vaccine.

[¶¶] The Community Tracing Collaborative is a multiorganization partnership that has supported COVID contact tracing and outbreak investigation in Massachusetts. https://www.mass.gov/info-details/learn-about-the-community-tracing-collaborative

FIGURE 1. SARS-CoV-2 infections (N = 469) associated with large public gatherings, by date of specimen collection and vaccination status* — Barnstable County, Massachusetts, July 2021



Abbreviation: MA DPH = Massachusetts Department of Public Health.

* Fully vaccinated was defined as ≥14 days after completion of state immunization registry–documented COVID-19 vaccination as recommended by the Advisory Committee on Immunization Practices.

Discussion

The SARS-CoV-2 Delta variant is highly transmissible (*1*), and understanding determinants of transmission, including human behavior and vaccine effectiveness, is critical to developing prevention strategies. Multipronged prevention strategies are needed to reduce COVID-19–related morbidity and mortality (*4*).

The findings in this report are subject to at least four limitations. First, data from this report are insufficient to draw conclusions about the effectiveness of COVID-19 vaccines against SARS-CoV-2, including the Delta variant, during this outbreak. As population-level vaccination coverage increases, vaccinated persons are likely to represent a larger proportion of COVID-19 cases. Second, asymptomatic breakthrough infections might be underrepresented because of detection bias. Third, demographics of cases likely reflect those of attendees at the public gatherings, as events were marketed to adult male participants; further study is underway to identify other population characteristics among cases, such as additional demographic characteristics and underlying health conditions including immunocompromising conditions.***

MA DPH, CDC, and affected jurisdictions are collaborating in this response; MA DPH is conducting additional case investigations, obtaining samples for genomic sequencing, and linking case information with laboratory data and vaccination history. Finally, Ct values obtained with SARS-CoV-2 qualitative RT-PCR diagnostic tests might provide a crude correlation to the amount of virus present in a sample and can also be affected by factors other than viral load.††† Although the assay used in this investigation was not validated to provide quantitative results, there was no significant difference between the Ct values of samples collected from breakthrough cases and the other cases. This might mean that the viral load of vaccinated and unvaccinated persons infected with SARS-CoV-2 is also similar. However, microbiological studies are required to confirm these findings.

Event organizers and local health jurisdictions should continually assess the need for additional measures, including limiting capacity at gatherings or event postponement, based on current rates of COVID-19 transmission, population vaccination coverage, and other factors.§§§ On July 27, CDC released

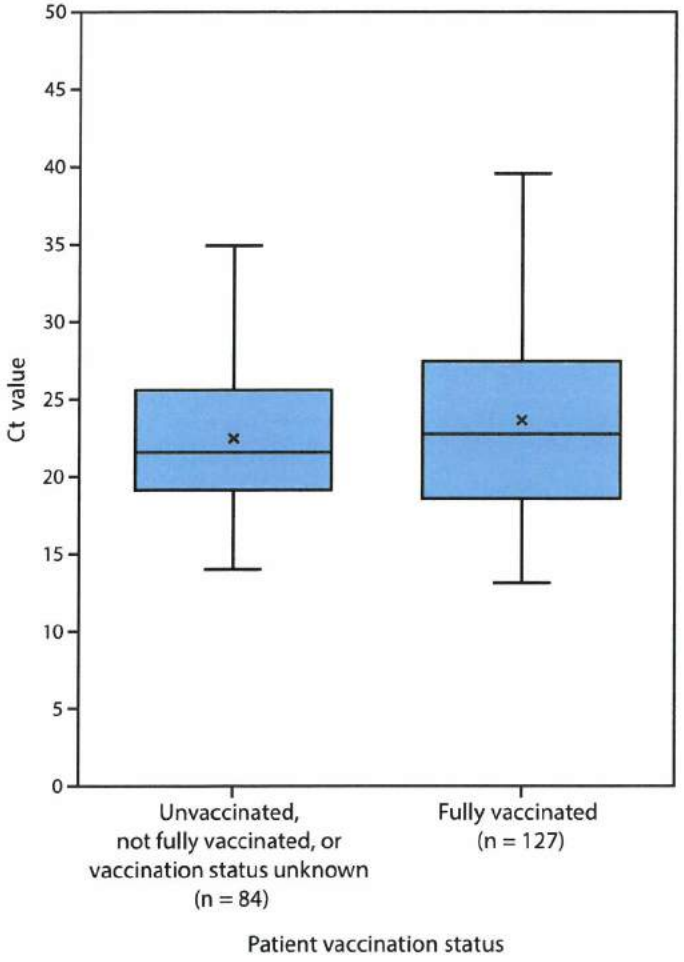
*** A preliminary analysis matching cluster-associated COVID-19 cases with the state HIV case surveillance data identified 30 (6%) cases with verified HIV infection; all were virally suppressed, and none were hospitalized as a result of infection with SARS-CoV-2.

††† https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html

§§§ https://www.cdc.gov/coronavirus/2019-ncov/community/large-events/considerations-for-events-gatherings.html

recommendations that all persons, including those who are fully vaccinated, should wear masks in indoor public settings in areas where COVID-19 transmission is high or substantial. Findings from this investigation suggest that even jurisdictions without substantial or high COVID-19 transmission might consider expanding prevention strategies, including masking in indoor public settings regardless of vaccination status, given the potential risk of infection during attendance at large public gatherings that include travelers from many areas with differing levels of transmission.

FIGURE 2. SARS-CoV-2 real-time reverse transcription–polymerase chain reaction cycle threshold values* for specimens from patients with infections associated with large public gatherings, by vaccination status† — Barnstable County, Massachusetts, July 2021§



Abbreviations: Ct = cycle threshold; RT-PCR = reverse transcription–polymerase chain reaction.

* Specimens were analyzed using a noncommercial real-time RT-PCR panel for SARS-CoV-2 performed under Emergency Use Authorization at the Clinical Research Sequencing Platform, Broad Institute of the Massachusetts Institute of Technology and Harvard University.

† Fully vaccinated was defined as ≥14 days after completion of state immunization registry–documented COVID-19 vaccination as recommended by the Advisory Committee on Immunization Practices.

§ Whiskers represent minimum and maximum observations; top of box represents the third quartile (Q3), bottom represents the first quartile (Q1), and box height represents the interquartile range. Midline is the median; "x" is the mean.

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Exhibit "S"

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

November 05, 2021

SENT VIA EMAIL

Elizabeth Brehm
Attorney
Siri & Glimstad
200 Park Avenue, 17th Floor
New York, New York 10166
foia@sirillp.com

This is **Exhibit "S"** referred to in the
Affidavit of Nadr Jomha
sworn (or affirmed) before me at
[redacted]
Barrister & Solicitor

2nd Letter Subject: Final Response Letter

Dear Ms. Brehm:

The Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) received your September 02, 2021, Freedom of Information Act (FOIA) request on September 02, 2021, seeking:

> "Documents reflecting any documented case of an individual who: (1) never received a COVID-19 vaccine; (2) was infected with COVID-19 once, recovered, and then later became infected again; and (3) transmitted SARS-CoV-2 to another person when reinfected."

A search of our records failed to reveal any documents pertaining to your request. The CDC Emergency Operations Center (EOC) conveyed that this information is not collected.

You may contact our FOIA Public Liaison at 770-488-6277 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

If you are not satisfied with the response to this request, you may administratively appeal by writing to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, Hubert H. Humphrey Building, 200 Independence Avenue, Suite 729H, Washington, D.C. 20201. You may also transmit your appeal via email to FOIARequest@psc.hhs.gov. Please mark both your appeal letter and envelope "FOIA Appeal." Your appeal must be postmarked or electronically transmitted by February 03, 2022.

Sincerely,

Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
Phone: (770) 488-6399
Fax: (404) 235-1852

#21-02152-FOIA

Exhibit "T"

Transmission potential of vaccinated and unvaccinated persons infected with the SARS-CoV-2 Delta variant in a federal prison, July—August 2021

Phillip P. Salvatore, PhD, SM – CDC COVID-19 Response Team
Christine C. Lee, PhD – CDC COVID-19 Response Team; Laboratory Leadership Service
Sadia Sleweon, MPH – CDC COVID-19 Response Team
David W. McCormick, MD, MPH – CDC COVID-19 Response Team; Epidemic Intelligence Service
Lavinia Nicolae, PhD – CDC COVID-19 Response Team
Kristen Knipe – CDC COVID-19 Response Team
Thomas Dixon – Bureau of Prisons, U.S. Department of Justice
Robert Banta, MSN – Bureau of Prisons, U.S. Department of Justice
Isaac Ogle, MSN – Bureau of Prisons, U.S. Department of Justice
Cristen Young – Bureau of Prisons, U.S. Department of Justice
Charles Dusseau – Bureau of Prisons, U.S. Department of Justice
Shawn Salmonson – Bureau of Prisons, U.S. Department of Justice
Charles Ogden, MPH – Bureau of Prisons, U.S. Department of Justice
Eric Godwin – Bureau of Prisons, U.S. Department of Justice
TeCora Ballom, DO – Bureau of Prisons, U.S. Department of Justice
Tara Ross – Bureau of Prisons, U.S. Department of Justice
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Hannah Browne – CDC COVID-19 Response Team
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Liesl M. Hagan, MPH – CDC COVID-19 Response Team

Disclaimer. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of Centers for Disease Control and Prevention (CDC).

Abstract

Background

The extent to which vaccinated persons who become infected with SARS-CoV-2 contribute to transmission is unclear. During a SARS-CoV-2 Delta variant outbreak among incarcerated persons with high vaccination rates in a federal prison, we assessed markers of viral shedding in vaccinated and unvaccinated persons.

Methods

Consenting incarcerated persons with confirmed SARS-CoV-2 infection provided mid-turbinate nasal specimens daily for 10 consecutive days and reported symptom data via questionnaire. Real-time reverse transcription-polymerase chain reaction (RT-PCR), viral whole genome sequencing, and viral culture was performed on these nasal specimens. Duration of RT-PCR positivity and viral culture positivity was assessed using survival analysis.

Results

A total of 978 specimens were provided by 95 participants, of whom 78 (82%) were fully vaccinated and 17 (18%) were not fully vaccinated. No significant differences were detected in duration of RT-PCR positivity among fully vaccinated participants (median: 13 days) versus those not fully vaccinated (median: 13 days; p=0.50), or in duration of culture positivity (medians: 5 days and 5 days; p=0.29). Among fully vaccinated participants, overall duration of culture positivity was shorter among Moderna vaccine recipients versus Pfizer (p=0.048) or Janssen (p=0.003) vaccine recipients.

Conclusions

As this field continues to develop, clinicians and public health practitioners should consider vaccinated persons who become infected with SARS-CoV-2 to be no less infectious than unvaccinated persons. These findings are critically important, especially in congregate settings where viral transmission can lead to large outbreaks.

Introduction

COVID-19 vaccines are highly effective in preventing severe illness and death from SARS-CoV-2 (the virus that causes COVID-19). However, because COVID-19 vaccines are not 100% effective in preventing infection, some infections among vaccinated persons are expected to occur. As global vaccination coverage increases, the role of vaccinated persons in transmission will be a critical determinant of the pandemic's future trajectory.[1] The extent to which vaccinated persons who become infected contribute to transmission of SARS-CoV-2, including the B.1.617.2 (Delta) variant, is not yet well understood. Some preprint manuscripts have reported comparable indicators of transmission potential regardless of vaccination status,[2] while others have reported reduced viability of virus isolated from vaccinated persons.[3]

The Delta variant has been associated with a peak in COVID-19 cases in the United States beginning in July 2021 that included large outbreaks among vaccinated and unvaccinated persons in crowded settings.[4-6] These findings are of particular concern for congregate living environments such as correctional and detention facilities and long-term care facilities because of the potential for rapid transmission of SARS-CoV-2 and the high prevalence of underlying health conditions associated with severe COVID-19.[7-9]

In a recent outbreak involving the Delta variant in a federal prison in Texas, the cumulative incidence of infection in two affected housing units was 74%; it was 93% and 70% among unvaccinated and vaccinated incarcerated persons, respectively.[6] Using serial mid-turbinate nasal specimens collected from a subset of incarcerated persons infected during this outbreak, this report assesses reverse transcription-polymerase chain reaction (RT-PCR) and viral culture characteristics as surrogate markers of transmission potential among persons fully vaccinated and those not fully vaccinated over time. This report is one of the first longitudinal investigations of viral shedding from vaccinated persons infected

with the Delta variant and contributes to the evidence base guiding infection prevention and control procedures across a variety of settings.

Methods

Investigational Setting

On July 12, 2021, an outbreak of SARS-CoV-2 among vaccinated and unvaccinated persons was detected in a federal prison in Texas. Staff from the Centers for Disease Control and Prevention (CDC) and Federal Bureau of Prisons (BOP) deployed to the prison to investigate the outbreak as previously reported.[6] As part of this outbreak investigation, a subset of incarcerated persons provided serial mid turbinate nasal specimens which were analyzed to evaluate the potential role of infected vaccinated and unvaccinated persons in transmission of SARS-CoV-2. This activity was reviewed and approved by the BOP Research Review Board and CDC and conducted consistent with applicable federal law and CDC policy.*

Participant Enrollment and Serial Specimen Collection

Incarcerated persons living in four housing units where COVID-19 cases had been identified were invited to participate in serial swabbing. Persons were eligible to enroll if they had tested positive for SARS-CoV-2 between July 12 (the start of the outbreak) and August 4, 2021. CDC and BOP staff held information sessions to explain the purpose of the project and to answer questions, including privacy protections and how results of the study would be made available to participants. All persons choosing to participate signed informed consent forms, which were provided in English and Spanish.

Specimen collection occurred during July 18—August 9, 2021. CDC and BOP staff collected one nasal mid-turbinate specimen daily for 10 consecutive days from participants who had tested positive,

beginning on July 19 or, for cases identified after July 19, beginning on the date of participants' first positive test. All incarcerated persons residing in housing units where cases were identified were placed under quarantine precautions. To assist in case-finding, consenting persons who were quarantined were tested every other day beginning on July 19 or on their first full day of quarantine; those who tested positive during quarantine were invited to participate in the 10 consecutive days of specimen collection. All participants were asked to provide a specimen on August 6 to provide data additional data on viral shedding, which corresponds to a late timepoint in infection for most participants (Figure 1).

On the tenth day of specimen collection, participants were asked to complete a paper-based questionnaire to report COVID-19-like symptoms during the course of their illness, including date of symptom onset and symptom duration. Information on demographic characteristics, COVID-19 vaccination history, previous positive SARS-CoV-2 diagnostic tests, and underlying medical conditions was extracted from BOP electronic medical records for all participants.

Laboratory Methods

Specimens were collected using nylon flocked minitip swabs, transferred into universal viral transport media (VTM) (Becton Dickinson, Franklin Lakes, NJ) immediately stored at 2-8°C and frozen at -20°C or colder within 72 hours, and sent to CDC for RT-PCR testing using the CDC Influenza SARS-CoV-2 Multiplex Assay. Remnant aliquots were stored at -70°C or below for viral culture. Due to capacity limitations, viral culture was performed on a subset of collected specimens. Specimens were included for viral culture if they had been collected 0, 3, 5, 7, or 9 days since onset and had an accompanying positive RT-PCR test with cycle threshold (Ct) value less than 35. For verification that this selected Ct cutoff did not exclude specimens containing culturable virus, viral culture was also performed on 25 of 102 specimens with Ct>35. (25/25 of these specimens were culture negative.) For more granular detail across the time-course of infection, viral culture was also performed on a subset of

specimens collected on other days (see Supplemental Figures 1-2 for details on specimens included for viral culture).

Specimens selected for culture were used to perform limiting-diluting inoculation of Vero CCL-81 cells expressing TMPRSS2, and cultures showing evidence of cytopathic effect were tested by RT-PCR for the presence of SARS-CoV-2 RNA. Viral recovery was as previously described.[10] Whole genome sequencing (WGS) was performed for one RT-PCR-positive specimen per participant with Ct less than 30 (per sequencing laboratory standard protocols).

Statistical Methods

Onset (used as time 0 in longitudinal analyses below) was defined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19,[11] or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. In two instances where a participant without symptoms had an initial positive test followed by at least 3 negative tests before subsequent positive tests, the date of second positive test was used.

Participants were considered fully vaccinated if ≥14 days had elapsed since they had completed all recommended doses of a COVID-19 primary vaccine series before the start of the outbreak. (No participant had completed a primary vaccine series <14 days before the outbreak.) Participants were considered not fully vaccinated if they had not received any doses of a vaccine or if they had not completed all doses of a vaccine series. Demographic characteristics of participants stratified by vaccination status were assessed using Fisher's exact tests.

Three surrogate markers for assessing transmission potential were analyzed as primary outcomes: RT-PCR positivity (an indicator of current/recent infection), RT-PCR Ct value (a semi-quantitative indicator of relative level of viral nucleic acid), and viral culture positivity (an indicator of viable/infectious virus). Dichotomous laboratory results (RT-PCR positivity and viral culture positivity)

were analyzed longitudinally with time 0 defined as the date of onset and the primary endpoints defined by a participant's last positive test. Specimens for which viral culture was not performed were presumed to be culture negative if an accompanying RT-PCR test was negative or was positive with Ct>35. To account for variation in the interval between onset and enrollment, and intermittent participation in specimen collection by some participants (which can result in interval and right censoring), survival analyses were performed using Turnbull estimation using the "interval" package implementation in R.[12] Hypothesis testing of survival functions was performed using the generalized Wilcoxon-Mann-Whitney method for interval-censored data.

As a post-hoc evaluation of potential interactions between vaccination status and known prior SARS-CoV-2 infections, a stratified analysis was conducted using Fisher's exact test to compare RT-PCR and viral culture results across these two variables among specimens collected on days with complete viral culture coverage (0, 3, 5, 7, and 9 days since onset).

Non-dichotomous laboratory results (RT-PCR Ct values) were characterized by days since onset using medians and interquartile ranges (IQRs). Because Ct values are semi-parametric, distributions were compared non-parametrically using the Mann-Whitney U test with ties (for dichotomous variables) or the Kruskal-Wallis test (for categorical variables with more than 2 levels); negative RT-PCR results were assigned higher ranks than any Ct value from positive RT-PCR results. To account for multiple hypothesis testing across days, α thresholds were adjusted using Bonferroni correction. All hypothesis tests performed are detailed in Supplementary Tables 1 and 2. All statistical analyses were performed in R version 4.0.2 (R Core Team, Vienna, Austria).

Results

Population Characteristics

Among 189 persons with SARS-CoV-2 infection eligible to enroll, a total of 96 persons consented to participate in serial specimen collection; one participant had a single positive diagnostic test (Ct=36.2) followed by seven negative diagnostic tests and reported no symptoms and was excluded as a non-case. Of the 95 included participants, 78 (82%) were documented as being fully vaccinated against SARS-CoV-2, 15 (16%) were unvaccinated and 2 (2%) were partially vaccinated and categorized as not fully vaccinated in further analyses (Table 1). Among fully vaccinated participants, a majority (57/78, 73%) received the Pfizer vaccine; smaller proportions received the Moderna vaccine (14/78, 18%) or Janssen vaccine (7/78, 9%). A majority (47/78, 60%) of fully vaccinated participants completed their vaccination series more than 120 days prior to the start of the outbreak (IQR: 81-140 days prior to start). Recipients of Pfizer vaccines completed their series earlier (IQR: 131-131 days) than recipients of Moderna (IQR: 81-82 days prior to start) or Janssen (IQR: 46-70 days prior to start) vaccines ($p<0.001$). A small number of participants (2/78 fully vaccinated, 3%, and 2/17 not fully vaccinated, 12%, $p=0.10$) had a documented prior SARS-CoV-2 infection. Based on symptom self-report at the end of sampling, 76% of participants reported at least one symptom in the COVID-19 case definition [CSTE 2021]. The most commonly reported symptoms were runny or stuffy nose (58%), loss of smell or taste (54%), and cough (45%). Of 95 specimens from 95 participants for which sequencing was attempted, 64 were successfully sequenced and passed quality metrics; all 64 (100%) belonged to the B.1.617.2 (Delta) lineage and AY.3 sublineage.

RT-PCR Positivity

From the 95 included participants, 978 specimens were collected for RT-PCR testing (825/978, 84% from fully vaccinated participants). Specimens were collected ranging from 13 days prior to onset (among participants tested during quarantine prior to diagnosis) to 32 days following onset. See Figure 1 for a diagrammatic representation of RT-PCR specimen collection from participants, and see

Supplemental Figure 1 for details of specimen collection by day since onset (stratified by vaccination status). A median of 6 days elapsed between onset and enrollment among fully vaccinated participants, compared with a median of 7 days among participants who were not fully vaccinated (p=0.33). Overall, 499 of the 978 (51%) specimens tested positive by RT-PCR.

No significant differences in time to last RT-PCR positive test were found. Median duration of RT-PCR positivity was 13 days among fully vaccinated participants versus 13 days among participants who were not fully vaccinated (p=0.50; Figure 2); and 10 days among participants with known history of prior SARS-CoV-2 infection (regardless of vaccination) versus 13 days among participants without any known prior infection (p=0.12). Among fully vaccinated participants, median duration of positivity was 10 days among Moderna vaccine recipients versus 13 days among Pfizer recipients and 13 days among Janssen recipients (p=0.39); and 13 days among participants fully vaccinated more than 120 days prior to the outbreak versus 11 days among participants vaccinated 120 days or less prior to the outbreak (p=0.32).

Ct Values

Ct values from specimens testing positive by RT-PCR increased with the number of days since onset (Figure 3). Among specimens from fully vaccinated participants, Ct values increased from a median of 26.4 (IQR: 23.5-28.4) on the day of onset to a median of 32.9 on day 10 (IQR: 30.5-34.6), while Ct values from specimens from participants who were not fully vaccinated increased from a median of 28.5 (IQR:24.8-31.8) on the day of onset to a median of 34.5 on day 10 (IQR: 29.4-35.2). Across the time-course of infection, no statistically significant difference was observed among Ct values by vaccination status on any day after Bonferroni correction (all p>0.0026, the Bonferroni-corrected α threshold). Additionally, no significant differences were observed among Ct values when stratified by vaccine product, time since vaccination, or known prior SARS-CoV-2 infection. While not statistically significant,

lower Ct values were observed early in the time-course of infection among Janssen vaccine recipients (day 3 median: 17.9; IQR: 17.6-19.4) than among Moderna (day 3 median: 27.4; IQR: 23.7-28.1) or Pfizer recipients (day 3 median: 24.8; IQR: 23.1-26.8; p=0.016 while Bonferroni α=0.0026).

Viral Culture Positivity

Of the 978 specimens collected, viral culture was performed on 286 (29%); an additional 556 (57%) were included as presumptive negative viral culture results due to an accompanying negative RT-PCR test (n=479) or a positive RT-PCR test with a Ct value greater than 35 (n=77). Viral culture capture by day since onset stratified by vaccination status is detailed in Supplementary Figure 2. Among the 842 specimens with a viral culture result, 75 (9%) had a positive viral culture. Virus was recovered from 57/690 (8%) of specimens from fully vaccinated participants, compared with 18/152 (12%) of specimens from participants who were not fully vaccinated (p=0.16).

No statistically significant difference was detected in the duration of viral culture positivity (Figure 4) between participants who were fully vaccinated (median: 5 days) compared with those who were not fully vaccinated (median: 5 days; p=0.29). (Viral culture results are illustrated as a function of days since onset and grouped by RT-PCR result in Supplementary Figure 4). Cumulative hazard functions indicate overall shorter culture positivity for fully vaccinated participants who received the Moderna vaccine than those who received Pfizer (p=0.048) or Janssen vaccines (p=0.003), but there was no significant difference between recipients of Pfizer and Janssen vaccines (p=0.12). No statistically significant differences in duration of culture positivity were detected when stratified according to time since vaccination (p=0.79) or known prior infection (p=0.99).

Factorial Stratification: Vaccination Status and History of Prior Infection

Figure 5 illustrates a post-hoc stratification of RT-PCR and viral culture results by vaccination status and prior SARS-CoV-2 infection. No statistically significant difference in RT-PCR or viral culture positivity was detected on any day; however, bivariate stratification resulted in small population sizes in some groups (n=2 participants each for those fully vaccinated with a known prior infection and those not fully vaccinated with a known prior infection), which limits the ability to draw conclusions about these groups.

Discussion

During a high-transmission outbreak of the SARS-CoV-2 Delta variant in a prison setting, we failed to find different durations of RT-PCR positivity, Ct values, or durations of viral culture positivity in fully vaccinated persons compared with persons who were not fully vaccinated. However, vaccinated persons who received the Moderna vaccine had a shorter duration of culture positivity compared with Pfizer or Janssen vaccine recipients. (However, Moderna vaccine recipients also were more recently vaccinated than Pfizer vaccine recipients.) Collectively, our findings suggest that, as evidence continues to emerge in this developing field, vaccinated persons who become infected should be regarded as not significantly less infectious than unvaccinated persons for the purposes of public health action.

As viral infections in vaccinated persons can result from either a failure to mount a protective immune response following initial vaccination or a gradual waning of immunological protection following initially robust protection, the infectiousness of vaccinated persons may be variable. It is plausible that some participants in this investigation who became infected despite vaccination had weak or waning vaccine-induced protection and were therefore similar to unvaccinated persons in the markers of transmission potential that we evaluated.

This report adds to a limited body of scientific literature evaluating the transmission potential of SARS-CoV-2 infections in vaccinated persons. Reports of infections in vaccinated persons have found mixed results using markers of transmission potential, and no longitudinal studies of viral culture characteristics in vaccinated persons with Delta infections have been published. A multi-site serial testing investigation involving Alpha (B.1.1.7) and Gamma (P.1) infections found that duration of culture positivity was shorter among vaccinated persons compared with unvaccinated persons.[13, 14] One report using surveillance data found lower Ct values among unvaccinated persons, but this difference was only observed for two of three RT-PCR probes and only during one of three months.[15] One cross-sectional report found no difference in Ct value by vaccination status.[2] However, extrapolating from cross-sectional and surveillance data may be challenging without data to account for timing of specimen collection in the course of infection. Nevertheless, this finding is corroborated by analysis of a clinical convenience sample which found vaccination did not impact Ct values and reduced viral recovery of Alpha variant but did not reduce recovery of Delta variant virus;[16] similar findings were mirrored by two retrospective health-system cohorts.[17, 18] A report of health system workers found that viral culture positivity was reduced in vaccinated persons despite similar Ct values as those in unvaccinated persons.[3] A separate report found that early in the clinical course of infection, Ct values were comparable between vaccinated and unvaccinated persons, but among individuals who presented to care later in their course of illness, Ct values were higher in vaccinated persons.[19] A study of household transmission of Delta infections found similar peak viral loads regardless of vaccination status, but noted faster declines in vaccinated persons.[20] Cumulatively, available data have not clearly or consistently identified markers of reduced transmission potential in vaccinated persons with SARS-CoV-2 infection. This report, which to our awareness represents the first longitudinal investigation of viral culture characteristics of vaccinated persons with Delta variant infections, further demonstrates the potential of vaccinated persons to contribute to SARS-CoV-2 transmission.

While our investigation did not find evidence of reduced transmission potential from vaccinated persons with infection, vaccination is known to reduce the risk of infection,[6, 21] which prevents secondary transmission. In addition, vaccination remains a strongly protective factor against morbidity and mortality due to SARS-CoV-2.[22] Protection against infection, morbidity, and mortality underscores the importance of maximizing vaccination coverage, particularly in settings where challenges to physical distancing can result in rapid, widespread transmission when infections do occur.

The evidence that vaccinated persons can transmit SARS-CoV-2 to others suggests that there is continued risk of widespread outbreaks when the virus is introduced into congregate settings, even when vaccination coverage is high. In particular, because of the potential for rapid transmission and high prevalence of underlying health conditions in incarcerated populations,[7, 8] persons living or working in correctional facilities should quarantine after exposure to SARS-CoV-2, regardless of vaccination status. Post-exposure quarantine is especially important where the risk of transmission is high (e.g., in dorm-style housing, and where staff and/or incarcerated persons frequently interact across housing units) or where the population is at high risk of severe outcomes from COVID-19. Facilities can continue to minimize the need for quarantine by enforcing consistent indoor masking to the extent possible, continuing recommended disinfection, cleaning, and ventilation, and maintaining routine test-based screening programs that can identify cases early and facilitate timely action (including isolation) to limit exposure to others. Facilities that implement routine test-based screening should continue to include vaccinated persons in their frame.

This report is subject to several limitations. Due to the small proportion of participants who were not fully vaccinated (19%), statistical comparisons on the basis of vaccination status were underpowered, and negative findings reported here warrant cautious interpretation. To increase the sample size of this group, two partially vaccinated participants were included, potentially diluting the characteristics of unvaccinated participants. However, our conclusions did not change when analyses

were performed excluding these two participants. Similarly, only four participants had known prior infection, of which a higher proportion occurred in those not fully vaccinated; therefore, these participants may appear to have slightly greater immunological protection than those without prior infection. On average, unvaccinated participants enrolled earlier in the outbreak and later in their course of infection than vaccinated participants; we utilized Turnbull estimation in survival analyses to account for the possibility of interval censoring in this population. All symptom data was self-reported and collected at the end of the specimen collection period, which may have impacted the accuracy of participants' recall related to the date of symptom onset. Ct values are semi-quantitative indicators of viral RNA levels and cannot be interpreted as quantitative markers of viral load or infectiousness. To avoid drawing quantitative conclusions around Ct values, we conservatively utilized non-parametric rank-based statistics (Mann-Whitney and Kruskal-Wallis) with Bonferroni correction to describe Ct values in this investigation. Information on prior SARS-CoV-2 infection was obtained from medical records; persons with earlier infections that were undiagnosed or diagnosed prior to incarceration and not documented in the BOP medical system may not have been correctly characterized. Finally, we did not attempt viral culture for 561 specimens with Ct>35 and classified them as presumptively negative. This decision was based on negative viral culture results from 25/25 specimens with Ct>35 for which viral culture was performed during this investigation, as well as previously published findings demonstrating an inability to recover viable virus from specimens that were RT-PCR negative.[23]

In this investigation, we found no statistically significant difference in transmission potential between vaccinated persons and persons who were not fully vaccinated. Therefore, our findings indicate that prevention and mitigation measures should be applied without regard to vaccination status for persons in high-risk settings or those with significant exposures. In congregate settings, and correctional and detention facilities in particular, post-exposure testing and quarantine remain essential tools to limit transmission when cases are identified, in addition to other recommended prevention

342 measures.[24] Our data add to a growing body of evidence characterizing transmission potential from
343 vaccinated persons. Future studies of transmission potential from vaccinated persons with infection,
344 incorporating similar laboratory-based markers as well as evidence of transmission from secondary
345 attack rates and network analysis, may help to further describe the contributions of vaccinated persons
346 in chains of transmission as the pandemic evolves and new variants emerge.

Conflict of Interest Statement

The authors have no conflicts of interest to report. All authors have completed the ICMJE Conflict of Interest declaration.

Acknowledgements

Mario Cordova, Torrey Haskins, Jennifer Jackson, Joshua Jett, Barbara Swopes, Tammy Winbush, Federal Bureau of Prisons.

Footnotes

* 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. Sect. 241(d); 5 U.S.C. Sect. 552a; 44 U.S.C. Sect. 3501 et seq.

359 **Table 1. Characteristics of enrolled participants who tested positive for SARS-CoV-2, Federal prison,**
360 **Texas, July 12—August 9, 2021**

	All participants		Fully vaccinated		Not fully vaccinated*		p-value†
	n	%	n	%	n	%	
Total	95	100%	78	81%	17	19%	
Sex							
Male	95	100%	78	100%	17	100%	
Age							0.4
18-29	5	5%	3	4%	2	12%	
30-39	22	23%	19	24%	3	18%	
40-49	28	29%	22	28%	6	35%	
50-59	25	26%	20	26%	5	29%	
≥ 60	15	16%	14	18%	1	6%	
Race/Ethnicity							**0.008**
American Indian/Alaska Native	2	2%	2	3%	0	0%	
Asian	1	1%	1	1%	0	0%	
Black	16	17%	8	10%	8	47%	
Hispanic	12	13%	10	13%	2	12%	
White	64	67%	57	73%	7	41%	
Country of birth							0.6
Non US-born	4	4%	3	4%	1	6%	
US-born	91	96%	75	96%	16	94%	
Vaccination status							
Fully vaccinated	78	82%	78	100%	0	0%	
Not fully vaccinated*	17	18%	0	0%	17	100%	
Partially vaccinated	2	2%	0	0%	2	12%	
Unvaccinated	15	16%	0	0%	15	88%	
Vaccine product received							
Janssen	7	7%	7	9%	0	0%	
Moderna	14	15%	14	17%	0	0%	
Pfizer	57	60%	57	74%	0	0%	
Time from full vaccination to outbreak (if fully vaccinated)							
≤120 days	31	33%	31	33%	0	0%	
>120 days	47	49%	47	61%	0	0%	
Medical comorbidities							
Overweight‡	31	33%	24	31%	7	41%	
Obesity‡	47	49%	42	54%	5	29%	0.3
Severe obesity ‡	7	7%	6	8%	1	6%	
History of smoking	46	48%	42	54%	4	24%	**0.03**
Hypertension	43	45%	38	49%	5	29%	0.1

Diabetes	15	16%	14	18%	1	6%	0.3
Moderate/severe asthma	10	11%	8	10%	2	12%	1.0
Chronic obstructive pulmonary disease	6	6%	6	8%	0	0%	0.6
Cancer	1	1%	1	1%	0	0%	1.0
Chronic kidney disease	2	2%	2	3%	0	0%	1.0
Immunocompromised state	2	2%	2	3%	0	0%	1.0
HIV	0	0%	0	0%	0	0%	
Serious cardiac conditions	0	0%	0	0%	0	0%	
Liver disease	0	0%	0	0%	0	0%	
Documented prior SARS-CoV-2 infection							0.1
No	91	96%	76	97%	15	88%	
Yes	4	4%	2	3%	2	12%	
COVID-19 disease outcomes							
Hospitalization	2	2%	1	1%	1	6%	
Death	0	0%	0	0%	0	0%	
Reported Symptoms							
Reported any symptoms in CSTE case definition§	66	70%	54	70%	12	71%	0.7
Reported any symptoms	72	76%	59	76%	13	76%	0.4
Runny/Stuffy Nose	55	58%	48	62%	7	41%	0.4
Loss of Smell or Taste	51	54%	43	55%	8	44%	1.0
Cough	43	45%	35	45%	8	47%	0.8
Headache	40	42%	33	42%	7	41%	1.0
Muscle Aches	40	42%	30	38%	10	59%	0.08
Subjective Fever	34	36%	27	35%	7	41%	0.6
Measured Fever	10	11%	6	8%	4	24%	0.06
Chills	29	31%	21	27%	8	47%	0.06
Sore Throat	24	25%	21	27%	3	18%	0.7
Shortness of Breath	20	21%	14	18%	6	35%	0.08
Abdominal Pain, Nausea, Vomiting	17	18%	12	15%	5	28%	0.2
Diarrhea	16	17%	11	14%	5	28%	0.1
Other	6	6%	6	8%	0	0%	1.0
None Reported ¶	23	24%	19	24%	4	24%	1.0

*Not fully vaccinated participants include 15 who have not received any dose of a SARS-CoV-2 vaccine and 2 who receive only the first dose of a two-dose SARS-CoV-2 vaccine series.

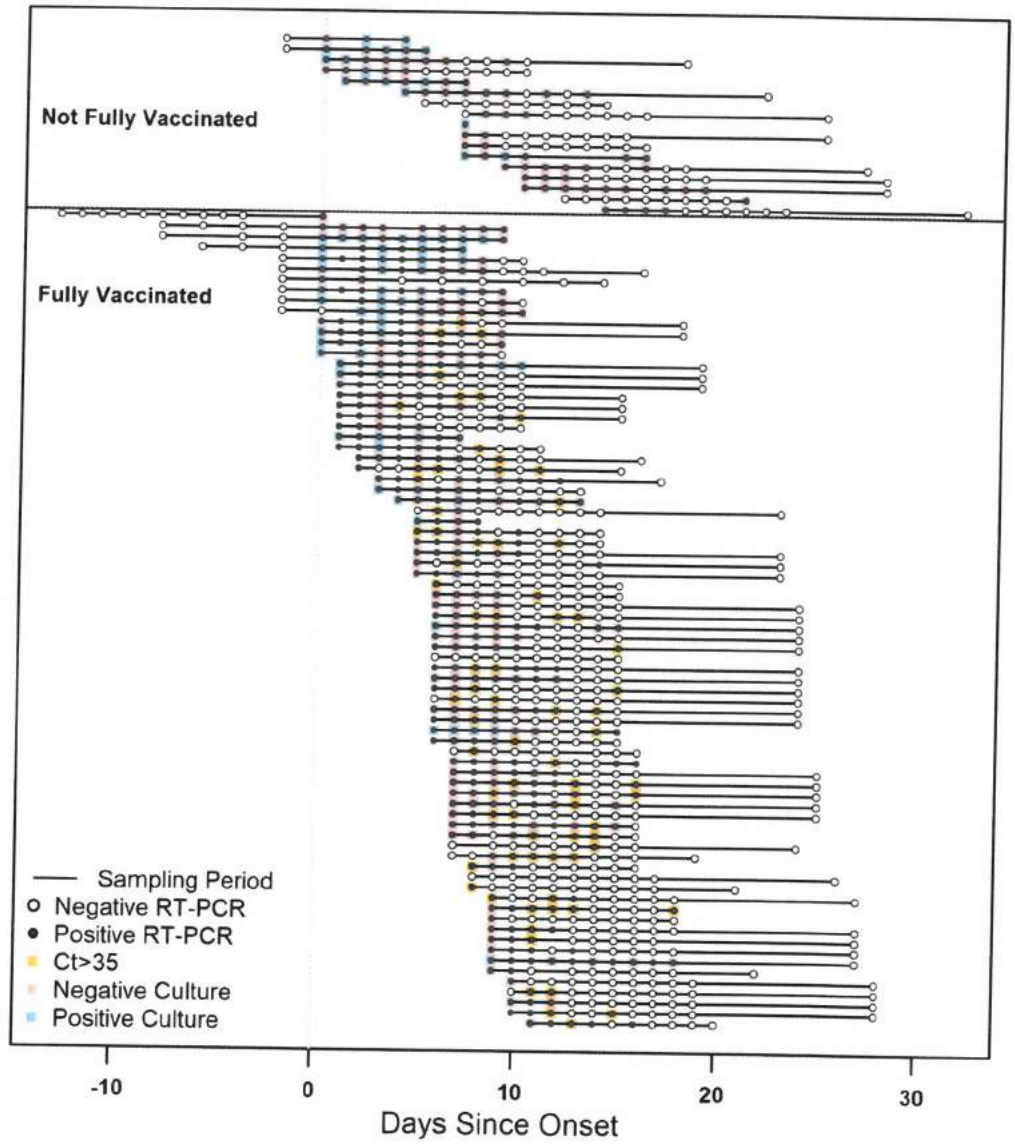
†P-values correspond to results of Fisher's exact tests.

‡Overweight was defined as a body mass index (BMI) >25 kg/m2 but <30 kg/m2; obesity was defined as BMI ≥30 kg/m2 but <40 kg/m2; severe obesity was defined as BMI ≥40 kg/m2.

§The COVID-19 case definition of the Council of State and Territorial Epidemiologists (CSTE) includes fever, chills, muscle aches, headache, sore throat, nausea/vomiting, diarrhea, fatigue, stuffy/runny nose, cough, shortness of breath, or loss of taste or smell. [CSTE 2021]

¶ 8 participants (5 fully vaccinated and 3 not fully vaccinated) declined to report symptoms in addition to 15 (14 and 1, respectively) who reported that they had no symptoms

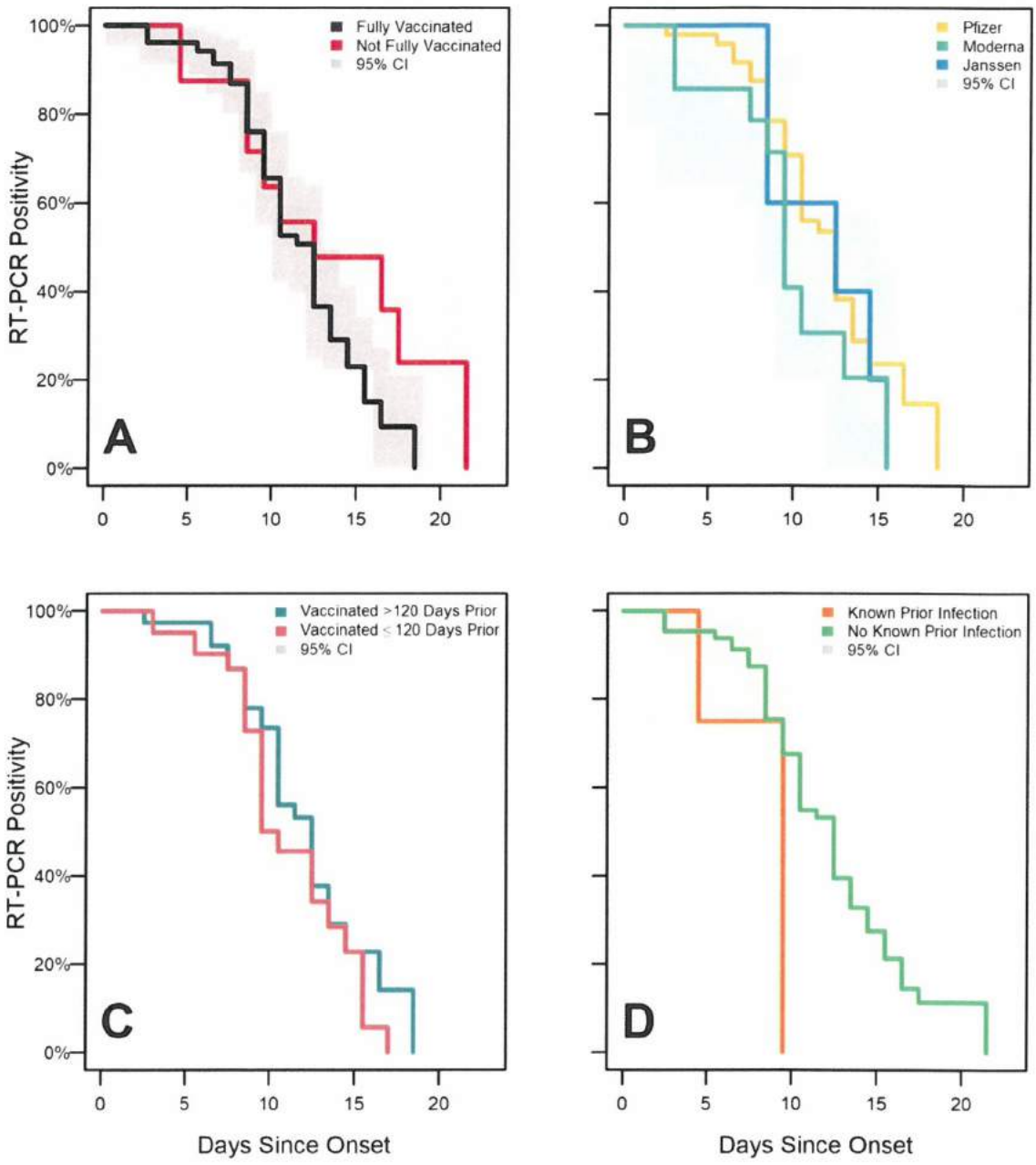
371 **Figure 1. Timelines and results of nasal mid-turbinate specimens collected from enrolled participants,**
372 **Federal prison, Texas, July 12—August 9, 2021**



373

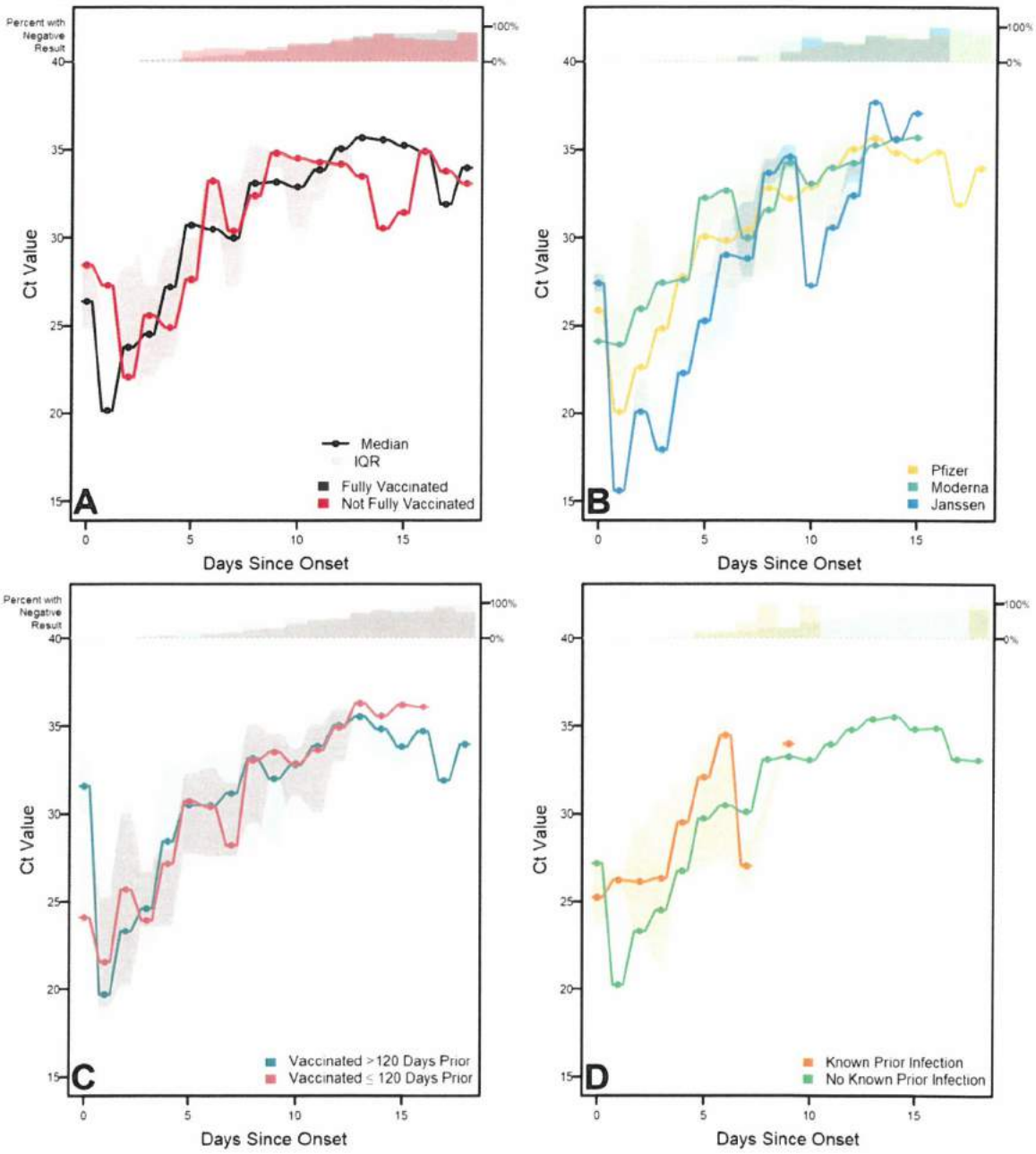
374 The timelines of specimen collection and laboratory results for 95 included participants are represented
375 diagrammatically, indexed by the day of onset. Onset was determined to be either a) date of first onset of self-
376 reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2
377 test, whichever occurred first. Each participant is represented by a horizontal line corresponding to the
378 investigation sampling period during their time-course of illness. Participants who were not fully vaccinated
379 (including 2 participants who received only the first dose of a two-dose COVID-19 vaccine series) are depicted at
380 the top of the figure, while fully vaccinated participants are depicted at the bottom. RT-PCR results are
381 represented by solid circles (positive results) or open circles (negative results). For specimens with positive RT-PCR
382 results for which viral culture was performed, culture results are indicated by overlaid blue boxes (positive culture
383 results) or red boxes (negative culture results). Specimens with positive RT-PCR results with a cycle threshold (Ct)
384 value greater than 35 for which viral culture was not performed are indicated by overlaid orange boxes (indicated
385 a presumptive negative viral culture result). Some participants provided specimens during case-finding testing
386 while in quarantine and may have RT-PCR negative specimens collected prior to onset.

Figure 2. SARS-CoV-2 RT-PCR test positivity survival curves for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the results of Turnbull estimation survival functions with a primary endpoint of last positive reverse transcription-polymerase chain reaction (RT-PCR) test result. Solid lines indicate nonparametric maximum likelihood estimates and shaded regions correspond to 95% confidence intervals estimated through modified bootstrap. Survival functions are plotted by Turnbull interval midpoints. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of known prior SARS-CoV-2 infection.

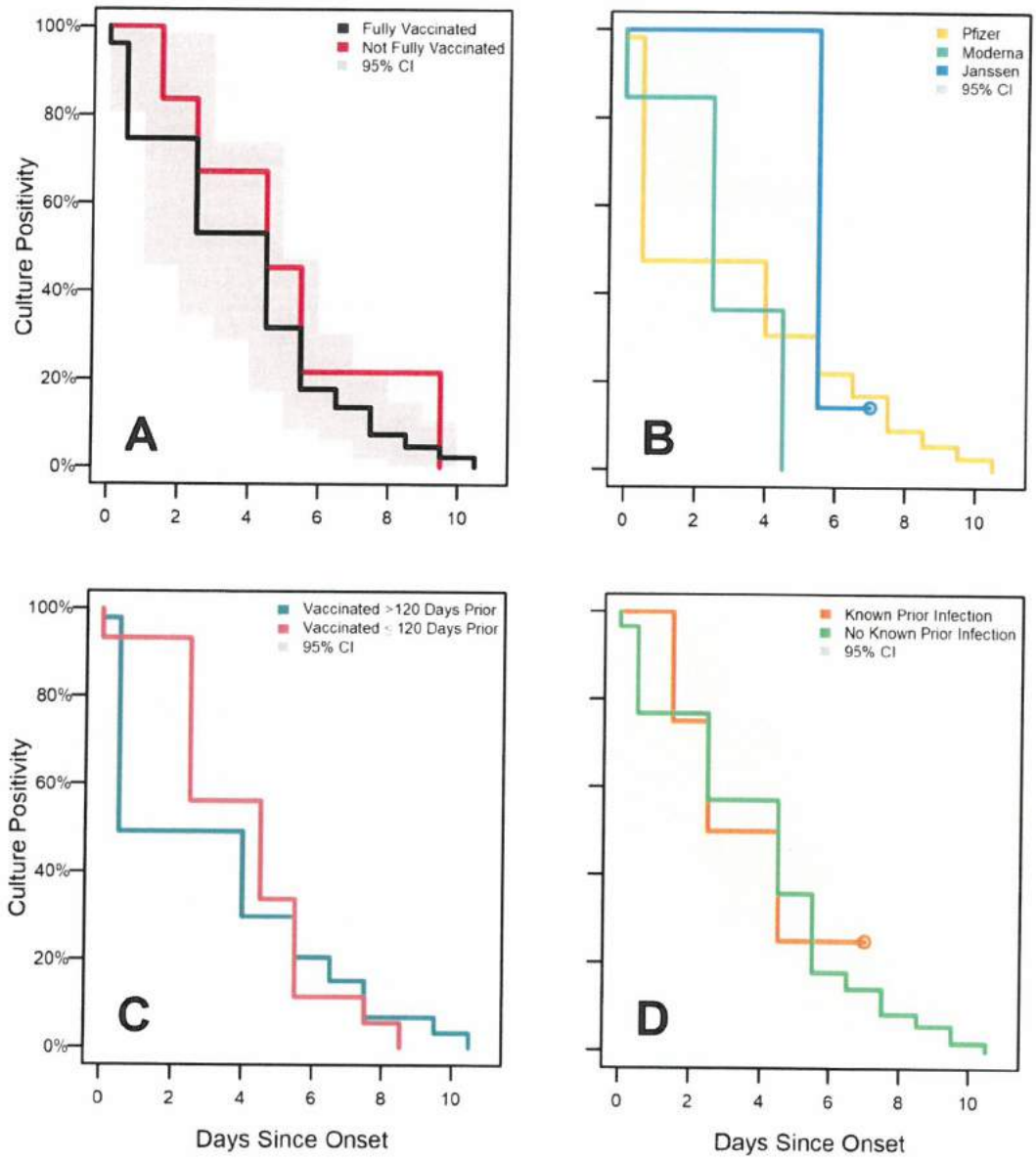
400 **Figure 3. RT-PCR Cycle Threshold distributions for enrolled participants with confirmed SARS-CoV-2**
401 **infection, Federal prison, Texas, July 12—August 9, 2021**



402

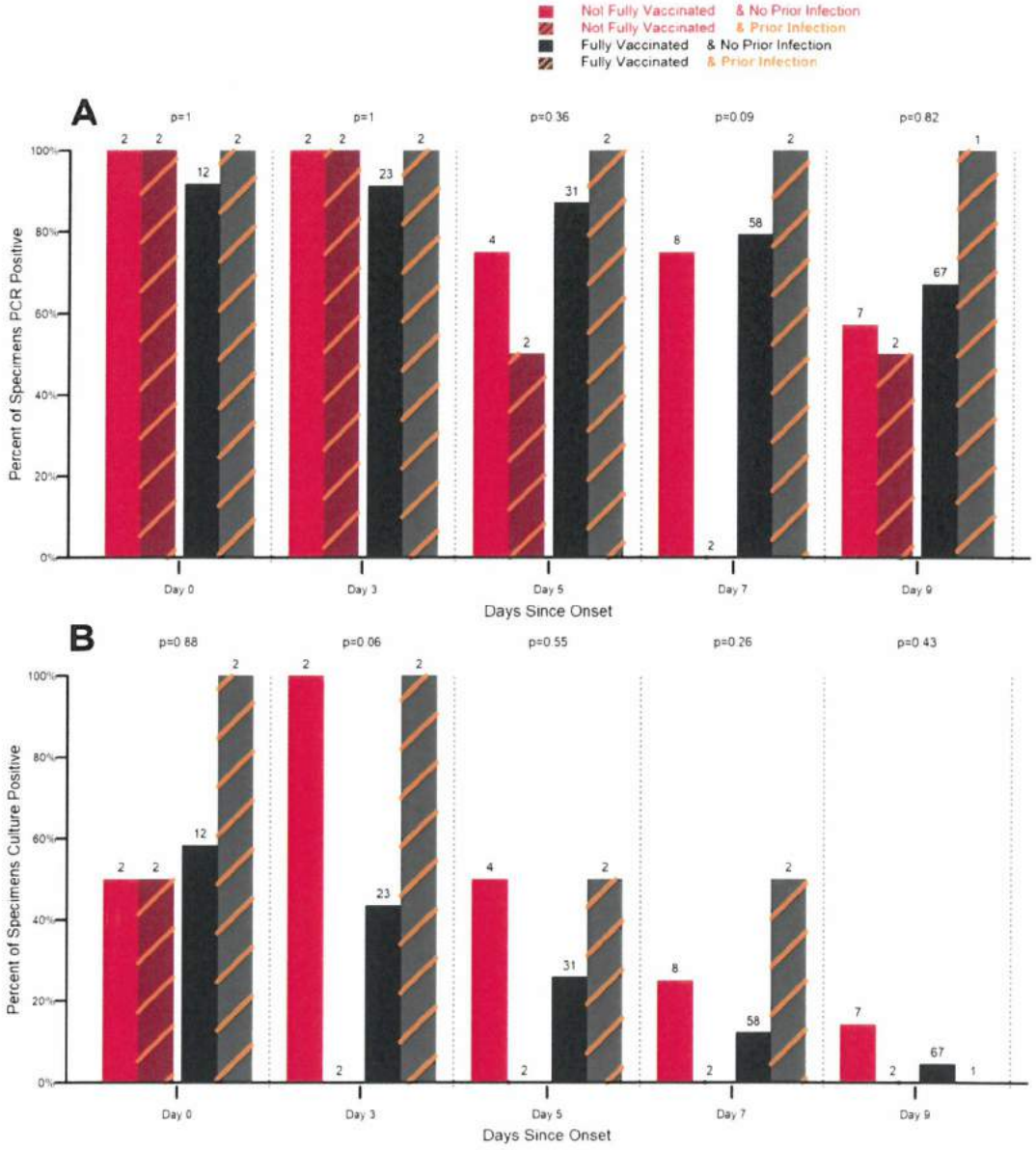
403 Panels illustrate daily medians and interquartile ranges (IQRs) for reverse transcription-polymerase chain reaction
404 (RT-PCR) cycle threshold (Ct) values among specimens with positive RT-PCR results. Solid lines indicate median Ct
405 values and shaded regions indicate IQRs. Percentages at the top of each panel indicate the proportion of
406 specimens with negative RT-PCR results each day Onset was determined to be either a) date of first onset of self-
407 reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2
408 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated
409 participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B
410 depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to
411 the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of
412 known prior SARS-CoV-2 infection.

Figure 4. SARS-CoV-2 viral culture test positivity survival curves for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the results of Turnbull estimation survival functions with a primary endpoint of last positive viral culture test result. Specimens were included as presumptive negative results if no culture was performed but were accompanied by negative RT-PCR results or positive RT-PCR results with Ct>35. Solid lines indicate nonparametric maximum likelihood estimates and shaded regions correspond to 95% confidence intervals estimated through modified bootstrap. Survival functions are plotted by Turnbull interval midpoints. When Turnbull intervals are bounded by positive infinity (resulting from right-censoring in subgroups), survival functions are truncated by open points at the rightmost non-infinite intervals. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Panel A depicts RT-PCR positivity by vaccination status (not fully vaccinated participants include 2 participants who received only the first dose of a two-dose COVID-19 vaccine series). Panel B depicts positivity by vaccine product among fully vaccinated participants. Panel C depicts positivity according to the time from completion of a COVID-19 vaccine/series to onset. Panel D depicts positivity according to history of known prior SARS-CoV-2 infection.

Figure 5. SARS-CoV-2 RT-PCR test positivity (A) and viral culture test positivity (B) stratified by vaccination status and prior infection status for enrolled participants, Federal prison, Texas, July 12—August 9, 2021



Panels illustrate the proportions of specimens for which RT-PCR test results (panel A) or viral culture test results (panel B) were positive, stratified by both vaccination status and history of prior SARS-CoV-2 infection. Solid bars indicate results for participants with no known prior infections, and striped bars indicate results for participants with documented prior infections. Specimens were included as presumptive negative results if no culture was performed but were accompanied by negative RT-PCR results or positive RT-PCR results with Ct>35. Onset was determined to be either a) date of first onset of self-reported symptom(s) meeting the case definition of COVID-19 or b) date of first positive diagnostic SARS-CoV-2 test, whichever occurred first. Results are depicted only for days 0, 3, 5, 7, and 9 since onset, representing days for which 100% of eligible specimens had viral culture performed. Bar labels indicate the number of specimens collected from participants in each group for each day. P-values are reported at the top of each daily grouping and correspond to Fisher's exact test of proportions across the four groups.

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526

Exhibit "U"

This is **Exhibit "U"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at

[redacted]

Eva Chipiuk
Barrister & Solicitor

Read The CDC Disclaimer

VAERS COVID Vaccine Adverse Event Reports

Reports from the Vaccine Adverse Events Reporting System. Our default data reflects all VAERS data including the "nondomestic" reports. ?

All VAERS COVID Reports | US/Territories/Unknown

894,143 Reports
Through November 12, 2021 ?

18,853
DEATHS

94,537
HOSPITALIZATIONS

99,470
URGENT CARE

ANAPHYLAXIS

11,229

BELL'S PALSY

2,996

Miscarriages

9,332

Heart Attacks

13,237

Myocarditis/Pericarditis

30,010

Permanently Disabled

4,387

Thrombocytopenia/
Low Platelet

10,455
Shingles

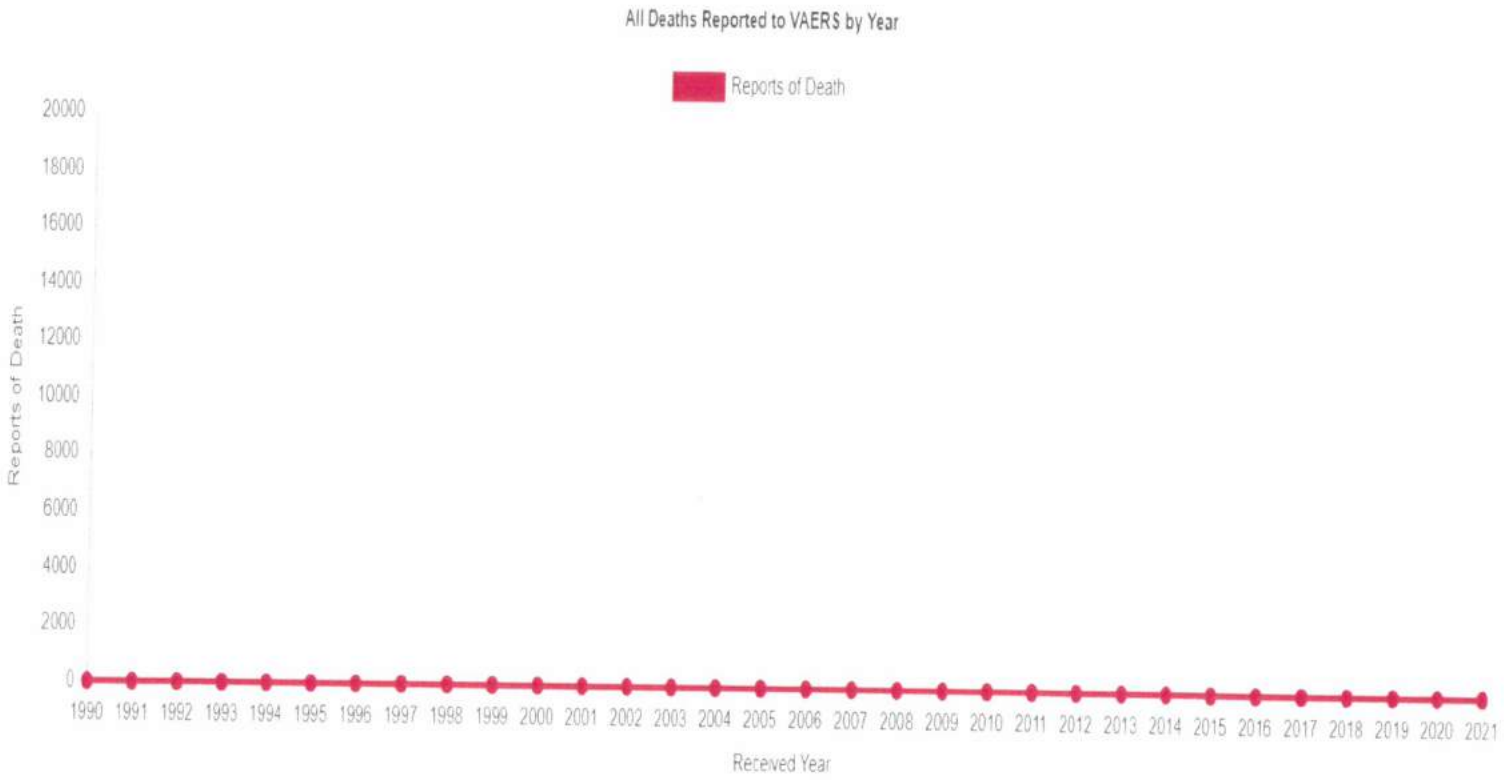

Exhibit "V"

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

COVID-19

Safety of COVID-19 vaccines

Table of contents

The European Medicines Agency (EMA) monitors the safety of COVID-19 vaccines authorised in the European Union (EU) extremely carefully. This enables the detection of any rare side effects that may emerge once many millions of people are vaccinated.

- Nearly 575 million doses of vaccines have been given to people in the EU and European Economic Area (EEA), as of the end of October 2021.
- The authorised COVID-19 vaccines are safe and effective. They were evaluated in tens of thousands of participants in clinical trials and have met EMA's scientific standards for safety, efficacy and quality.
- The safety of COVID-19 vaccines is continuously monitored and evaluated.
- Monthly safety updates give an overview of the PRAC's regular safety evaluation.
- The vast majority of known side effects of COVID-19 vaccines are mild and short-lived.
- Serious safety problems are extremely rare.

Latest safety information

The latest information on the safety of each vaccine is in the vaccine's monthly safety update, available via the links below.

The figures below provide overall numbers of **suspected side effects** that individuals and healthcare professionals have reported after using a COVID-19 vaccine in the EU and EEA.

Reported cases concern suspected side effects, i.e. medical events that have been observed after vaccination, but which are **not necessarily related to or caused by the vaccine**. The problem may have happened anyway, for example, because of an unrelated health issue.

The nature of spontaneous reporting also means that some people may have not reported their side effects, particularly if they were mild.

For more information on these reports see:

- European suspected adverse drug reactions database (www.adrreports.eu)
- Important information on how to interpret the data

Comirnaty

(BioNTech and Pfizer)

Status as of 28/10/2021

428,000,000

Doses given to people in the EU/EEA

412,571*

Reports of suspected side effects in the EU/EEA (see www.adrreports.eu)

* Reported cases concern suspected side effects, i.e. medical events that have been observed after vaccination, but which are not necessarily related to or caused by the vaccine.

Read latest safety update

All Comirnaty safety updates ›

Vaxzevria

(AstraZeneca)

Status as of 28/10/2021

68,800,000

Doses given to people in the EU/EEA

214,528*

Reports of suspected side effects in the EU/EEA (see www.adrreports.eu)

* Reported cases concern suspected side effects, i.e. medical events that have been observed after vaccination, but which are not necessarily related to or caused by the vaccine.

Read latest safety update

All Vaxzevria safety updates >

Spikevax

(Moderna)

Status as of 28/10/2021

61,600,000

Doses given to people in the EU/EEA

94,636*

Reports of suspected side effects in the EU/EEA (see www.adrreports.eu)

* Reported cases concern suspected side effects, i.e. medical events that have been observed after vaccination, but which are not necessarily related to or caused by the vaccine.

Read latest safety update

All Spikevax safety updates >

COVID-19 Vaccine Janssen

Status as of 28/10/2021

16,300,000

Doses given to people in the EU/EEA

28,244*

Reports of suspected side effects in the EU/EEA (see www.adrreports.eu)

* Reported cases concern suspected side effects, i.e. medical events that have been observed after vaccination, but which are not necessarily related to or caused by the vaccine.

Read latest safety update

All Janssen safety updates >

How EMA monitors vaccine safety

The EU safety monitoring plan for COVID-19 vaccines requires EMA to monitor suspected side effects reported by individuals and healthcare professionals in the EU.

An EU database called EudraVigilance holds these reports. The European suspected adverse drug reactions database provides public access to these data in a number of ways, while taking account of EU data protection law.

EMA's PRAC and the national competent authorities continuously monitor EudraVigilance to identify any new safety issues that require investigation. These are known as **safety signals**.

When assessing a safety signal, the PRAC looks for any unusual or unexpected patterns, such as a medical event occurring in vaccinated people at a higher rate than in the general population.

They review other sources of evidence, such as clinical studies, epidemiology and pharmacoepidemiology studies, the medical literature and information from regulators outside the EU.

The PRAC then conducts a robust assessment of all combined **safety data** before concluding on how the signal affects the vaccine's safety and its benefit-risk balance.

If needed, EMA may decide to update the vaccine's product information to provide the right advice to healthcare professionals and patients, require the manufacturer to conduct additional studies, or restrict the use of the vaccine.

Patients and healthcare professionals should report any suspected side effects after receiving a COVID-19 vaccine to their national competent authority. For information on how to do this see:

- Reporting a suspected side effect

Related content

- COVID-19 vaccines: Monitoring vaccine safety and use in real life
- Monitoring of COVID-19 medicines

External links

- European suspected adverse drug reactions database
- European Vaccination Information Portal: Monitoring vaccine safety and reporting side effects
- European Commission: Safe COVID-19 vaccines for Europeans
- European Council: COVID-19: the EU's contribution to global vaccine solidarity
- European Centre for Disease Prevention and Control (ECDC): COVID-19 vaccine tracker

Topics

- COVID-19
- Pharmacovigilance
- Vaccines

CONTACT

European Medicines Agency
Domenico Scarlattilaan 6
1083 HS Amsterdam
The Netherlands

Tel: +31 (0)88 781 6000

How to find us

Postal address and deliveries

Business hours and holidays

For the United Kingdom, as of 1 January 2021, European Union law applies only to the territory of Northern Ireland (NI) to the extent foreseen in the Protocol on Ireland / NI.

European Union agencies network

An agency of the European Union

Research and analysis

Coronavirus vaccine - weekly summary of Yellow Card reporting

Updated 2 December 2021

Contents

This publication is available at https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting

This report covers the period 9 December 2020 to 24 November 2021.

Summary

At the time of this report, over 144,432 people across the UK have died within 28 days of a positive test for coronavirus (COVID-19). Vaccination is the single most effective way to reduce deaths and severe illness from COVID-19. A national immunisation campaign has been underway since early December 2020.

Three COVID-19 vaccines, the COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca and COVID-19 Vaccine Moderna, are currently being used in the UK. All have been authorised for supply by the Medicines and Healthcare products Regulatory Agency (MHRA) following a thorough review of safety, quality and efficacy information from clinical trials. In clinical trials (https://www.gov.uk/government/collections/mhra-guidance-on-coronavirus-covid-19#vaccines-and-vaccine-safety), the vaccines showed very high levels of protection against symptomatic infections with COVID-19. Data are now available (https://www.gov.uk/government/publications/phe-monitoring-of-the-effectiveness-of-covid-19-vaccination) on the impact of the vaccination campaign in reducing infections and illness in the UK.

The MHRA confirmed on 9 September 2021 that the COVID-19 vaccines made by Pfizer and AstraZeneca can be used as safe and effective booster doses. Following review of data for the COVID-19 Vaccine Moderna, the MHRA and CHM experts also concluded that this vaccine can be used as a safe and effective booster dose.

All vaccines and medicines have some side effects. These side effects need to be continuously balanced against the expected benefits in preventing illness.

The COVID-19 Pfizer/BioNTech Vaccine was evaluated in clinical trials involving more than 44,000 participants. The most frequent adverse reactions (https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19/information-for-healthcare-professionals-on-pfizerbiontech-covid-19-vaccine) in trials were pain at the injection site, fatigue, headache, myalgia (muscle pains), chills, arthralgia (joint pains), and fever; these were each reported in more than 1 in 10 people. These reactions were usually mild or moderate in intensity and resolved within a few days after vaccination. Adverse reactions were reported less frequently in older adults (over 55 years) than in younger people.

The COVID-19 Vaccine AstraZeneca was evaluated in clinical trials involving more than 23,000 participants. The most frequently reported adverse reactions (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-astrazeneca/information-for-healthcare-professionals-on-covid-19-vaccine-astrazeneca) in these trials were injection-site tenderness, injection-site pain, headache, fatigue, myalgia, malaise, pyrexia (fever), chills, arthralgia, and nausea; these were each reported in more than 1 in 10 people. The majority of adverse reactions were mild to moderate in severity and usually resolved within a few days of vaccination. Adverse reactions reported after the second dose were milder and reported less frequently than after the first dose. Adverse reactions were generally milder and reported less frequently in older adults (65 years and older) than in younger people.

The COVID-19 Vaccine Moderna was evaluated in clinical trials involving more than 30,000 participants. The most frequent adverse reactions in these trials were pain at the injection site, fatigue, headache, myalgia (muscle pains), arthralgia (joint pains), chills, nausea/vomiting, axillary swelling/tenderness (swelling/tenderness of glands in the armpit), fever, injection site swelling and redness; these were each reported in more than 1 in 10 people. These reactions were usually mild or moderate in intensity and resolved within a few days after vaccination. Adverse reactions were reported less frequently in older adults (over 65 years) than in younger people.

The MHRA continually monitors safety during widespread use of a vaccine. We have in place a proactive strategy to do this (https://www.gov.uk/government/publications/report-of-the-commission-on-human-medicines-expert-working-group-on-covid-19-vaccine-safety-surveillance). We also work closely with our public health partners in reviewing the effectiveness and impact of the vaccines to ensure the benefits continue to outweigh any possible side effects.

Part of our monitoring role includes reviewing reports of suspected side effects. Any member of the public or health professional can submit suspected side effects through the Yellow Card scheme (https://yellowcard.mhra.gov.uk/). The nature of Yellow Card reporting means that reported events are not always proven side effects. Some events may have happened anyway, regardless of vaccination. This is particularly the case when millions of people are vaccinated, and especially when vaccines are being given to the most elderly people and people who have underlying illness.

This safety update report is based on detailed analysis of data up to 24 November 2021. As of 24 November 2021, an estimated 24.5 million first doses of the COVID-19 Pfizer/BioNTech Vaccine and 24.9 million first doses of the COVID-19 Vaccine AstraZeneca had been administered, and around 20.8 million and 24.1 million second doses of COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine AstraZeneca respectively. An approximate 1.5 million first doses and approximately 1.3 million second doses of the COVID-19 Vaccine Moderna have also now been administered.

As of 24 November 2021, for the UK, 136,582 Yellow Cards have been reported for the COVID-19 Pfizer/BioNTech Vaccine, 238,086 have been reported for the COVID-19 Vaccine AstraZeneca, 19,101 for the COVID-19 Vaccine Moderna and 1,280 have been reported where the brand of the vaccine was not specified.

For the COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca and COVID-19 Vaccine Moderna the overall reporting rate is around 3 to 7 Yellow Cards per 1,000 doses administered.

In the week since the previous summary for 17 November 2021 we have received a further 2,631 Yellow Cards for the COVID-19 Pfizer/BioNTech Vaccine, 599 for the COVID-19 Vaccine AstraZeneca, 985 for the COVID-19 Vaccine Moderna and 26 where the brand was not specified.

It is important to note that Yellow Card data cannot be used to derive side effect rates or compare the safety profile of COVID-19 vaccines as many factors can influence ADR reporting.

For all COVID-19 vaccines, the overwhelming majority of reports relate to injection-site reactions (sore arm for example) and generalised symptoms such as 'flu-like' illness, headache, chills, fatigue (tiredness), nausea (feeling sick), fever, dizziness, weakness, aching muscles, and rapid heartbeat. Generally, these happen shortly after the vaccination and are not associated with more serious or lasting illness.

These types of reactions reflect the normal immune response triggered by the body to the vaccines. They are typically seen with most types of vaccine and tend to resolve within a day or two. The nature of reported suspected side effects is broadly similar across age groups, although, as was seen in clinical trials and as is usually seen with other vaccines, they may be reported more frequently in younger adults.

A number of detailed assessments of safety topics have been undertaken and we have updated our advice on these topics accordingly. Overall, our advice remains that the benefits of the vaccines outweigh the risks in the majority of people. Further comments on use in specific populations and details on the specific safety topics can be found within Section 3.

Conclusion

- Vaccines are the best way to protect people from COVID-19 and have already saved thousands of lives. Everyone should continue to get their vaccination when asked to do so unless specifically advised otherwise.
- As with all vaccines and medicines, the safety of COVID-19 vaccines is being continuously monitored.
- The expected benefits of the vaccines in preventing COVID-19 and serious complications associated with COVID-19 far outweigh any currently known side effects in the majority of patients.

Further information on the type of suspected adverse reactions (ADRs) reported for the COVID-19 Pfizer/BioNTech Vaccine, the COVID-19 Vaccine AstraZeneca and the COVID-19 Vaccine Moderna is provided in Annex 1. It is important to read the attached guidance notes to ensure appropriate interpretation of the data.

1. Introduction

The MHRA is the executive Agency of the Department of Health and Social Care that acts to protect and promote public health and patient safety, by ensuring that medicines and medical devices meet appropriate standards of safety, quality and efficacy.

The MHRA operates the Yellow Card scheme (https://yellowcard.mhra.gov.uk/) on behalf of the Commission on Human Medicines (CHM). The scheme collects and monitors information on suspected safety concerns or incidents involving vaccines, medicines, medical devices, and e-cigarettes. The scheme relies on voluntary reporting of suspected adverse incidents by healthcare professionals and members of the public (patients, users, or carers). The purpose of the scheme is to provide an early warning that the safety of a product may require further investigation. Further information about the Yellow Card scheme, including its contribution to identifying safety issues can be found on the Yellow Card website (https://yellowcard.mhra.gov.uk/the-yellow-card-scheme/).

The MHRA is playing an active role in responding to the coronavirus pandemic. In relation to COVID-19 vaccines, the MHRA has authorised their supply following a rigorous review of their safety, quality and efficacy. The clinical trials of COVID-19 vaccines have shown them to be effective and acceptably safe; however, as part of its statutory functions, the MHRA is responsible for monitoring these vaccines on an ongoing basis to ensure their benefits continue to outweigh any risks. This is a requirement for all authorised medicines and vaccines in the UK. This monitoring strategy is continuous, proactive and based on a wide range of information sources, with a dedicated team of scientists reviewing information daily to look for safety issues or unexpected rare events.

This report summarises information received via the Yellow Card scheme and will be published regularly to include other safety investigations carried out by the MHRA under the COVID-19 Vaccine Surveillance Strategy (https://www.gov.uk/government/publications/report-of-the-commission-on-human-medicines-expert-working-group-on-covid-19-vaccine-safety-surveillance).

What is a Yellow Card?

The Yellow Card scheme is a mechanism by which anybody can voluntarily report any suspected adverse reactions or side effects to the vaccine. It is very important to note that a Yellow Card report does not necessarily mean the vaccine caused that reaction or event. We ask for any suspicions to be reported, even if the reporter isn't sure if it was caused by the vaccine. Reports to the scheme are known as suspected adverse drug reactions (ADRs).

Many suspected ADRs reported on a Yellow Card do not have any relation to the vaccine or medicine and it is often coincidental that symptoms occurred around the same time as vaccination. The reports are continually reviewed to detect possible new side effects that may require regulatory action, and to differentiate these from things that would have happened regardless of the vaccine or medicine being administered, for instance due to underlying or undiagnosed illness.

It is therefore important that the suspected ADRs described in this report are not interpreted as being proven side effects of COVID-19 vaccines. A list of the possible side effects of the COVID-19 Pfizer/BioNTech Vaccine (https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19), COVID-19 Vaccine AstraZeneca (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-astrazeneca) vaccine and COVID-19 Vaccine Moderna (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-moderna/information-for-healthcare-professionals-on-covid-19-vaccine-moderna) are provided in the product information document for healthcare professionals and the UK recipient information. These can also be found on the Coronavirus Yellow Card reporting site (https://coronavirus-yellowcard.mhra.gov.uk/).

This public summary provides an overview of all UK suspected ADRs associated with the new coronavirus (COVID-19) vaccines (COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca and COVID-19 Vaccine Moderna), and MHRA's analysis of the data, between 9 December 2020 and 24 November 2021 (inclusive). A glossary of key terms is provided in Annex 2.

If identified, information on new and emerging safety concerns will be provided in future editions of this report together with details of any resulting regulatory action or changes to advice on use of the vaccines.

2. Yellow Card reports

Vaccine doses administered

Data from the UK Public Health agencies (https://coronavirus.data.gov.uk/details/vaccinations) show that at least 50,852,133 people have received their first vaccination in the UK by 24 November 2021, with 46,232,258 second doses administered. The priority groups of the immunisation campaign for this period included people aged 12 years and over, the clinically vulnerable, care home residents and workers, and frontline health and social care workers. Individuals are also being invited for their booster vaccination if it has been 152 days (5 months) since their second dose and they are either aged 50 and over or are aged 16 and over with a health condition that puts them at high risk from COVID-19. On 29 November 2021 the JCVI announced those aged 18 to 39 will also be eligible for a booster when the NHS calls them forward.

Table 1: Number of people who have received the first dose of a vaccination for COVID-19 in the UK between 8 December 2020 and end of 24 November 2021.

Country	Number of doses
England	42,638,518
Wales	2,465,562
Northern Ireland	1,360,946
Scotland	4,342,107

Table 2: Number of people who have received the second dose of a vaccination for COVID-19 in the UK between 8 December 2020 and end of 24 November 2021.

Country	Number of doses
England	38,764,212
Wales	2,260,038
Northern Ireland	1,265,081
Scotland	3,942,927

As of 24 November, an estimated 24.5 million first doses of the COVID-19 Pfizer/BioNTech Vaccine and 24.9 million first doses of the COVID-19 Vaccine AstraZeneca had been administered, and around 20.8 million and 24.1 million second doses of the COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine AstraZeneca respectively. An approximate 1.5 million first doses and approximately 1.3 million second doses of the COVID-19 Vaccine Moderna have also now been administered. These figures are based on numbers of exposures reported individually by the individual nations which are extrapolated to produce an estimate of the total number of doses. Data are not always reported weekly and can be updated for historical dates when vaccinations are recorded on the relevant system. Therefore, data for this may be incomplete and the resulting estimates are approximate.

The estimated number of doses administered differs from the estimated number of people vaccinated due to the different data sources used.

As of 24 November 2021, an estimated 16,383,575 people had received their booster or third vaccination in the UK. The priority groups being offered a booster dose of coronavirus (COVID-19) vaccine for this part of the vaccination campaign include people aged 40 years and over, health and social care workers and the clinically vulnerable. On 29 November 2021 the JCVI announced those aged 18 to 39 will also be eligible for a booster when the NHS calls them forward.

Table 3: Number of people who have received the third or booster dose of a vaccination for COVID-19 in the UK between 8 December 2020 and end of 24 November 2021.

Country	Number of doses
England	13,697,204
Wales	806,297
Northern Ireland	344,451
Scotland	1,535,623

Yellow Card reporting trends

A report of a suspected ADR to the Yellow Card scheme does not necessarily mean that it was caused by the vaccine, only that the reporter has a suspicion it may have been. Underlying or previously undiagnosed illness unrelated to vaccination can also be factors in such reports. The relative number and nature of reports should therefore not be used to compare the safety of the different vaccines. The MHRA may also refer to 'cases' as opposed to 'reports' within the analysis of the Yellow Card data; these typically refer to ADR reports that have undergone medical assessment and are considered to meet certain criteria for diagnosis of the reported event and have at least a plausible association with the vaccine. All cases and reports are kept under continual review in order to identify possible new risks.

Up to and including 24 November 2021, the MHRA received and analysed 136,582 UK Yellow Cards from people who have received the COVID-19 Pfizer/BioNTech Vaccine. These reports include a total of 388,618 suspected reactions (i.e. a single report may contain more than one symptom). The first report was received on 9 December 2020.

Up to and including 24 November 2021, the MHRA received and analysed a total of 238,086 UK reports of suspected ADRs to the COVID-19 Vaccine AstraZeneca. These reports include a total of 844,212 suspected reactions (a single report may contain more than one symptom). The first report was received on 4 January 2021.

Up to and including 24 November 2021, the MHRA received and analysed a total of 19,101 UK reports of suspected ADRs to the COVID-19 Vaccine Moderna. These include a total 62,126 suspected reactions (a single report may contain more than one symptom). The first report was received on 7 April 2021.

Additionally, up to and including 24 November 2021, the MHRA received 1,280 Yellow Card reports where the brand of vaccine was not specified by the reporter.

In the week since the previous summary for 17 November 2021 we have received a further 2,631 Yellow Cards for the COVID-19 Pfizer/BioNTech Vaccine, 599 for the COVID-19 Vaccine AstraZeneca, 985 for the COVID-19 Vaccine Moderna and 26 where the brand was not specified.

It is important to note that Yellow Card data cannot be used to derive side effect rates or compare the safety profile of COVID-19 vaccines as many factors can influence ADR reporting.

Table 4: Number of suspected ADR reports received in the UK up to and including 17 November 2021.

Country	Number of reports COVID-19 Pfizer/BioNTech Vaccine	Number of reports COVID-19 Vaccine/AstraZeneca	Number of reports COVID-19 Vaccine Moderna	Number of reports Brand unspecified
England	106,282	196,744	15,584	768
Wales	6,926	10,513	534	70
Northern Ireland	2,537	2,913	132	16
Scotland	10,423	16,966	2,019	140

The figures in Table 4 are based upon the postcode provided by the reporter. The sums of the reports in the table will not equal the total reports received for each vaccine as postcode may not have always been provided or may have been entered incorrectly. It is important to note that the number of reports received for each country does not directly equate to the number of people who may have experienced adverse reactions and therefore cannot be used to determine the incidence of reactions. ADR reporting rates are influenced by many aspects, including the extent of use.

We are working with public health bodies and encouraging all healthcare professionals and patients alike to report any suspected ADRs to the Yellow Card scheme. As expected, reports gradually increase in line with an increase in doses administered.

The overall reporting rate is in the order of 3 to 7* Yellow Cards per 1,000 doses administered for the COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca and COVID-19 Vaccine Moderna. It is known from the clinical trials that the more common side effects for all vaccines can occur at a rate of more than one in 10 doses (for example, local reactions or symptoms resembling transient flu-like symptoms).

3. Analysis of data

One of the MHRA's main roles is to continually monitor the safety of medicines and vaccines during widespread use, and we have in place a proactive strategy to do this (https://www.gov.uk/government/publications/report-of-the-commission-on-human-medicines-expert-working-group-on-covid-19-vaccine-safety-surveillance) for COVID-19 vaccines. We also work closely with our public health partners in reviewing the effectiveness and impact that the vaccines are having to ensure benefits continue to outweigh any possible side effects. In addition, we work with our international counterparts to gather information on the safety of vaccines in other countries.

Given the huge scale of the COVID-19 immunisation programme, with many millions of doses of vaccines administered over a relatively short time period, vigilance needs to be continuous, proactive and as near real-time as is possible. The importance of this is two-fold. First we need to rapidly detect, confirm, and quantify any new risks and weigh these against the expected benefits. We then can take any necessary action to minimise risks to individuals.

Secondly, we need to very quickly establish if any serious medical events which are temporally-related to vaccination are merely a coincidental association. These associations are likely while we are still in the midst of a major national vaccination programme, and because many of the millions of people offered the vaccine in the early phase of a vaccination campaign were elderly and/or had underlying medical conditions, which increases the likelihood of unrelated illnesses occurring soon after vaccination. As mentioned above, the nature of Yellow Card reporting means that reported events are not always proven adverse reactions, and some may have happened regardless of vaccination.

Yellow Card reports of suspected ADRs are evaluated, together with additional sources of evidence, by a team of safety experts to identify any new safety issues or side effects. We apply statistical techniques that can tell us if we are seeing more events than we would expect to see, based on what is known about background rates of illness in the absence of vaccination. This aims to account for factors such as coincidental illness. We also look at the clinical characteristics to see if new patterns of illness are emerging that could indicate a new safety concern.

We supplement this form of safety monitoring with other epidemiology studies including analysis of data on national vaccine usage, anonymised GP-based electronic healthcare records and other healthcare data to proactively monitor safety. We also take into account the international experience based on data from other countries using the same vaccines. These combined safety data enable the MHRA to detect side effects or safety issues associated with COVID-19 vaccines. As well as confirming new risks, an equally important objective of monitoring will be to quickly rule out risks – in other words to confirm that the vaccine is not responsible for a suspected side effect and to provide reassurance on its safety.

Overall safety

As with any vaccine, COVID-19 vaccines will cause side effects in some people. The total number and the nature of the majority of Yellow Cards received so far is not unusual for a new vaccine for which members of the public and healthcare professionals are encouraged to report any suspected adverse reaction.

As highlighted above, it is known from the clinical trials that the most common side effects for all vaccines can occur at a rate of more than one per 10 doses (such as local reactions, symptoms resembling transient flu-like symptoms). Overall, Yellow Card reporting is therefore lower than the reporting rate of possible side effects from the clinical trials, although we generally do not expect all suspected side effects to be reported on Yellow Cards. The primary purpose of Yellow Card reporting is to detect new safety concerns.

For all COVID-19 vaccines, detailed review of all reports has found that the overwhelming majority relate to injection site reactions (sore arm for example) and generalised symptoms such as a 'flu-like' illness, headache, chills, fatigue (tiredness), nausea (feeling sick), fever, dizziness, weakness, aching muscles, and rapid heartbeat. Generally, these happen shortly after the vaccination and are not associated with more serious or lasting illness. These types of reaction reflect the acute immune response triggered by the body to the vaccines, are typically seen with most types of vaccine and tend to resolve within a day or two. The nature of reported suspected ADRs across all ages is broadly similar, although, as seen in the clinical trials and as is usually seen with other vaccines, they may be reported more frequently in younger adults.

As we receive more reports of these types of reactions with more exposure to the COVID-19 vaccines, we are building a picture of how individuals are experiencing them and the different ways that side effects may present in people. Some people have reported a sudden feeling of cold with shivering/shaking accompanied by a rise in temperature, often with sweating, headache (including migraine-like headaches), nausea, muscle aches and feeling unwell, starting within a day of having the vaccine. Similar to the flu like illness reported in clinical trials, these effects may last a day or two.

It is important to note that it is possible to have caught COVID-19 and not realise until after vaccination. If other COVID symptoms are experienced or fever is high and lasts longer than two or three days, vaccine recipients should stay at home and arrange to have a test.

A number of detailed assessments of safety topics have been undertaken and we have updated our advice on these topics accordingly. Overall, our advice remains that the benefits of the vaccines outweigh the risks in the majority of people. Further comments on use in specific populations and details on the following safety topics can be found below.

Comments on safety in specific populations

Safety of COVID-19 vaccines in pregnancy

The MHRA closely monitors the safety of COVID-19 vaccine exposures in pregnancy, including published information as well as Yellow Card reports for COVID-19 vaccines used in pregnancy. These reports have been reviewed by the independent experts of the Commission on Human Medicines' COVID-19 Vaccines Benefit Risk Expert Working Group and by the Medicines for Women's Health Expert Advisory Group (MWHEAG).

Pregnant women have the same risk of getting COVID-19 as non-pregnant women but they may be at an increased risk of becoming severely ill, particularly if they get infected in the third trimester or if they also have underlying medical problems, compared to non-pregnant women. The current advice of the Joint Committee on Vaccination and Immunisation (JCVI) is that the COVID-19 vaccines, including booster doses, should be offered to those who are pregnant at the same time as non-pregnant individuals based on their age and clinical risk group. The COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna vaccines are currently the preferred vaccines for use during pregnancy and can be given at any stage in pregnancy.

The numbers of Yellow Card reports for pregnant women are low in relation to the number of pregnant women who have received COVID-19 vaccines to date (more than 104,000 up to end of September 2021 in England, Scotland and Wales). Pregnant women have reported similar suspected reactions to the vaccines as people who are not pregnant. Reports of miscarriage and stillbirth are also low in comparison to how commonly these events occurred in the UK outside of the pandemic. A few reports of commonly occurring congenital anomalies and obstetric events have also been received. There is no pattern from the reports to suggest that any of the COVID-19 vaccines used in the UK, or any reactions to these vaccines, increase the risk of miscarriage, stillbirths, congenital anomalies or birth complications.

Sadly, miscarriage is estimated to occur in about 20 to 25 in 100 pregnancies in the UK and most occur in the first 12 to 13 weeks of pregnancy (the first trimester). Newly published studies from the USA (1) and Norway (2) have compared miscarriage rates for vaccinated and unvaccinated women who were pregnant over the same time periods. The studies included data from a large number of women (more than 15,000) who received the COVID-19 Pfizer/BioNTech Vaccine or COVID-19 Vaccine Moderna. Both studies found that the occurrence of miscarriage was equally likely amongst unvaccinated women as amongst women at the same stage of pregnancy who were vaccinated in the previous 3 to 5 weeks. These studies provide strong evidence for no increased risk of miscarriage in association with the mRNA vaccines in current use. Data on the COVID-19 Vaccine AstraZeneca is less extensive but is consistent with these findings.

Evidence for pregnancy outcomes other than miscarriage is accumulating as more pregnancies reach full term. Currently available evidence does not suggest any increased risks of pregnancy complications, stillbirths, preterm births or adverse neonatal outcomes following vaccination in later pregnancy.

Stillbirths are sadly estimated to occur in about 1 in 200 pregnancies in the UK. Information from surveillance by UKHSA (formerly Public Health England) has found similar rates of stillbirth amongst (more than 24,000) women who were vaccinated during pregnancy and those who gave birth over the same period and were unvaccinated. Likewise, surveillance by Public Health Scotland has found similar rates of perinatal mortality (including stillbirths) amongst (more than 3,800) women who were vaccinated during pregnancy and those who gave birth over the same period and were unvaccinated.

Although, like most vaccines and medicines, clinical trials of COVID-19 vaccines in pregnant women were not carried out prior to use of the vaccines in the general population, there is now growing evidence from clinical use which provides reassurance on the safety of the vaccines in pregnancy. This adds to the evidence from non-

clinical studies of the COVID-19 vaccines which have not raised any concerns about safety in pregnancy. The COVID-19 vaccines do not contain organisms that can multiply in the body, so they cannot infect an unborn baby in the womb.

The MHRA will continue to closely monitor safety data for use of the COVID-19 vaccines in pregnancy, including through evaluation of electronic healthcare record data.

(1) Kharbanda EO, et al. Spontaneous abortion following COVID-19 vaccination during pregnancy. JAMA. doi:10.1001/jama.2021.15494 (2) Magnus, MC et al. Covid-19 Vaccination during Pregnancy and First-Trimester Miscarriage N Engl J Med 2021; 385:2008-2010 DOI: 10.1056/NEJMc2114466

Safety of COVID-19 vaccines in those breastfeeding

The MHRA closely monitors the safety of COVID-19 vaccines during breastfeeding, including evaluation of Yellow Card reports for COVID-19 vaccines from breastfeeding women. These reports have been reviewed by the independent experts of the Commission on Human Medicines' COVID-19 Vaccines Benefit Risk Expert Working Group, by paediatric and breastfeeding experts.

There is no current evidence that COVID-19 vaccination while breastfeeding causes any harm to breastfed children or affects the ability to breastfeed.

COVID-19 vaccines do not contain live components and there is no known risk associated with being given a non-live vaccine whilst breastfeeding. The current advice of the Joint Committee on Vaccination and Immunisation (JCVI) is that breastfeeding parents may be offered any suitable COVID-19 vaccine depending on their age.

We have received about 3,500 Yellow card reports from women breastfeeding at the time of vaccination. Most of these women reported only suspected reactions in themselves which were similar to reports for the general population, with no effects reported on their milk supply or in their breastfed children.

A small number of women have reported decreases in their milk supply, most of which were transient, or possible reactions in their breastfed child. A number of factors can affect milk supply and infant behaviour, including general maternal health, amount of sleep, and anxiety. The symptoms reported for the children (high temperature, rash, diarrhoea, vomiting and general irritability) are common conditions in children of this age, so some of the effects reported may have occurred by coincidence.

A small number of women may experience a reduction in their breast milk production and it may be helpful for breastfeeding women to know how to maintain their breast milk supply, particularly if they are feeling unwell. The NHS website has a good resource for this: https://www.nhs.uk/start4life/baby/breastfeeding/ (https://www.nhs.uk/start4life/baby/breastfeeding/).

Suspected side effects reported in individuals under 18 years old

The MHRA closely monitors the safety of COVID-19 vaccine exposures in individuals under 18 years old, including Yellow Card reports for COVID-19 vaccines used in this age group.

To date there have been an estimated 2.6 million first doses and 417, 500 second doses of the COVID-19 Pfizer/BioNTech Vaccine given to under 18s, approximately 11,500 first doses and 10,000 second doses of the COVID-19 Vaccine AstraZeneca given to this population and 17,800 first doses and 13,600 second doses of the COVID-19 Vaccine Moderna given to individuals under 18.

The MHRA has received 1,873 UK reports of suspected ADRs for the COVID-19 Pfizer/BioNTech Vaccine in which the individual was reported to be under 18, 243 reports for the COVID-19 Vaccine AstraZeneca, 6 for the COVID-19 Vaccine Moderna and 8 where the brand of vaccine was unspecified.

The experience reported in under 18s is similar to that identified in the general population and a review of these reports does not raise any additional safety topics specific to this age group.

There has been a small number of reports for myocarditis and pericarditis (inflammation of the heart) in individuals under 18 years both in the UK and internationally. This is a recognised potential risk with the COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna and the MHRA is closely monitoring these events. Further information surrounding these very rare reports of myocarditis and pericarditis within this population can be found within the specific section on this safety topic later in the summary. We will continue to closely monitor the safety of the COVID-19 vaccines in those under 18 years old.

Suspected side effects reported in individuals receiving a booster vaccination

Safety monitoring plans have been agreed to ensure action can be taken on any emerging safety concerns from supplementary or booster doses.

As of 24 November 2021, over 16 million COVID-19 third doses and booster doses have been administered in the UK.

The MHRA has received 10,460 UK reports of suspected ADRs where the COVID-19 Pfizer/BioNTech Vaccine was reported to be the booster dose, 145 reports where the COVID-19 Vaccine AstraZeneca was reported to be the booster dose, 1,575 reports where the COVID-19 Vaccine Moderna was reported to be the booster dose and 68 reports where the brand of vaccine booster was unspecified. Overall, this represents an overall reporting rate of less than 1 report per 1,000 third or booster doses. This is lower than the reporting rate for COVID-19 vaccines for all vaccine doses combined, which is between 3 to7 reports per 1,000 doses.

The nature of events reported with third and booster doses is similar to that reported for the first two doses of the COVID-19 vaccines, and the vast majority of reports relate to expected reactogenicity events.

Review of third and booster dose reports does not raise any new safety concerns. As part of the MHRA's booster safety monitoring strategy, reports of suspected adverse events following COVID-19 boosters given at the same time as seasonal flu vaccines have been closely monitored, and no new safety concerns have been identified in this data either.

There have been a small number of reports of suspected myocarditis and pericarditis (inflammation of the heart) following booster doses with Pfizer/BioNTech and Moderna COVID-19 vaccines. This is a recognised potential risk with the COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna and the MHRA is closely monitoring these events. The reports after booster doses are extremely rare and there is no indication that these events are more serious after boosters. Further information surrounding these very rare reports of suspected myocarditis and pericarditis can be found within the specific section on this safety topic later in the summary.

We will continue to closely monitor the safety of booster and third doses of the COVID-19 vaccines.

Comments on specific safety topics

The following reports reflect data up to 24 November 2021. The glossary provides an explanation of the clinical terms used.

Anaphylaxis (severe allergic reactions)

On 9 December 2020, the MHRA issued preliminary guidance on severe allergic reactions after administration of the COVID-19 Pfizer/BioNTech Vaccine due to early reports of anaphylaxis. Following further detailed review, this advice was amended on 30 December to the current advice. The advice is that people with a previous history of severe allergic reactions to any ingredients of the vaccine should not receive it. People who receive the vaccine should be monitored for at least 15 minutes afterwards. Similar advice was introduced for the Covid-19 Vaccine Moderna as it uses a similar technology to the Pfizer vaccine.

Widespread use of the vaccine now suggests that severe allergic reactions to the COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna are very rare. Anaphylaxis can also be a very rare side effect associated with most other vaccines.

The MHRA continues to monitor reports of severe allergic reactions with the COVID-19 Pfizer/BioNTech Vaccine and has received 562 UK spontaneous adverse reactions associated with anaphylaxis or anaphylactoid reactions. Severe allergic reactions to the COVID-19 Pfizer/BioNTech Vaccine remain very rare. The MHRA's guidance remains that those with a previous history of allergic reactions to the ingredients of the vaccine should not receive it.

The MHRA is closely monitoring reports of anaphylaxis with the COVID-19 Vaccine Moderna and has received 47 reports of anaphylaxis in association with the vaccine. Anaphylaxis is a potential side effect of the vaccine, and it is recommended that those with known hypersensitivity to the ingredients of the vaccine should not receive it.

The MHRA also closely monitors reports of anaphylaxis or anaphylactoid reactions with the COVID-19 Vaccine AstraZeneca and has received 845 UK spontaneous adverse reactions associated with anaphylaxis or anaphylactoid reactions reported and such reports are very rare. The product information reflects the fact that reports of anaphylaxis have been received for the COVID-19 Vaccine AstraZeneca.

Bell's Palsy

MHRA continues to review reports of suspected Bell's Palsy and to analyse them against the number expected to occur by chance in the absence of vaccination (the 'natural rate'). The number of reports of facial paralysis received so far is similar to the expected natural rate and does not currently suggest an increased risk following the vaccines. We will continue to monitor these events, including through evaluation of electronic healthcare record data.

Thrombo-embolic (blood clotting) events with concurrent low platelets

The MHRA has undertaken a thorough review into UK cases of an extremely rare and unlikely to occur specific type of blood clot in the brain, known as cerebral venous sinus thrombosis (CVST) occurring together with low levels of platelets (thrombocytopenia) following vaccination with the COVID-19 Vaccine AstraZeneca. It has also considered other blood clotting reports (thromboembolic events) alongside low platelet levels.

This scientific review concluded that the evidence of a link with COVID-19 Vaccine AstraZeneca is likely and an announcement was made on 7 April 2021 with a further statement on 7 May 2021. We have continued to publish the latest breakdown of all cases of these extremely rare side effects on a weekly basis.

Anyone who experienced cerebral or other major blood clots occurring with low levels of platelets after their first vaccine dose of COVID-19 Vaccine AstraZeneca should not have further doses. Anyone who did not have these side effects should come forward for their second dose when invited.

The MHRA has also confirmed that the evidence to date does not suggest that the COVID-19 Vaccine AstraZeneca causes venous thromboembolism which occurred in the absence of a low platelet count.

Anyone who experiences any of the following from around 4 days after vaccination should seek medical advice urgently:

- a severe headache that is not relieved with simple painkillers or gets worse or feels worse when you lie down or bend over
- an unusual headache that may be accompanied by blurred vision, confusion, difficulty with speech, weakness, drowsiness or seizures (fits)
- rash that looks like small bruises or bleeding under the skin beyond the injection site

- shortness of breath, chest pain, leg swelling or persistent abdominal (tummy) pain.

Up to 24 November 2021, the MHRA had received Yellow Card reports of 427 cases of major thromboembolic events (blood clots) with concurrent thrombocytopenia (low platelet counts) in the UK following vaccination with COVID-19 Vaccine AstraZeneca. Forty-seven of the 426 reports have been reported after a second dose. Of the 427 reports, 215 occurred in females, and 208 occurred in males aged from 18 to 93 years. The overall case fatality rate was 17% with 74 deaths, six of which occurred after the second dose.

Cerebral venous sinus thrombosis was reported in 155 cases (average age 46 years) and 272 had other major thromboembolic events (average age 54 years) with concurrent thrombocytopenia. The estimated number of first doses of COVID-19 Vaccine AstraZeneca administered in the UK by 24 November was 24.9 million and the estimated number of second doses was 24.1 million.

The overall incidence after first or unknown doses was 15.3 per million doses. Taking into account the different numbers of patients vaccinated with COVID-19 Vaccine AstraZeneca in different age groups, the data indicates that there is a higher reported incidence rate in the younger adult age groups following the first dose compared to the older groups (21.1 per million doses in those aged 18-49 years compared to 11.0 per million doses in those aged 50 years and over). The number of first doses given to those in the 18-49 years age group is estimated to be 8.5 million while an estimated 16.3 million first doses have been given to patients aged 50+ years. The MHRA advises that this evidence should be taken into account when considering the use of the vaccine. There is some evidence that the reported incidence rate is higher in females compared to men although this is not seen across all age groups and the difference remains small.

The overall incidence of thromboembolic events with concurrent low platelets after second doses was 2.0 cases per million doses. Taking into account the different numbers of patients vaccinated with COVID-19 Vaccine AstraZeneca in different age groups, the data indicates that there is a lower reported incidence rate in younger adult age groups following the second dose compared to the older groups (1.0 per million doses in those aged 18-49 years compared to 2.0 per million doses in those aged 50 years and over). The number of second doses given to those in the 18-49 years age group is estimated to be 8.2 million while an estimated 15.9 million second doses have been given to patients aged 50+ years. These rates after second doses should not be directly compared to the incidence rates reported after the first dose as the time for follow-up and identification of cases after second doses is more limited and differs across age groups. However, the data are reassuring, particularly regarding younger recipients where there is a significantly lower incidence after the second dose compared to the first, and there is overall no indication of an increased risk of these events after the second dose in any age group. Anyone who did not have these side effects should come forward for their second dose when invited.

These cases have also been analysed by the government's independent advisory body, the COVID-19 Vaccines Benefit Risk Expert Working Group, which includes lay representatives and advice from leading haematologists. On the basis of this ongoing review, the advice remains that the benefits of the vaccine outweigh the risks in the majority of people.

Table 5: Number of suspected thrombo-embolic events with concurrent thrombocytopenia ADR cases received for the Oxford University/AstraZeneca vaccine in the UK up to and including 10 November 2021

Country	Number of cases
England	331
Wales	13
Northern Ireland	10

Country	Number of cases
Scotland	35
Unknown	38

Table 6: Number of UK suspected thrombo-embolic events with concurrent thrombocytopenia ADR cases received for the COVID-19 Vaccine AstraZeneca by patient age up to and including 24 November 2021.

Age range (years)	Number of cases	Number of fatal cases
18-29	30	7
30-39	50	11
40-49	108	13
50-59	101	19
60-69	61	10
70-79	40	7
80-89	6	3
90-99	2	1
Unknown	29	3
Total	427	74

Table 7: Number of UK suspected thrombo-embolic events with concurrent thrombocytopenia ADR cases received for the COVID-19 Vaccine AstraZeneca by patient sex up to and including 24 November 2021.

Sex	Number of cases	Number of fatal cases
Male	208	33
Female	215	41
Unknown	4	0
Total	427	74

Up to 24 November 2021, the MHRA had received Yellow Card reports of 27 cases of major thromboembolic events (blood clots) with concurrent thrombocytopenia (low platelet counts) in the UK following use of the COVID-19 Pfizer/BioNTech vaccine. These events occurred in 10 females, and 17 males aged from 18 to 91

years, and the overall case fatality rate was 15% with four deaths reported.

Up to 24 November 2021, the MHRA had received Yellow Card reports of 3 cases of major thromboembolic events (blood clots) with concurrent thrombocytopenia (low platelet counts) in the UK following the use of COVID-19 vaccine Moderna. The 3 events occurred in adult males under the age of 60, and there have been no fatal cases reported.

To note, direct comparison of the summary provided here and the analysis profiles is not possible. This is because this summary includes reports of CVST or other thrombo-embolic events with concurrent thrombocytopenia. Yellow Card reports may contain more than one reported reaction and the analysis profiles are listed by individual reactions rather than whole reports. Therefore, summing the reactions listed in the profiles will not equate to the total cases included within this summary.

Capillary Leak Syndrome

The MHRA has received 14 reports of suspected capillary leak syndrome (a condition where fluid leaks from the small blood vessels into the body) in the context of more than 48.9 million doses of COVID-19 Vaccine AstraZeneca given. Of these reports, 3 people had a history of capillary leak syndrome. This is an extremely rare relapsing-remitting condition and triggers for relapses are not well understood. As a precautionary measure, the MHRA is advising that COVID-19 Vaccine AstraZeneca is not used in people who have previously experienced episodes of capillary leak syndrome. The product information has been updated to reflect this advice.

Menstrual disorders (period problems) and unexpected vaginal bleeding

The MHRA is reviewing reports of suspected side effects menstrual disorders (period problems) and unexpected vaginal bleeding following vaccination against COVID-19 in the UK. These reports are also being reviewed by the independent experts of the Commission on Human Medicines' COVID-19 Vaccines Benefit Risk Expert Working Group and the Medicines for Women's Health Expert Advisory Group. The rigorous evaluation completed to date does not support a link between changes to menstrual periods and related symptoms and COVID-19 vaccines.

A total of 42,325 suspected reactions relating to a variety of menstrual disorders have been reported after all three of the COVID-19 vaccines including heavier than usual periods, delayed periods and unexpected vaginal bleeding. These suspected reactions have been reported in 33,006 individual Yellow Card reports (as each report may contain more than one suspected reaction). This is following approximately 50.2 million COVID-19 vaccine doses administered to women up to 24 November 2021. The number of reports of menstrual disorders and vaginal bleeding is low in relation to both the number of people who have received COVID-19 vaccines to date and how common menstrual disorders are generally.

The menstrual changes reported are mostly transient in nature. There is no evidence to suggest that COVID-19 vaccines will affect fertility and your ability to have children.

Whilst uncomfortable or distressing, period problems are extremely common and stressful life events can disrupt menstrual periods. Changes to the menstrual cycle have also been reported following infection with COVID-19 and in people affected by long-COVID. General advice about period problems and/or unexpected vaginal bleeding is available from the NHS website (https://www.nhs.uk/). It is important that anyone experiencing changes to their periods that are unusual for them, persist over time, or has any new vaginal bleeding after the menopause, following COVID-19 vaccination, should contact their doctor. Anyone presenting with menstrual disorders and/or unexpected vaginal bleeding following COVID-19 vaccination should be treated according to clinical guidelines for these conditions, as usual.

The MHRA continues to closely review reports of suspected side effects of menstrual disorders and unexpected vaginal bleeding.

Myocarditis and pericarditis (Inflammation of the heart)

The MHRA has undertaken a thorough review of both UK and international reports of suspected myocarditis and pericarditis following vaccination against COVID-19. There has been a consistent pattern of higher reporting of these suspected events with the COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna, and of these occurring more frequently in males. In the UK the body of evidence shows that for Pfizer in particular, there is similar frequency of reporting after the first and second dose, with suspected events typically occurring within a short time after vaccination. These reports have also been analysed by the government's independent advisory body, the Commission on Human Medicines (CHM) and its COVID-19 Vaccines Benefit Risk Expert Working Group. Following their advice, the product information for the COVID-19 Vaccine Moderna and the COVID-19 Pfizer/BioNTech Vaccine was updated to inform of these reports and advise healthcare professionals and patients to be aware of important symptoms for myocarditis and pericarditis.

These reports are extremely rare, and the events reported are typically mild with individuals usually recovering within a short time with standard treatment and rest. People should come forward for their first and second vaccination when invited to do so, unless advised otherwise.

It is important that anyone who experiences new onset of symptoms such as chest pain, shortness of breath or feelings of having a fast-beating, fluttering, or pounding heart seeks medical attention.

Up to and including 24 November 2021, we have received 450 reports of myocarditis and 341 reports of pericarditis following use of the COVID-19 Pfizer/BioNTech Vaccine, as well as four reports of carditis, three reports each of viral pericarditis, and viral myocarditis, two reports for infective pericarditis, and one report each of constrictive pericarditis, non-infective endocarditis and streptococcal endocarditis. For COVID-19 Vaccine AstraZeneca there have been 161 reports of myocarditis and 192 reports of pericarditis following vaccination up to and including 24 November 2021 as well as seven eight reports for endocarditis, five reports for viral pericarditis, two reports each for endocarditis bacterial, carditis, viral myocarditis and acute endocarditis, and one report each for infectious myocarditis and autoimmune myocarditis. There have been 103 reports of myocarditis, 59 reports of pericarditis and one report each of hypersensitivity myocarditis and endocarditis following use of COVID-19 Vaccine Moderna up to the same date. Three fatal events have been reported with the COVID-19 Pfizer/BioNTech Vaccine and two fatal events with the COVID-19 Vaccine AstraZeneca. There have been no fatal myocarditis or pericarditis events reported with the COVID-19 Vaccine Moderna to date. Fatal events are being monitored closely and are carefully followed up to gather relevant information. The majority of fatal reports describe underlying illnesses in these patients that could provide alternative explanations for the events reported.

Based on reports of suspected ADRs in the UK, the overall reporting rate across all age groups for suspected myocarditis (including viral myocarditis), after both first and second dose, is 10 reports per million doses of COVID-19 Pfizer/BioNTech Vaccine and for suspected pericarditis (including viral pericarditis and infective pericarditis) the overall reporting rate is 7 reports per million doses. For COVID-19 Vaccine Moderna, the overall reporting rate for suspected myocarditis is 37 per million doses and for suspected pericarditis is 21 per million doses. For COVID-19 Vaccine AstraZeneca the overall reporting rate for suspected myocarditis (including viral myocarditis and infectious myocarditis) is 3 per million doses and for suspected pericarditis (including viral pericarditis) is 4 per million doses. It should be noted that more than one event can be included in each report.

When the reporting rate is calculated by age group (see Table 8) the reporting rate for suspected myocarditis and pericarditis is highest in the 18-29-year age group for the Pfizer/BioNTech and Moderna COVID-19 vaccines. A more even spread in reporting rates across the age groups is seen for AstraZeneca COVID-19 vaccine. For all vaccines there is a trend for decreased reporting in the older age groups. Pfizer/BioNTech is currently the preferred COVID-19 vaccine for the under 18s age group in the UK vaccination programme, and for this vaccine there is no indication in the current data that there is an increased reporting rate of suspected myocarditis and pericarditis in this age group compared to young adults. There are largely similar reporting rates between the first and second doses of the Pfizer/BioNTech and AstraZeneca COVID-19 vaccines. There is greater variability between first and second dose reporting rates with Moderna however the reporting rate estimates for Moderna may lack precision due to the more limited experience with Moderna in the UK and small numbers of suspected reports. This introduces more uncertainty into the data.

It is important not to compare the reporting rates between the different COVID-19 vaccines as many factors can influence ADR reporting. These reporting rates may also be subject to change as more experience is gathered in the UK.

Table 8: Reporting rates per million doses for UK ADR reports of suspected myocarditis and pericarditis associated with COVID-19 vaccines by patient age, and dose, up to and including 24 November 2021.

Age range (years)	COVID-19 Pfizer/BioNTech Vaccine First or unknown dose	COVID-19 Pfizer/BioNTech Vaccine Second dose	COVID-19 Vaccine Moderna First or unknown dose	COVID-19 Vaccine Moderna Second dose	COVID-19 Vaccine AstraZeneca reporting rate First or unknown dose	COVID-19 Vaccine AstraZeneca Second dose
< 18 years	11	Not calculated*	Not applicable**	Not applicable**	Not applicable**	Not applicable**
18-29	21	22	50	70	8	14
30-39	18	20	39	51	10	9
40-49	16	16	38	22	11	7
50-59	5	13	31	34	7	6
60-69+	4	10	Not applicable**	Not applicable**	6	5
70+	3	4	Not applicable**	Not applicable**	4	4

*There is currently insufficient data to calculate a reliable estimate of the reporting rate post-dose 2 in under 18-year-olds in the UK due to, as yet, relatively limited exposure of dose 2 in these individuals. This estimate will be included once sufficient exposure has accumulated.

**There have been no reports of suspected heart inflammation events received for individuals in these age groups

Table 9: Number of UK ADR reports associated with suspected myocarditis, pericarditis and other related terms received for the COVID-19 Vaccine AstraZeneca, COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna by patient age up to and including 24 November 2021.

	Number of reports	Number of reports	Number of reports
Age range (years)	COVID-19 Pfizer/BioNTech Vaccine	COVID-19 Vaccine Moderna	COVID-19 Vaccine AstraZeneca
under 18 years	34	0	0

Age range (years)	Number of reports COVID-19 Pfizer/BioNTech Vaccine	Number of reports COVID-19 Vaccine Moderna	Number of reports COVID-19 Vaccine AstraZeneca
19-29	249	74	26
30-39	199	46	36
40-49	77	14	90
50-59	47	2	83
60+	86	2	85
Unknown	84	20	37
Total	776	158	357

Table 10: Number of UK ADR reports associated with suspected myocarditis, pericarditis and other related terms received for the COVID-19 Vaccine AstraZeneca, COVID-19 Pfizer/BioNTech Vaccine and COVID-19 Vaccine Moderna by patient sex up to and including 24 November 2021.

Age range (years)	Number of reports COVID-19 Pfizer/BioNTech Vaccine	Number of reports COVID-19 Vaccine Moderna	Number of reports COVID-19 Vaccine AstraZeneca
Female	308	43	163
Male	442	109	185
Unknown	26	6	9
Total	776	158	357

Myocarditis and pericarditis happen very rarely in the general population, and it is estimated that in the UK there are about 60 new cases of myocarditis diagnosed per million patients per year and about 100 new cases of pericarditis diagnosed per million patients per year.

The MHRA will continue to closely monitor reports of suspected myocarditis and pericarditis with all of the currently authorised COVID-19 vaccines.

Delayed hypersensitivity reactions

The MHRA has been reviewing reports of skin reactions occurring around the vaccination site that appear a little while after vaccination. These reactions are suggestive of a delayed hypersensitivity reaction that occurs 4-11 days after vaccination. The reactions are characterized by a rash, swelling and tenderness that can cover the

whole upper arm and may be itchy and/or painful and warm to the touch. The majority of the reports received have been with COVID-19 Vaccine Moderna and the product information for this vaccine has been updated to highlight the possibility of delayed injection site reactions.

The reactions are usually self-limiting and resolve within a day or two, although in some patients it can take slightly longer to disappear. Individuals who experience this reaction after their first dose may experience a similar reaction in shorter timeframe following the second dose, however, none of the reports received have been serious and people should still take their second dose when invited. Those who experience delayed skin reactions after their COVID-19 vaccination which do not resolve within a few days should seek medical advice.

Guillain-Barré Syndrome

Guillain-Barré Syndrome is a very rare condition which causes inflammation of the nerves and can lead to numbness, weakness and pain, usually in the feet, hands and limbs and can spread to the chest and face. Guillain-Barré Syndrome tends to affect both sides of the body at once. This condition is known to be associated with certain infectious diseases.

Up to and including the 24 November 2021, the MHRA has received 463 reports of Guillain-Barré Syndrome with the COVID-19 Vaccine AstraZeneca and 26 reports of a related disease called Miller Fisher syndrome. Up to the same date, the MHRA has received 68 reports of Guillain-Barre Syndrome following use of the COVID-19 Pfizer/BioNTech Vaccine and 1 report of Miller Fisher syndrome and for the COVID-19 Vaccine Moderna there have been seven reports of Guillain-Barré Syndrome.

The MHRA has been closely monitoring and assessing reports of suspected Guillain-Barré Syndrome received following administration of the COVID-19 vaccine. Following the most recent review of the available data the evidence of a possible association has strengthened. Therefore, following advice from the government's independent advisory body, the Commission on Human Medicines (CHM) and its COVID-19 Vaccines Benefit Risk Expert Working Group, the product information for the COVID-19 Vaccine AstraZeneca was further updated to include GBS in the tabulated list of adverse reactions associated with the COVID-19 Vaccine AstraZeneca and to encourage healthcare professionals and the public to look out for signs of GBS.

The MHRA will continue to review reports of Guillain-Barré Syndrome received following vaccination with COVID-19 vaccines to further assess a possible association between Guillain-Barré Syndrome and COVID-19 vaccines, with independent advice from its Vaccine Benefit-Risk Working Group.

Swelling of the vaccinated limb

There have been rare reports of extensive swelling of the vaccinated limb after receiving the COVID-19 Pfizer/BioNTech Vaccine. The product information has been updated to include "extensive swelling of the vaccinated limb" as a side effect of the vaccine. This type of swelling is also recognised to occur with other (non-COVID-19) vaccines.

Facial swelling in those with a history of facial dermal fillers

Rare reports of facial swelling occurring 1-2 days after vaccination in vaccine recipients with a history of injection of facial dermal fillers were observed in the clinical trials for the COVID-19 Vaccine Moderna. Information about this possible side effect has been included in the product information for the COVID-19 Vaccine Moderna since it was first authorised for use.

The MHRA has also received Yellow Card reports of facial swelling in those with a history of injection of facial dermal fillers for the COVID-19 Pfizer/BioNTech Vaccine. A recent review of the world-wide ADR data for the COVID-19 Pfizer/BioNTech Vaccine vaccine found that, in most instances, the facial swelling was mild, transient and was localised to the site of the dermal filler. The product information for the COVID-19 Pfizer/BioNTech Vaccine has been updated to include facial swelling in those with a history of injection of facial dermatological fillers as a side effect of the vaccine.

Events with a fatal outcome

Vaccination and surveillance of large populations means that, by chance, some people will experience and report a new illness or events in the days and weeks after vaccination. A high proportion of people vaccinated early in the vaccination campaign were very elderly, and/or had pre-existing medical conditions. Older age and chronic underlying illnesses make it more likely that coincidental adverse events will occur, especially given the millions of people vaccinated. It is therefore important that we carefully review these reports to distinguish possible side effects from illness that would have occurred irrespective of vaccination.

Part of our continuous analysis includes an evaluation of natural death rates over time, to determine if any specific trends or patterns are occurring that might indicate a vaccine safety concern. Based on age-stratified all-cause mortality in England and Wales taken from the Office for National Statistics death registrations (https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/datasets/deathsregisteredinenglandandwalesseriesdrreferencetables), several thousand deaths are expected to have occurred, naturally, within 7 days of the many millions of doses of vaccines administered so far, mostly in the elderly.

The MHRA has received 628 UK reports of suspected ADRs to the COVID-19 Pfizer/BioNTech Vaccine in which the patient died shortly after vaccination, 1,136 reports for the COVID-19 Vaccine AstraZeneca, 19 for the COVID-18 Vaccine Moderna and 32 where the brand of vaccine was unspecified. The majority of these reports were in elderly people or people with underlying illness. Usage of the vaccines has increased over the course of the campaigns and as such, so has reporting of fatal events with a temporal association with vaccination. However, this does not mean that there is a link between vaccination and the fatalities reported. Review of specific fatal reports is provided in the summaries above. The pattern of reporting for all other fatal reports does not suggest the vaccines played a role in these deaths.

A range of other isolated events or series of reports of non-fatal, serious suspected ADRs have been reported. These all remain under continual review, including thorough analysis of expected rates in the absence of vaccine. There are currently no indications of specific patterns or rates of reporting that would suggest the vaccine has played a role.

4. Conclusion

At the time of this report, over 144,432 people across the UK have died within 28 days of a positive test for coronavirus.

Vaccination is the single most effective way to reduce deaths and severe illness from COVID-19. A national immunisation campaign has been underway since early December 2020.

In clinical trials (https://www.gov.uk/government/collections/mhra-guidance-on-coronavirus-covid-19#vaccines-and-vaccine-safety), the COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca and COVID-19 Vaccine Moderna have demonstrated very high levels of protection against symptomatic infection. Data are available (https://www.gov.uk/government/publications/phe-monitoring-of-the-effectiveness-of-covid-19-vaccination) on the impact of the vaccination campaign in reducing infections and illness in the UK.

All vaccines and medicines have some side effects. These side effects need to be continuously balanced against the expected benefits in preventing illness.

Following widespread use of these vaccines across the UK, the vast majority of suspected adverse reaction reports confirm the safety profile seen in clinical trials. Most reports relate to injection-site reactions (sore arm for example) and generalised symptoms such as a 'flu-like' illness, headache, chills, fatigue, nausea, fever, dizziness, weakness, aching muscles, and rapid heartbeat. Generally, these reactions are not associated with more serious illness and likely reflect an expected, normal immune response to the vaccines.

The expected benefits of the vaccines in preventing COVID-19 and serious complications associated with COVID-19 far outweigh any currently known side effects. As with all vaccines and medicines, the safety of COVID-19 vaccines is continuously monitored and benefits and possible risks remain under review.

We take every report of a suspected ADR seriously and encourage everyone to report through the Yellow Card scheme.

Annex 1: Vaccine Analysis Print

The attached Vaccine Analysis Prints contain a complete listing of all suspected adverse reactions that have been reported to the MHRA via the Yellow Card scheme for the COVID-19 Pfizer/BioNTech Vaccine, COVID-19 Vaccine AstraZeneca, COVID-19 Vaccine Moderna and where the brand of the vaccine was not specified. This includes all reports received from healthcare professionals, members of the public, and pharmaceutical companies.

This information does not represent an overview of the potential side effects associated with the vaccines. A list of the recognised adverse effects of COVID-19 vaccines is provided in the information for healthcare professionals (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-astrazeneca/information-for-healthcare-professionals-on-covid-19-vaccine-astrazeneca) and the recipient information (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-astrazeneca/information-for-uk-recipients-on-covid-19-vaccine-astrazeneca). These can also be found on the Coronavirus Yellow Card reporting site (https://coronavirus-yellowcard.mhra.gov.uk/). Conclusions on the safety and risks of the vaccines cannot be made on the data shown in the Profile alone.

When viewing the vaccine analysis profile you should remember that:

- Reporters are asked to submit Yellow Card reports even if they only have a suspicion that the medicine or vaccine may have caused the adverse reaction. The existence of an adverse reaction report in the profile does not necessarily mean that the vaccine has caused the suspected reaction.
- It may be difficult to tell the difference between something that has occurred naturally and a suspected adverse reaction. Sometimes these events can be part of the condition being treated rather than being caused by the vaccine.
- Many factors have to be considered when assessing whether the vaccine has caused a reported adverse reaction. When monitoring the safety of vaccines and medicines, MHRA staff carry out careful analysis of these factors.

For a medicine or vaccine to be considered safe, the expected benefits will be greater than the risk of having harmful reactions. It is important to note that most people take medicines and vaccines without having any serious side effects.

Vaccine Analysis Print - COVID-19 Pfizer/BioNTech Vaccine
(https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1038175/COVID-19_mRNA_Pfizer-BioNTech_Vaccine_Analysis_Print_DLP_24.11.2021.pdf)

Vaccine Analysis Print - COVID-19 Vaccine AstraZeneca
(https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1038176/COVID-19_AstraZeneca_Vaccine_Analysis_Print_DLP_24.11.2021.pdf)

Vaccine Analysis Print - COVID-19 Vaccine Moderna
(https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1038177/COVID-19_Moderna_Vaccine_Analysis_Print_DLP_24.11.2021.pdf)

Vaccine Analysis Print - Brand unspecified
(https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1038178/COVID-19_Brand_unspecified_Vaccine_Analysis_Print_DLP_24.11.2021.pdf)

Annex 2 Glossary

Anaphylaxis or anaphylactoid reactions

Anaphylaxis is a severe and potentially life-threatening allergic reaction. These reactions can occur after an exposure to a trigger, such as a certain ingredient in foods or medicines or an insect sting. Anaphylaxis and anaphylactoid reactions can be treated with adrenaline.

Bell's palsy

Bell's palsy is a condition that causes temporary weakness or paralysis (lack of movement) of the muscles in one side of the face. It is the most common cause of facial paralysis. For most people, the facial paralysis is temporary. Viral infections such as those with herpes viruses have been linked to Bell's palsy.

Booster dose/vaccination

A COVID-19 booster vaccine dose helps improve the protection obtained from the first two doses of the vaccine. It helps give longer-term protection against getting seriously ill from COVID-19.

Capillary Leak Syndrome (CLS)

Capillary Leak Syndrome (CLS) occurs when fluid leaks from the small blood vessels into the body.

Cerebral venous sinus thrombosis (CVST)

Cerebral venous sinus thrombosis occurs when the brain's venous sinuses or the smaller veins draining into them are partially or completely blocked by a blood clot. This prevents blood from draining out of the brain. As a result, the oxygen supply to nerve cells may be impaired and blood cells can leak into the brain tissue causing damage to the brain (haemorrhagic infarction).

Clinical Practice Research Datalink (CPRD)

Clinical Practice Research Datalink (https://www.cprd.com/) (CPRD) is a real-world research service to support public health and clinical studies. CPRD is jointly sponsored by the Medicines and Healthcare products Regulatory Agency and the National Institute for Health Research (NIHR), as part of the Department of Health and Social Care. CPRD collects anonymised patient data from a network of GP practices across the UK.

Commission on Human Medicines (CHM)

The Commission on Human Medicines (https://www.gov.uk/government/organisations/commission-on-human-medicines) (CHM) advises ministers on the safety, efficacy and quality of medicinal products. For COVID-19 vaccines, the CHM has a COVID-19 Vaccines Safety Surveillance Methodologies Expert Working Group and a COVID-19 Vaccines Benefit Risk Expert Working Group.

Endocarditis

Endocarditis is inflammation of the inner lining of the heart (endocardium).

Epidemiology studies

Epidemiological studies include large numbers of people and are designed to compare the risk of a particular event in an exposed population, in this case those who have received a vaccine, to those who have not. They attempt to account for differences in the different groups to help us understand if any difference in risk is caused by the exposure. Epidemiological studies measure the risk of illness or death in an exposed population compared to that risk in an identical, unexposed population.

Guillain-Barré Syndrome

Guillain-Barré Syndrome is inflammation of the nerves and can lead to numbness, weakness and pain, usually in the feet, hands and limbs and can spread to the chest and face. This syndrome has been associated with viral infections such as the flu.

Miller-Fisher Syndrome

Miller-Fisher syndrome is a variation of Guillain-Barré Syndrome that affects the nervous system and can cause weakness in the face and a lack of balance and co-ordination. Similar to Guillain-Barré Syndrome, this syndrome has been associated with viral infections such as the flu.

Miscarriage

The loss of a pregnancy during the first 23 weeks.

Myocarditis

Myocarditis is the inflammation of the heart muscle (myocardium).

Non-clinical studies

Non-clinical studies refers to studies that are not performed on the human body. These are largely done before clinical trials in humans and can include animal safety and efficacy studies, human tissue sample studies or toxicology.

Pericarditis

Pericarditis is inflammation of the pericardium, the protective sac that surrounds your heart.

Regulation 174 authorisation

Temporary authorisation for supply of a medicine or vaccine by the UK Department of Health and Social Care and the Medicines and Healthcare products Regulatory Agency. This temporary authorisation grants permission for a medicine (vaccine) to be used for active immunisation to prevent COVID-19 disease caused by SARS-CoV-2 virus. Authorisation is subject to a number of conditions. These are available for each vaccine on the MHRA website.

Suspected adverse reactions

Also known as side effects. All medicines or vaccines can cause adverse reactions in some people. Adverse drug reactions reported to the MHRA are looked at and used to assess the balance of risks and benefits of medicines and vaccines.

Stillbirth

A stillbirth is when a baby is born dead after 24 completed weeks of pregnancy. If the baby dies before 24 completed weeks, it's known as a miscarriage.

Temporal Association

Events occurring following vaccination but may or may not be caused by the vaccine.

Third dose/vaccination

A COVID-19 third vaccine is being offered to those who had a weakened immune system when they had the first two doses of the COVID-19 vaccination. The third dose may help to improve immune response and give better protection.

Thrombocytopenia

Thrombocytopenia is where the blood contains a lower than normal number of platelets. Platelets are the smallest of the blood cells and are involved in the clotting process.

Yellow Card scheme

The MHRA's scheme for healthcare professionals and members of the public to report suspected adverse reactions for a medicine or vaccine, as well as medical devices and other products. The dedicated Coronavirus Yellow Card reporting site (https://coronavirus-yellowcard.mhra.gov.uk/) was launched in May 2020 specifically for medicines and medical devices used in COVID-19, as well as COVID-19 vaccines when authorised.

Exhibit "W"

Self-Reported Real-World Safety and Reactogenicity of COVID-19 Vaccines: A Vaccine Recipient Survey

Article *in* Life · March 2021

DOI: 10.3390/life11030249

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Ray Borrow
Public Health England
606 PUBLICATIONS 20,814 CITATIONS

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This is **Exhibit "W"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at

[redacted]

life

MDPI

Brief Report

Self-Reported Real-World Safety and Reactogenicity of COVID-19 Vaccines: A Vaccine Recipient Survey

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Abstract: An online survey was conducted to compare the safety, tolerability and reactogenicity of available COVID-19 vaccines in different recipient groups. This survey was launched in February 2021 and ran for 11 days. Recipients of a first COVID-19 vaccine dose ≥ 7 days prior to survey completion were eligible. The incidence and severity of vaccination side effects were assessed. The survey was completed by 2002 respondents of whom 26.6% had a prior COVID-19 infection. A prior COVID-19 infection was associated with an increased risk of any side effect (risk ratio 1.08, 95% confidence intervals (1.05–1.11)), fever (2.24 (1.86–2.70)), breathlessness (2.05 (1.28–3.29)), flu-like illness (1.78 (1.51–2.10)), fatigue (1.34 (1.20–1.49)) and local reactions (1.10 (1.06–1.15)). It was also associated with an increased risk of severe side effects leading to hospital care (1.56 (1.14–2.12)). While mRNA vaccines were associated with a higher incidence of any side effect (1.06 (1.01–1.11)) compared with viral vector-based vaccines, these were generally milder ($p < 0.001$), mostly local reactions. Importantly, mRNA vaccine recipients reported a considerably lower incidence of systemic reactions (RR < 0.6) including anaphylaxis, swelling, flu-like illness, breathlessness and fatigue and of side effects requiring hospital care (0.42 (0.31–0.58)). Our study confirms the findings of recent randomised controlled trials (RCTs) demonstrating that COVID-19 vaccines are generally safe with limited severe side effects. For the first time, our study links prior COVID-19 illness with an increased incidence of vaccination side effects and demonstrates that mRNA vaccines cause milder, less frequent systemic side effects but more local reactions.

Keywords: Coronavirus disease 2019; COVID-19; COVID-19 vaccine; safety; reactogenicity; tolerability; adverse events

1. Introduction

Coronavirus Disease 2019 (COVID-19) rapidly became a leading cause of death and short and long-term morbidity among people over the age of 45 [1,2], posing an unprecedented burden to healthcare systems with worldwide economic consequences and prolonged lockdowns [3]. Vaccines currently being rolled out are anticipated to significantly modify these trends. While their effectiveness and safety have been proven in recent studies [4–6], data in specific groups remain lacking. Generally, people with a previous history of COVID-19 in whom vaccination is currently advised [7] were excluded from the clinical trials [4–6]. Whilst it is accepted that prior infection with COVID-19 induces a natural immunity potentially lasting for at least six months [8], it is unknown if previous infection may be associated with a greater number of vaccination side effects. Moreover, the safety and reactogenicity of the different types of vaccines (mRNA or viral vector-based) have not been compared head-to-head. This anonymized online survey was conducted to compare the safety profiles of available COVID-19 vaccines and evaluate their side effects in different groups of vaccine recipients.

2. Materials and Methods

This online survey, developed in plain English language and piloted by experts and lay people, captured basic epidemiological data, details on COVID-19 exposure, vaccination history and the incidence and severity of the respective side effects (Appendix A: Table A1). More specifically, we enquired about the following symptoms: localized reactions (pain, swelling, tenderness, redness, itching or other), fever, skin rash, shortness of breath, tingling in the mouth, face, body/extremities, swelling in the face or mouth, generalized swelling, anaphylaxis (severe allergic reaction with face swelling and breathlessness), tiredness or fatigue, flu-like illness or any other side effects. It was launched via Google Forms on 3 February 2021 for 11 days and was shared within the institutions of the investigators through professional contacts and social media. The only inclusion criterion was the receipt of the first dose of any COVID-19 vaccine at least seven days prior to survey completion.

The main objectives were to evaluate the differences in the incidence and severity of vaccination side effects among (i) people with versus without previously reported COVID-19 infection and (ii) those who received different vaccine types. Moreover, we explored the differences in self-reported side effects between the first and second vaccine dose among different ethnicities and among those with different preconceptions toward the vaccine. Finally, we explored the impact of the interval between COVID-19 exposure and vaccination and the incidence of side effects.

For our main analysis, a positive COVID-19 history was considered in cases of (a) a self-reported history of symptoms consistent with COVID-19 disease provided that COVID-19 was not excluded by a negative PCR test, (b) a positive COVID-19 PCR test or (c) a positive COVID-19 antigen test. In a sensitivity analysis, a COVID-19 infection was only considered valid if it was confirmed by PCR or antigen testing while patients with an uncertain exposure (clinical history not confirmed by laboratory testing) were excluded.

Between group differences were assessed using chi-squared and Mann–Whitney U tests for dichotomous and continuous variables, respectively, after a Shapiro–Wilk test excluded the normal distribution of the latter. Between group differences in the incidence of side effects are presented as risk ratios (RR) with the respective 95% confidence intervals (CI). Predictors of the incidence and severity of side effects were evaluated in univariate followed by multivariate binomial logistic regression and cumulative link models for ordinal data, respectively. Age, gender, ethnicity, vaccine type, prophylactic analgesia or other medication use prior to vaccination, vaccine preconceptions and prior COVID-19 exposure were evaluated as potential confounding factors. Unless otherwise specified, the analyses were based on side effect profiles from the first dose of the vaccine.

Ethics approval was not necessary for this anonymized survey.

3. Results

Within 11 days, this online survey was completed by 2002 participants (Table A2, Figure A1), mostly health professionals of a working age (median: 45, interquartile range [IQR]: 35–50 years). A total of 532 (26.6%) had a history of a previous COVID-19 infection of whom 366 (68.8%) were confirmed by PCR (n = 273) and/or antigen testing (n = 162). A COVID-19 infection preceded the first vaccination dose by a median of 87 (IQR: 47–223) days. The majority of respondents were Caucasians (88.3%) mostly from the UK (78.6%) and Greece (16.6%). As anticipated, a prior history of a COVID-19 infection was more prevalent among frontline workers, health professionals and people from the UK where a very high incidence of COVID-19 was documented [9]. Moreover, recipients of a viral vector-based vaccine (mainly the AstraZeneca vaccine) were relatively older (Figure A2, $p < 0.001$) and were mostly based in the UK (89.7% compared with 76.4% of those that received viral mRNA vaccines, $p < 0.001$). Finally, doctors were more likely to have received an mRNA-based vaccine compared with the other groups ($p < 0.001$).

A prior COVID-19 infection was associated with an 8% increase in the risk of having any side effects after the first vaccine dose (RR 1.08, 95% CI (1.05–1.11), Table 1, Figure 1). We also observed a significantly increased risk of self-reported fever (2.24 (1.86–2.70)), breathlessness (2.05 (1.28–3.29)), flu-like illness (1.78 (1.51–2.10)), fatigue (1.34 (1.2–1.49)), local reactions (1.10 (1.06–1.15)) and "other" side effects (1.46 (1.16–1.82)). Among those experiencing side effects, a prior COVID-19 infection was associated with an increased severity of any side effect, local side effects or fatigue ($p < 0.001$). More importantly, a prior COVID-19 infection was associated with the risk of experiencing a severe side effect requiring hospital care (1.56 (1.14–2.12)). These observations remained significant in multivariate analyses and our sensitivity analysis (Table A3). A similar increase in the risk of any side effects following the second dose in those with a prior COVID-19 infection was also noted (1.08 (1.05–1.11)), although the lack of significant associations with specific side effects may have resulted from the limited sample included in this analysis.

Table 1. Differences in the incidence and severity of side effects after the first dose of the COVID-19 vaccine among participants who had or did not have a prior COVID-19 infection.

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (p-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (p-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (p-Value)
Any side effect	1.08 (1.05–1.11)	0.575 (0.004)	<0.001	<0.001
Localized reaction	1.10 (1.06–1.15)	0.45 (0.003)	<0.001	0.003
Fever	2.24 (1.86–2.70)	0.876 (<0.001)	NS	NS
Flu-like illness	1.78 (1.51–2.10)	0.658 (<0.001)	NS	NS
Shortness of breath	2.05 (1.28–3.29)	0.651 (0.011)	NS	NS
Skin rash	1.04 (0.54–2.00)	NS	NS	NS
Tingling	1.26 (0.83–1.91)	NS	NS	NS
Swelling	1.00 (0.32–3.14)	NS	NS	NS
Generalized swelling	1.84 (0.94–3.60)	NS	NS	NS
Anaphylaxis	0.55 (0.06–4.72)	NS	NS	NS
Fatigue or tiredness	1.34 (1.2–1.49)	0.418 (<0.001)	<0.001	<0.001
Other	1.46 (1.16–1.82)	0.349 (0.013)	NS	NS
		Worse outcomes associated with a prior COVID-19 infection		

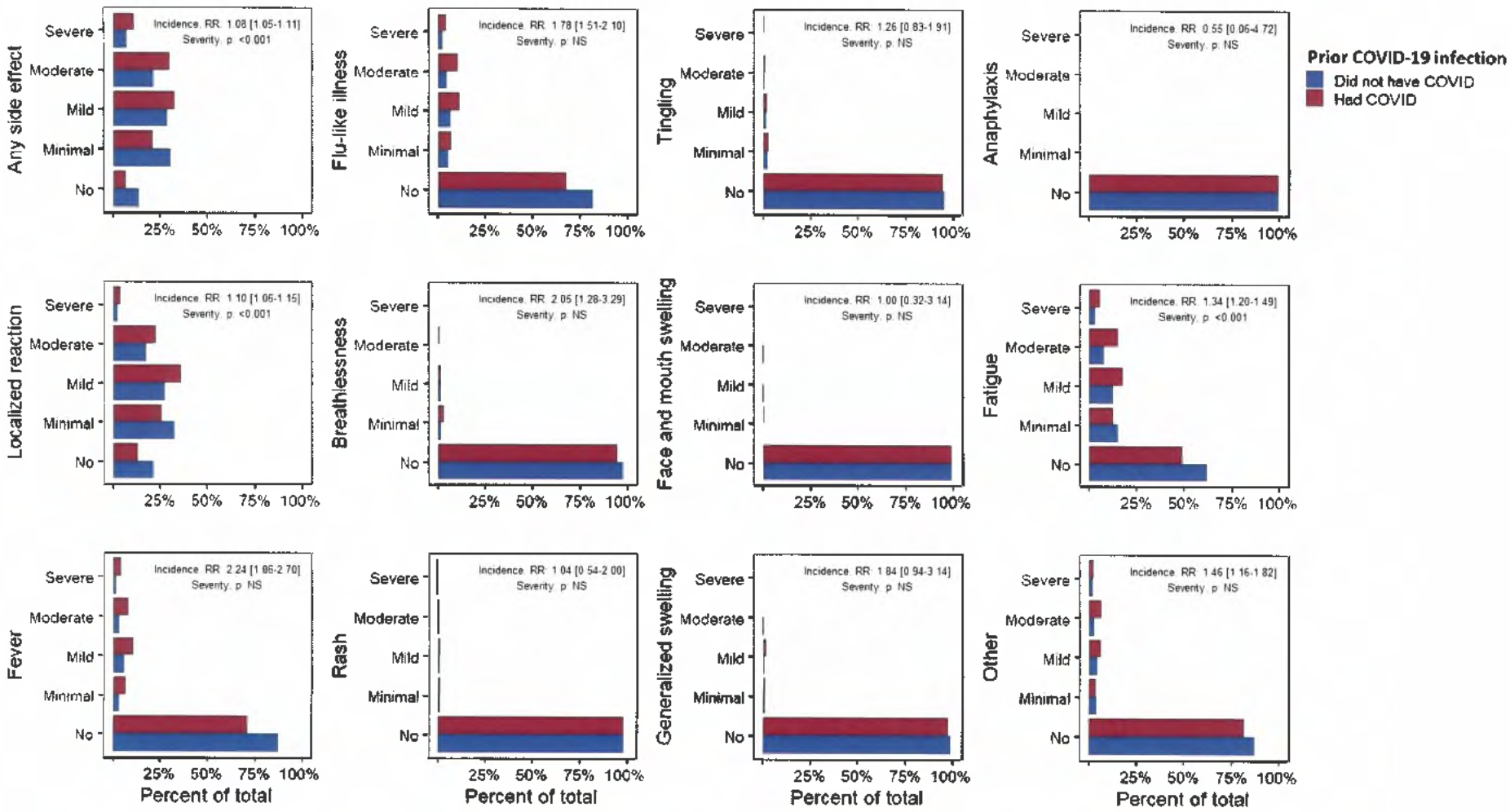

Figure 1. Incidence and severity of self-reported side effects after the first dose of the COVID-19 vaccine among participants who had or did not have a known prior COVID-19 infection. Risk ratios less than 1 favoured those that did not have a prior COVID-19 infection.

Furthermore, significant differences were observed between the side effect profiles of mRNA versus viral vector vaccines (predominantly Pfizer versus AstraZeneca, Table 2, Figure 2). Overall, the recipients of mRNA vaccines reported a higher incidence of any self-reported side effects (1.06 (1.01–1.11)), which were, however, of significantly milder severity compared with those who received viral vector vaccines. While mRNA vaccines were associated with an increased incidence of reported local reactions (1.29 (1.19–1.40)), they were associated with a considerably lower incidence of self-reported systemic side effects including anaphylaxis (0.19 (0.04–0.62)), fever (0.28 (0.24–0.34)), swelling in the face or mouth (0.29 (0.10–0.80)) or generalized swelling (0.29 (0.15–0.56)), flu-like illness (0.34 (0.29–0.40)), breathlessness (0.43 (0.26–0.70)), fatigue (0.56 (0.51–0.62)) or other side effects (0.67 (0.52–0.86)). These observations were corroborated by multivariate analyses. Most importantly, mRNA vaccines were associated with a significantly lower incidence of severe side effects (requiring hospital care, RR 0.42 (0.31–0.58)).

Table 2. Differences in the incidence and severity of side effects among people who received an mRNA or a viral vector vaccine.

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (*p*-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (*p*-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (*p*-Value)
Any side effect	1.06 (1.01–1.11)	NS	<0.001	<0.001
Localized reaction	1.29 (1.19–1.40)	0.892 (<0.001)	NS	NS
Fever	0.28 (0.24–0.34)	−1.993 (<0.001)	<0.001	NS
Flu-like illness	0.34 (0.29–0.40)	−1.795 (<0.001)	<0.001	NS
Shortness of breath	0.43 (0.26–0.70)	−0.853 (0.002)	NS	NS
Skin rash	0.86 (0.40–1.83)	NS	NS	NS
Tingling	0.68 (0.43–1.09)	NS	NS	NS
Swelling	0.29 (0.10–0.80)	−1.326 (0.015)	NS	NS
Generalized swelling	0.29 (0.15–0.56)	−1.423 (<0.001)	NS	NS
Anaphylaxis	0.19 (0.04–0.94)	−1.890 (0.024)	NS	NS
Fatigue or tiredness	0.56 (0.51–0.62)	−1.331 (<0.001)	< 0.001	NS
Other	0.67 (0.52–0.86)	−0.471 (0.004)	NS	NS
		mRNA vaccines superiority		
		Viral vector vaccines superiority		

In general, the second dose of the vaccine was associated with a higher incidence of side effects (Table 3). More specifically, respondents reported experiencing more frequently any side effects (1.04 (1.01–1.07)), skin rash (2.25 (1.4–3.62)), fever (1.72 (1.46–2.02)), flu-like illness (1.67 (1.45–1.91)) and fatigue (1.40 (1.28–1.53)). In addition, a multivariate regression demonstrated that participants who had side effects after the first vaccine dose were at a significantly higher risk of having the same side effects after the second dose. Among those experiencing side effects, the severity did not significantly differ between the two doses. However, the likelihood of having a severe side effect requiring hospital care was significantly decreased (0.58 (0.38–0.88)).

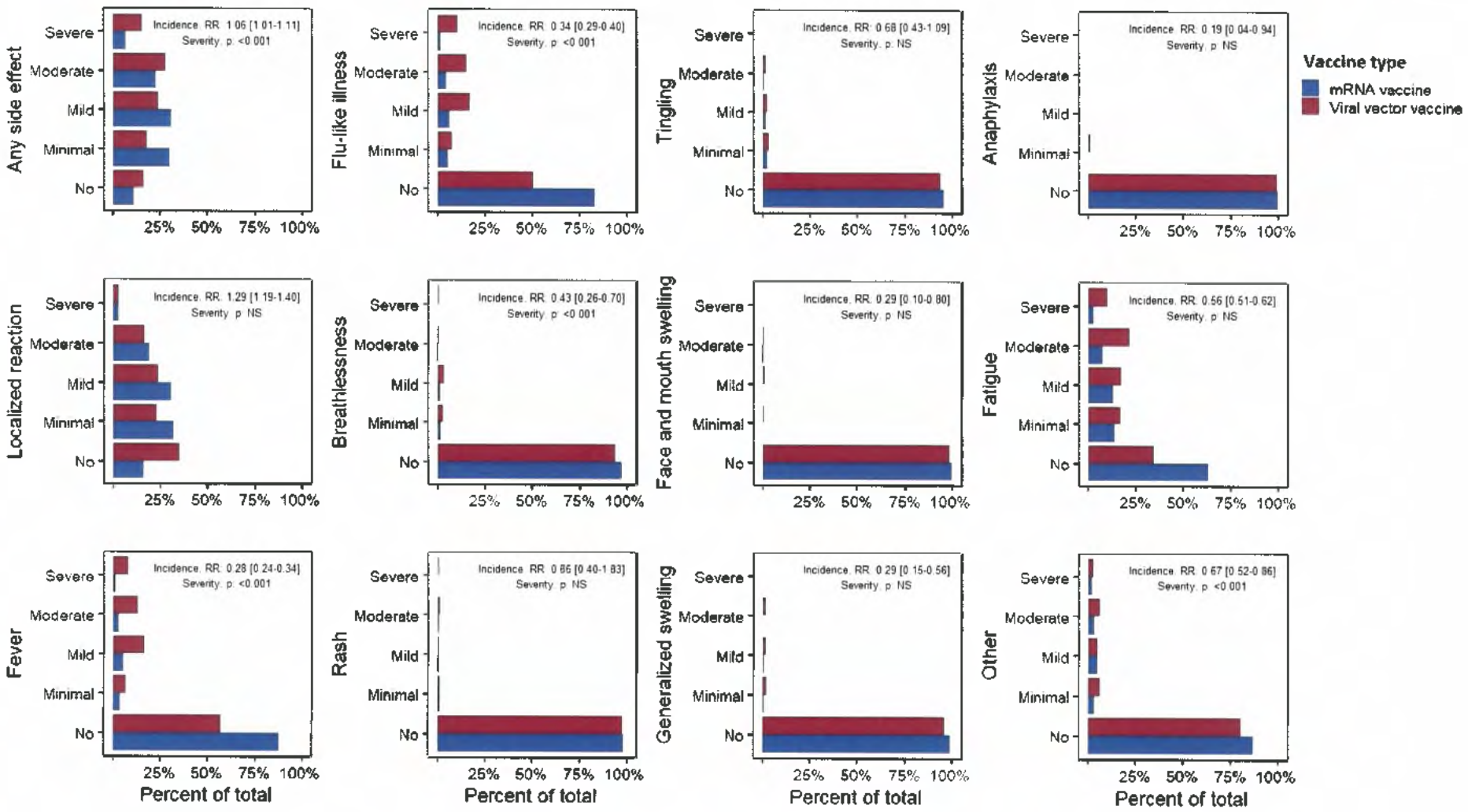

Figure 2. Incidence and severity of side effects after the first dose of (1) an mRNA or (2) a viral vector vaccine. Risk ratios less than 1 favoured the mRNA vaccine.

Table 3. Differences in the incidence and severity of side effects after the second or the first dose of the vaccine.

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (*p*-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (*p*-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (*p*-Value)
Any side effect	1.04 (1.01–1.07)	NS	NS	NS
Localized reaction	0.98 (0.94–1.03)	2.469 (<0.001)	NS	NS
Fever	1.72 (1.46–2.02)	1.3 (<0.001)	NS	NS
Flu-like illness	1.67 (1.45–1.91)	0.979 (0.001)	NS	NS
Shortness of breath	0.95 (0.57–1.61)	4.491 (<0.001)	NS	NS
Skin rash	2.25 (1.4–3.62)	4.297 (<0.001)	0.05	NS
Tingling	1.31 (0.89–1.92)	3.096 (<0.001)	NS	NS
Swelling	2.03 (0.87–4.77)	NS	NS	NS
Generalized swelling	1.2 (0.61–2.34)	4.925 (<0.001)	NS	NS
Anaphylaxis	2.54 (0.72–8.98)	4.747 (0.012)	NS	NS
Fatigue or tiredness	1.4 (1.28–1.53)	0.868 (<0.001)	NS	NS
Other	1.05 (0.83–1.32)	2.104 (<0.001)	NS	NS
		Worse outcomes after the second COVID-19 vaccine dose		

Stratification by ethnicity revealed that white participants reported a lower incidence of fever (0.62 (0.48–0.79)) and flu-like illness (0.78 (0.62–0.97)) compared with the remaining participants (Table A4). Finally, those reporting a pre-vaccination concern about the safety of the vaccine reported more often tingling (2.23 (1.45–3.42)), breathlessness (1.73 (1.00–2.98)) and fatigue (1.17 (1.03–1.34)) (Table A5).

Multivariate analyses also revealed a strong negative association between age and the self-reporting of any side effect, local reactions, fever, flu-like illness, rash, tingling, generalized swelling and fatigue ($p < 0.01$). Finally, a history of allergy was associated with an increased incidence of self-reported breathlessness and rash ($p < 0.01$). However, as described in the previous paragraphs and tables, most of the associations observed in univariate analyses remained significant in multivariate analyses accounting for these and other potential confounding factors.

4. Discussion

People with a prior COVID-19 exposure were largely excluded from the vaccine trials [4–6] and, as a result, the safety and reactogenicity of the vaccines in this population have not been previously fully evaluated. For the first time, this study demonstrated a significant association between a prior COVID-19 infection and a significantly higher incidence and severity of self-reported side effects after a vaccination for COVID-19. Consistently, compared with the first dose of the vaccine, we found an increased incidence and severity of self-reported side effects after the second dose when recipients had been previously exposed to viral antigen, probably because they had already developed an immunity against the antigens. This was supported by recent studies demonstrating that seropositive individuals developed rapid immune responses with higher antibody titres after the first vaccination dose compared with those without a previous COVID-19 infection [10,11]. In view of the rapidly accumulating data demonstrating that COVID-19 survivors generally have an adequate natural immunity for at least six months, it may be appropriate to re-evaluate the recommendation for the immediate vaccination of this group. In the meantime, taking into account our findings as well as studies demonstrating higher antibody titres among individuals with a prior COVID-19 infection, it might be appropriate for a note to be included in the vaccine information sheets highlighting that these people are more likely to experience non-serious side effects

Moreover, this is the first head-to-head real-world comparison of the self-reported safety of viral vector versus mRNA vaccines with the latter associated with a 58% decreased incidence of self-reported severe side effects requiring hospital care. While a greater number of recipients of mRNA vaccines reported at least one (any) side effect, the difference was predominantly driven by the frequent local reactions. The incidence of the systemic side effects evaluated (flu-like illness, pyrexia and fatigue), which are more burdensome to the recipients, was significantly reduced. The recipients of the viral vector-based vaccines were relatively older. However, differences in the incidence of adverse events were confirmed in multivariate analyses accounting for the age of the respondents as a covariate. Moreover, given that older people reported side effects less frequently, a potential bias due to age difference would be expected to favour viral vector-based vaccines. These findings may have an impact on vaccine choice and health policies. The cause of the observed imbalance between the safety profiles of mRNA-based versus viral-vector vaccines was unclear and should be evaluated in future studies.

The main strengths of our study included a large study population that better reflected real-life compared with the populations studied in the clinical trials, the availability of adequate details about the participants and the safety profiles of the vaccines and very limited missing data. The potential bias of respondents is the main limitation of any survey and as this survey was shared though social media, we were not able to estimate the non-response rate. However, the bias of respondents was more likely to affect the absolute incidence of side effects, which we did not evaluate here, rather than the relative incidence and severity across different groups of people. Potential recall bias should also be mentioned although all participants had been vaccinated within 10 weeks prior to completing the survey. As noted, most respondents were from the UK and Greece due to the ability of the investigators to establish contacts quickly to publicise this survey. The UK has also been successful in rolling out COVID-19 vaccines quickly leading to more of those invited being eligible to participate. It is not surprising that the Pfizer vaccine was the most delivered vaccine as it was the first vaccine to be licensed within the UK, with more individuals receiving it in total when the survey was circulated.

In conclusion, this extensive survey of over 2000 recipients of COVID-19 vaccines confirmed the findings of recent randomised controlled trials (RCTs) demonstrating that COVID-19 vaccines are generally safe with limited severe side effects. Moreover, it linked previous COVID-19 illnesses with an increased incidence of vaccination side effects. It also demonstrated that mRNA vaccines caused milder, less frequent systemic side effects but more local reactions. These findings will need to be validated in clinical studies, preferably randomized controlled trials including patients from multiple groups.

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Institutional Review Board Statement: An ethical review and approval were waived for this anonymous online survey. This was confirmed by the Research & Development Directorate of the Salford Royal NHS Foundation Trust.

Informed Consent Statement: Patient consent was implied by the completion of this online survey. Written consent was waived because this online survey was completely anonymous. This was confirmed by the Research & Development Directorate of the Salford Royal NHS Foundation Trust.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Appendix A

Table A1. Definitions of the severity of side effects.

Severity	Definition
Minimal	Negligible impact
Mild	No treatment needed
Moderate	Needed treatment or advice from healthcare professional outside the hospital
Severe	Needed hospital care

Table A2. Baseline characteristics of the study participants. Continuous variables are presented as medians (IQR) and categorical as *n* (%). Between group differences were anticipated and explained by the incidence of COVID-19 in different subgroups. Characteristically, a higher incidence of a prior COVID-19 infection was observed among frontline workers, health professionals and among British people (a very high incidence of COVID-19 was documented in the UK).

Characteristics	Participants with a Prior COVID-19 Infection (n = 532)	Participants with No Known Prior COVID-19 Infection (n = 1470)	Missing Data	Between Group Differences (p-Value)
Gender (Female)	393 (73.9%)	1051 (71.5%)	0.7%	NS
Age ≥ 60 (%) *	56 (10.5%)	202 (13.7%)	0.5%	NS
Weight (kg)	75 (64–88)	74 (64–85)	4.0%	NS
Height (cm)	168 (163–173)	168 (162–175)	2.2%	NS
Country				
Europe				
UK	472 (88.7%)	1100 (74.8%)		
Greece	38 (7.1%)	294 (20%)	0.6%	<0.001
Other European countries	10 (1.9%)	30 (2.0%)		
Americas	5 (0.9%)	17 (1.2%)		
Asia	5 (0.9%)	17 (1.2%)		
Africa	0 (0%)	1 (0.1%)		
Ethnicity				
White	464 (87.2%)	1303 (88.6%)		
Asian	35 (6.6%)	63 (4.3%)	1.8%	NS
Arab	21 (3.9%)	45 (3.1%)		
Other	7 (1.3%)	28 (1.9%)		
Role				
Doctor	140 (26.3%)	486 (33.1%)		
Nurse	125 (23.5%)	188 (12.8%)	3.2%	<0.001
Other health professional	161 (30.3%)	382 (26.0%)		
Not a health professional	105 (19.7%)	401 (27.8%)		
Frontline workers	372 (69.9%)	795 (54.1%)	0.6%	<0.001
COVID-19 prior to vaccination			0%	

Laboratory confirmed exposure	366 (68.8%)	NA		
Consistent symptoms, not tested	166 (31.2%)	NA		
No known exposure	NA	1470 (100%)		
Vaccine type				
Pfizer	443 (83.3%)	1230 (83.7%)		
Oxford AstraZeneca	80 (15.0%)	202 (13.7%)	0.5%	NS
Other	4 (0.8%)	20 (1.4%)		
Unknown	2 (0.4%)	3 (0.2%)		
Vaccine preconception				
Positive	343 (64.5%)	1027 (69.9%)	0.8%	NS
Neutral	76 (14.3%)	174 (11.8%)		
Negative	110 (20.7%)	259 (17.6%)		
Second vaccine dose received	114 (21.4%)	411 (28.0%)	0%	0.004
Past medical history				
Chronic cardiac disease	9 (1.7%)	25 (1.7%)		NS
Chronic respiratory disease	74 (13.9%)	171 (11.6%)		NS
Chronic kidney disease	4 (0.8%)	9 (0.6%)		NS
Chronic liver disease	1 (0.2%)	6 (0.4%)		NS
Chronic neurological disease	8 (1.5%)	17 (1.2%)		NS
Active cancer	1 (0.2%)	9 (0.6%)	7.7%	NS
Asplenia	1 (0.2%)	4 (0.3%)		NS
Allergy	56 (10.5%)	134 (9.1%)		NS
Diabetes	17 (3.2%)	49 (3.3%)		NS
Hay fever, eczema	114 (21.4%)	251 (17.1%)		0.04
Immunosuppression	14 (2.6%)	49 (33.3%)		NS
Transplantation history	0 (0%)	0 (0%)		NS
None	282 (53.0%)	825 (56.1%)		NS

* Participants with a prior COVID-19 exposure were younger compared with those without a prior exposure. See Figure A1.

Table A3. Differences in the incidence and severity of side effects after the first dose of a COVID-19 vaccine among participants who had or did not have a prior self-reported COVID-19 infection. Sensitivity analysis only included participants with a prior COVID-19 infection confirmed with a consistent PCR or antibody test (n = 366) versus those without any suspicion of a prior COVID-19 infection (n = 1470).

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (p-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (p-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (p-Value)
Any side effect	1.09 (1.05–1.12)	0.581 (0.015)	<0.001	0.004
Localized reaction	1.11 (1.06–1.16)	0.411 (0.019)	0.002	NS
Fever	2.45 (2.01–3)	0.902 (<0.001)	NS	NS
Flu-like illness	1.92 (1.61–2.29)	0.691 (<0.001)	NS	NS
Shortness of breath	2.06 (1.22–3.49)	0.564 (0.043)	NS	NS
Skin rash	1.38 (0.7–2.71)	NS	NS	NS
Tingling	1.22 (0.75–1.98)	NS	NS	NS
Swelling	0.73 (0.16–3.28)	NS	NS	NS
Generalized swelling	1.72 (0.8–3.73)	NS	NS	NS

Anaphylaxis	0.8 (0.09–6.85)	NS	NS	NS
Fatigue or tiredness	1.39 (1.24–1.56)	0.459 (<0.001)	<0.001	0.002
Other	1.45 (1.12–1.87)	0.288 (0.069)	NS	NS
		Worse outcomes associated with a prior COVID-19 infection		

Table A4. Differences in the incidence and severity of side effects among different ethnicities (white or other).

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (*p*-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (*p*-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (*p*-Value)
Any side effect	1.05 (0.99–1.11)	NS	NS	NS
Localized reaction	1.04 (0.97–1.12)	NS	NS	NS
Fever	0.62 (0.48–0.79)	–0.546 (0.003)	NS	NS
Flu-like illness	0.78 (0.62–0.97)	NS	NS	NS
Shortness of breath	1.16 (0.54–2.5)	NS	NS	NS
Skin rash	0.7 (0.32–1.56)	NS	NS	NS
Tingling	1.69 (0.79–3.61)	NS	NS	NS
Swelling	0.86 (0.2–3.81)	NS	NS	NS
Generalized swelling	0.64 (0.27–1.53)	NS	NS	NS
Anaphylaxis	0.66 (0.08–5.67)	NS	NS	NS
Fatigue or tiredness	0.88 (0.76–1.02)	NS	NS	NS
Other	1.38 (0.94–2.03)	0.446 (0.049)	NS	NS
		Worse outcomes: non-white ethnicity		
		Worse outcomes: white ethnicity		

Table A5. Differences in the incidence and severity of side effects among people with a different preconception toward the vaccine prior to vaccination and those who were keen to receive the vaccine versus those who were concerned about receiving the vaccine.

Side Effect	Incidence of Side Effects: Risk Ratio (95% CI)	Incidence of Side Effects: Multivariate Logistic Regression, Coefficient (*p*-Value)	Severity of Side Effects: Univariate Cumulative Risk Models (*p*-Value)	Severity of Side Effects: Multivariate Cumulative Risk Models (*p*-Value)
Any side effect	1.01 (0.97–1.06)	NS	< 0.001	0.025
Localized reaction	0.99 (0.93–1.05)	NS	0.002	NS
Fever	1.19 (0.93–1.53)	NS	0.009	NS
Flu-like illness	1.07 (0.86–1.34)	NS	< 0.001	NS
Shortness of breath	1.73 (1.00–2.98)	–0.085 (0.03)	NS	NS
Skin rash	1.25 (0.59–2.65)	NS	NS	NS
Tingling	2.23 (1.45–3.42)	–0.114 (0.001)	NS	NS
Swelling	0.4 (0.05–3.03)	NS	NS	NS
Generalized swelling	0.72 (0.26–2.04)	NS	NS	NS
Anaphylaxis	NA	NS	NS	NS
Fatigue or tiredness	1.17 (1.03–1.34)	NS	0.009	NS
Other	1.26 (0.96–1.66)	–0.043 (0.045)	NS	NS
		Worse outcomes: concerned		

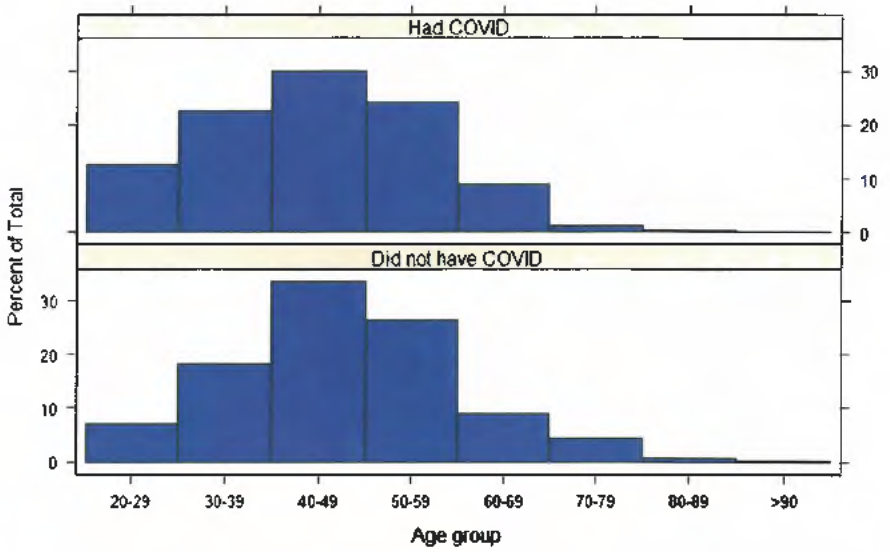

Figure A1. Age of the participants stratified by whether they had or did not have a previous COVID-19 infection.

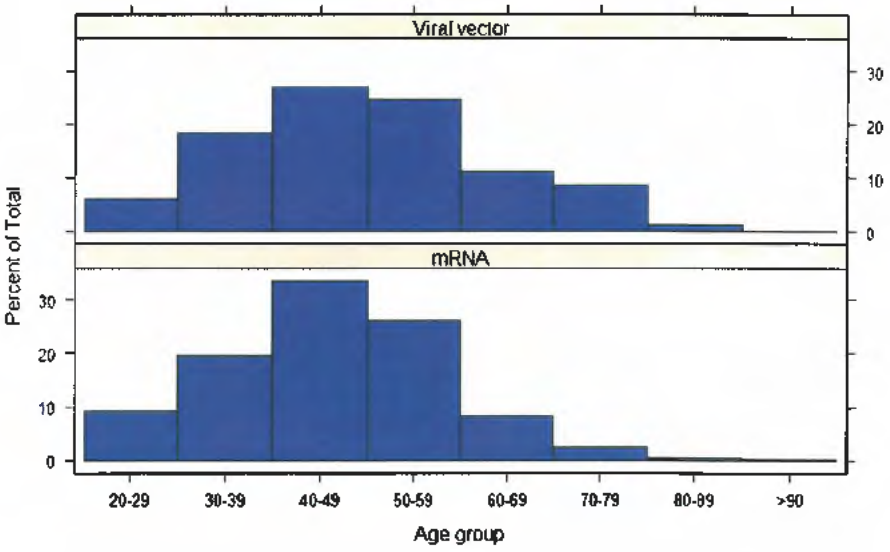

Figure A2. Age of the participants stratified by the type of vaccine they received.

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Exhibit "X"

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Vaccine- and natural infection-induced mechanisms that could modulate vaccine safety

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ABSTRACT

A degraded/dysfunctional immune system appears to be the main determinant of serious/fatal reaction to viral infection (for COVID-19, SARS, and influenza alike). There are four major approaches being employed or considered presently to augment or strengthen the immune system, in order to reduce adverse effects of viral exposure. The three approaches that are focused mainly on augmenting the immune system are based on the concept that pandemics/outbreaks can be controlled/prevented while maintaining the immune-degrading lifestyles followed by much of the global population. The fourth approach is based on identifying and introducing measures aimed at strengthening the immune system intrinsically in order to minimize future pandemics/outbreaks.

Specifically, the four measures are: 1) restricting exposure to virus; 2) providing reactive/tactical treatments to reduce viral load; 3) developing vaccines to prevent, or at least attenuate, the infection; 4) strengthening the immune system intrinsically, by a) identifying those factors that contribute to degrading the immune system, then eliminating/reducing them as comprehensively, thoroughly, and rapidly as possible, and b) replacing the eliminated factors with immune-strengthening factors.

This paper focuses on vaccine safety. A future COVID-19 vaccine appears to be the treatment of choice at the national/international level. Vaccine development has been accelerated to achieve this goal in the relatively near-term, and questions have arisen whether vaccine safety has been/is being/will be compromised in pursuit of a shortened vaccine development time. There are myriad mechanisms related to vaccine-induced, and natural infection-induced, infections that could adversely impact vaccine effectiveness and safety. This paper summarizes many of those mechanisms.

1. Introduction

1.1. Background

Over the past two decades, there have been at least three major coronavirus-based infectious disease outbreaks/epidemics/pandemics: Severe Acute Respiratory Syndrome (SARS), 2002–2003; Middle East Respiratory Syndrome (MERS), starting in 2012; COVID-19, starting in December 2019 [1]. There are a number of similarities among these three infectious diseases, including abnormal values of selected biomarkers (e.g., neutrophils, lymphocytes, albumin, CRP, TNF-alpha, etc.), pulmonary inflammation, pulmonary damage, etc. The most

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important similarity among these infectious diseases is the demographic affected most severely [2]. This demographic tends to be the elderly, with comorbidities and degraded/dysfunctional immune systems, and others with degraded/dysfunctional immune systems [1–13]. While there is some decline in the immune system with age, comorbidity is a stronger predictor of impaired immunity than chronological age in older adults [14,15].

There are also similarities between COVID-19 and influenza: "Both (COVID-19 and influenza) cause fever, cough, body aches and fatigue; sometimes vomiting and diarrhea; can be mild or severe, even fatal in rare cases; can result in pneumonia" [16]. Additionally, "Neither virus is treatable with antibiotics, which only work on bacterial infections; both are treated by addressing symptoms, such as reducing fever; severe cases may require hospitalization and support such as mechanical ventilation" [16,17]. Both COVID-19 and influenza share the demographic affected most severely, as well.

The main measures being taken to control the spread of the SARS-CoV-2 coronavirus (the virus mainly associated with COVID-19) are conceptually those that were taken to control the spread of the SARS-CoV coronavirus in 2002–2003: good hygiene and quarantine (lockdown). The difference is the scale of these measures. Currently, many countries are on lockdown (at different levels of severity), restricting many activities and businesses that involve gatherings of large numbers of people in close proximity. As of early October 2020, it is unknown how long these restrictions will be in place.

In addition to identifying short-term adverse vaccine effects related to the mechanisms, this paper identifies potential mid-and long-term adverse vaccine effects that cannot be identified in short-term human clinical trials characteristic of vaccine efficacy testing. To ensure vaccine safety, long-term human testing under real-life conditions (exposures to multiple toxic stimuli) is required. There is an incompatibility between the accelerated vaccine development times being pursued by government and industry and the long times required for validation of vaccine safety.

In summary, it is difficult to see how safe COVID-19 vaccines can be developed and fully tested for safety on development time scales of one or two years, as proposed presently. The only real protection against a future COVID-19 pandemic or any other viral pandemic/outbreak is the one that was demonstrated to work in the SARS, MERS, and COVID-19 pandemics/outbreaks, and in the annual influenza pandemics/outbreaks: a healthy immune system capable of neutralizing incoming viruses as nature intended.

1.2. Potential treatments

There are myriad efforts being pursued to develop treatments and preventative measures for COVID-19. Some of these will now be outlined.

If treatments are defined as a set of actions that improve health, then (at least) two types of treatments are possible.

The first type can be defined as positive treatments. They can be subdivided into high-tech treatments and low-tech treatments.

The high-tech are the classical treatments where drugs (or supplements) and/or radiation and/or surgery are implemented, and symptoms are alleviated. These high-tech positive treatments are basically a reactive tactical response to abnormal markers of health. They can be applied for the short-term (e.g., antibiotics for bacterial infections, antivirals for viral infections, etc.) [18,19], or for the long-term (e.g., statins, blood thinners, antihypertensives, etc.) [20]. The low-tech treatments involve dietary supplements or natural bioactive compounds [21–23], sleep, and other behavioral changes shown to impact the immune system positively (see section A4-C of our previous COVID-19 monograph [13] for a bibliography of low-tech immune system strengthening factors). For long-term benefit, these low-tech treatments need to be maintained indefinitely [24]. On average, the high-tech treatments have greater risk than the low-tech treatments.

The second type can be defined as negative-negative treatments, where those factors that contribute to disease are first identified and then removed. The name derives from the mathematics world, where a negative of a negative is a positive [25]. These negative-negative treatments are basically a proactive strategic response to abnormal markers of health, and typically involve long-term changes in lifestyle and harmful exposures for improved health [26–29].

1.3. Tactical treatments

Much of the effort to help the most vulnerable COVID-19 demographic at this time has been searching for, and experimenting with, treatments that were/are used to combat other (mainly) viral diseases (aka repurposed treatments). These treatments include, but are not limited to: Actemra/Tocilizumab; Avigan/Favipiravir; Azithromycin; Baricitinib/Olumiant;

Bevacizumab/Avastin; Calquence/Acalabrutinib; Chloroquine; Colcrys/Colchicine; Convalescent Plasma; EIDD-2801; Fingolimod/Gilenya; Galidesivir; Hydroxychloroquine; Ilaris/Canakinumab; Ivermectin; Jakafi/Ruxolitinib; Kaletra/Lopinavir/Ritonavir; Kevzara/Sarilumab;

Kineret/Anakinra; Leronlimab; Mavrilimumab; Methylprednisolone; Olumiant/Baricitinib; Otezla/Apremilast; Remdesivir; Tamiflu/Oseltamivir; Umifenovir/Arbidol; Xeljanz/Tofacitinib [30–34].

Other novel tactical treatments could be identified using our Literature-Related Discovery and Innovation (LRDI)-based treatment repurposing methodology [35,36].

1.4. Strategic treatments

Strategic treatments were the focus of our previous COVID-19 monograph [13]. Their identification is a two-step process. First, markers of immune system health (ranging from specific biomarkers to more general descriptors) are selected. Second, those substances (e.g., smoking, excess alcohol, pesticides, etc.) behaviors (e.g., sedentary lifestyle, substance abuse, etc.), and other toxic stimuli that degrade the levels of these markers (i.e., lead to immune dysfunction, immunotoxicity, immunosuppression, etc.) are then identified and recommended for elimination [37]. The strategic treatments identified in the previous monograph are those contained within the immune system core literature. Additional novel strategic treatments could also be identified using our LRDI-based treatment repurposing methodology [35,36].

1.5. Reactive tactical vs proactive strategic treatments

The reactive tactical treatment approach for countering infections from viral exposure improves biomarker levels and reduces symptoms (if successful), but ordinarily does little to improve the body's resistance to disease. For viral infections, the tactical treatments will do little to strengthen the degraded/dysfunctional immune (and other) system. After tactical treatments for one viral infection, people with degraded/dysfunctional immune systems will again be vulnerable to serious infectious consequences from exposure to the next harmful virus they encounter.

The proactive strategic treatment approach will strengthen the immune (and other) system by removing those critical factors that contribute to disease and a degraded/dysfunctional immune system (unless irreversible damage has been done to the immune system, or individuals possess congenital or other hereditary damage to their immune system) [38–40]. These strategic treatments tend to require long-term adherence by their recipients. In turn, these recipients of strategic treatments will be less vulnerable to infection from exposure to the next pathogenic virus they encounter (SARS-CoV-2 or otherwise). Like many healthy people who were exposed to SARS-CoV and SARS-CoV-2, these people who follow the (typically) long-term proactive strategic treatment regimen successfully may not even be aware they have been exposed to, or infected by, the coronavirus. The only

indication of their infection will be coronavirus antibodies in their serum.

2. Methodology

A hybrid methodology was used to identify references showing potential long-term adverse effects of vaccines and vaccine/infection-induced mechanisms that could contribute to these adverse effects. Based on reading myriad vaccine adverse effects review articles, terms showing mechanisms were extracted (e.g., antibody-dependent enhancement, viral interference, route of infection, original antigenic sin, etc), and used as a Medline query to retrieve potentially relevant articles. All these retrieved articles were read, and the most relevant ones extracted. Their titles were entered into the Web of Science, and the citation network was explored (citing papers, cited papers, papers that shared common references, etc). Those records were read, and the most relevant ones extracted for this monograph.

3. Results

3.1. Overview

The main body of our previous COVID-19 monograph [12] addressed the first type of strategic treatment: 1) identification and removal of factors contributing to weakening the immune system (see section A4-A of the previous monograph [12] for a table of these contributing factors), and 2) identification and addition of factors contributing to strengthening the immune system (see section A4-C of the previous monograph [12] for a bibliography of low-tech immune system strengthening factors). The present section addresses the second type of strategic treatment: development and implementation of a COVID-19 vaccine. The prospects for such a vaccine will be addressed from three criteria perspectives: development time, efficacy, and safety.

Calina et al. evaluated the ongoing approaches to COVID-19 vaccine development, and stated: "Normally, the period of development of a vaccine is 12-15 years" [41]. Against this backdrop, SARS-CoV-2 vaccines are targeted for accelerated development, safety testing, manufacturing, and distribution by an order of magnitude [42]. Each of the accelerated steps [41] has drastically reduced the time required from normal development. Some of the potential adverse vaccine effects shown on the right of Fig. 1 may take years to emerge, well after the initial abbreviated vaccine safety testing period.

3.2. Past coronavirus vaccine development history

There have been two prior coronavirus outbreaks in the 21 st century: SARS in 2002–2003, and MERS starting in 2012. Vaccine development for each started/accelerated during the height of each outbreak.

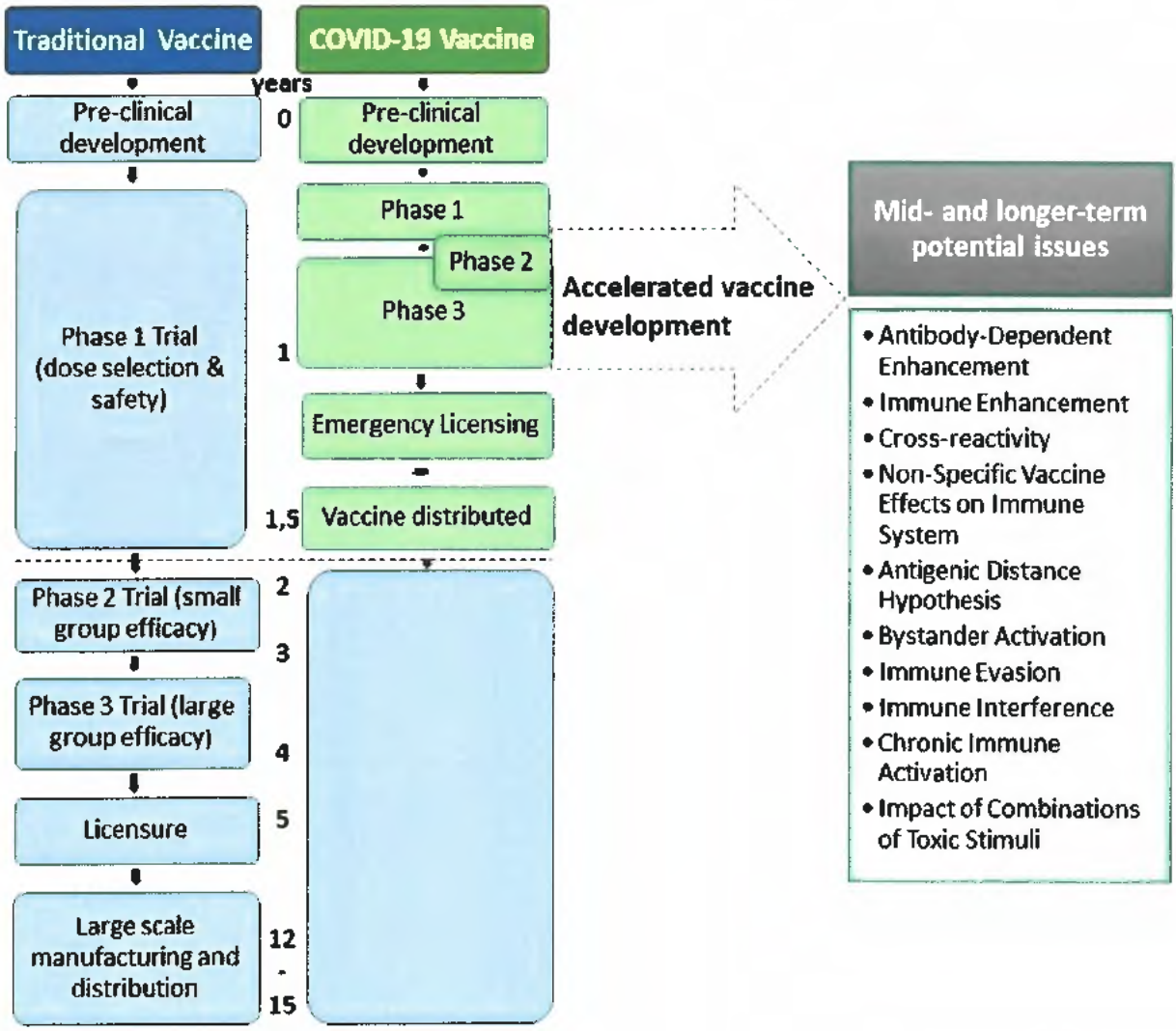

Fig. 1. Compares the traditional vaccine development schedule with the accelerated COVID-19 vaccine development schedule.

What have been the results of these prior coronavirus vaccine development efforts?

According to a comprehensive 2019 article on MERS vaccine development [43], "To date, there is no specific treatment proven effective against this viral disease. In addition, no vaccine has been licensed to prevent MERS-CoV infection thus far … In general, the potential vaccine candidates can be classified into six types: viral vector-based vaccine, DNA vaccine, subunit vaccine, nanoparticle-based vaccine, inactivated-whole virus vaccine and live-attenuated vaccine"

According to a comprehensive 2020 article on SARS and MERS vaccine development [44], "As of April 2020, no vaccine is commercially available for these coronavirus strains". The rationale for lack of a vaccine is given by the following: "Reasons for the lack of commercial and effective vaccines for SARS and MERS are varied. In the case of MERS, it is likely that the vaccine development was delayed because of the scarcity of suitable and cost-effective small animal models during pre-clinical experimentation. In addition, it is probable that a vaccine has not been delivered because of the low interest in investing in a vaccine for a disease that has produced relatively low and geographically centralized cases (compared with other more global and persistent infectious diseases such as influenza, HIV and tuberculosis). This last factor might have also contributed to the lack of a vaccine for SARS, in the sense that it was considered pointless to continue investing in a vaccine for a disease whose cases ceased to be reported in 2004."

While interest in a vaccine may have waned after the SARS pandemic/outbreak seemed to have terminated, research on such a vaccine persisted. References in the above article showed SARS vaccine research continued for a decade or more after the pandemic had ended [45,46].

Based on the experiences with SARS and MERS, successful vaccine development was not achieved after about a decade of research, or even more. That does not bode well for COVID-19 coronavirus vaccine development/safety testing/distribution for the one-year timescales being projected.

3.3. Challenges for successful vaccine development - overview

The main challenges facing successful coronavirus vaccine development can be summarized as time to development, efficacy of the vaccine and, most importantly, safety of the vaccine. A complementary perspective on some of the problems listed in [41] can be stated as follows:

First, although the virus's spike protein is a promising immunogen for protection, optimizing antigen design is critical to ensure optimal immune response. Debate continues over the best approach — for example, targeting the full-length protein or only the receptor-binding domain.

Second, preclinical experience with vaccine candidates for SARS and the Middle East respiratory syndrome (MERS) have raised concerns about exacerbating lung disease, either directly or as a result of antibody-dependent enhancement. Such an adverse effect may be associated with a type 2 helper T-cell (Th2) response. Hence, testing in a suitable animal model and rigorous safety monitoring in clinical trials will be critical" [47].

3.4. Vaccine mechanisms with uncertain consequences

Numerous mid- and longer-term potential issues concerning vaccines have been identified. Their themes are summarized initially, followed by excerpts from specific cited references.

1) Antibody-Dependent Enhancement (where enhanced virus entry and replication in a number of cell types is enabled by antibodies) [47–54];
   - -1a) Intrinsic Antibody-Dependent Enhancement (where non-neutralizing antibodies raised by natural infection with one virus may enhance infection with a different virus) [55–61];
   - -1b) Immune Enhancement (enhancement of secondary infections via immune interactions) [62–65];
   - -1c) Cross-reactivity (an antibody raised against one specific antigen has a competing high affinity toward a different antigen.) [66,67]
   - -1d) Cross-Infection Enhancement (infection enhancement of one virus by antibodies from another virus) [68,69]
2) Vaccine-associated Virus Interference (where vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-specific immunity associated with natural infection) [70–75];
3) Vaccine-Associated Imprinting Reduction (where vaccinations could also reduce the benefits of 'imprinting', a protection conferred upon children who experienced infection at an early age) [76,77];
4) Non-Specific Vaccine Effects on Immune System (where previous infections can alter an individual's susceptibility to unrelated diseases) [78,79];
5) Impact of Infection Route on Immune System (where immune protection can be influenced by the route of exposure/delivery) [80–82];
6) Impact of Combinations of Toxic Stimuli (where people are exposed over their lifetime to myriad toxic stimuli that may impact the influence of any vaccine) [78; 83, 84];
7) Antigenic Distance Hypothesis (negative interference from prior season's influenza vaccine (v1) on the current season's vaccine (v2) protection may occur when the antigenic distance is small between v1 and v2 (v1 ≈ v2) but large between v1 and the current epidemic (e) strain (v1 ≠ e).) [85–87];
8) Bystander Activation (activation of T cells specific for an antigen X during an immune response against antigen Y) [88–90];
9) Gut Microbiota (Impact of gut microbial composition on vaccine response) [91–95];
10) Homologous Challenge Infection Enhancement (the strain of challenge virus used in the testing assay is very closely related to the seed virus strain used to produce the vaccine that a subject received) [96–98];
11) Immune Evasion (evasion of host response to viral infection) [99–102];
12) Immune Interference (interference from circulating antibody to the vaccine virus) [103,104];
    - 12a) Original antigenic sin (propensity of the body's immune system to preferentially utilize immunological memory based on a previous infection when a second slightly different version of that foreign entity (e.g. a virus or bacterium) is encountered.) [105–108];
13) Prior Influenza Infection/Vaccination (effects of prior influenza infection/vaccination on severity of future disease symptoms) [109–116];
14) Timing between Viral Exposures (elapsed time between viral exposures) [117–120];
15) Vaccine-Associated Enhanced Respiratory Disease (where vaccination enhances respiratory disease) [121–123];
16) Chronic Immune Activation (continuous innate immune responses) [124–126].

3.5. Vaccine effectiveness

The previous section addressed mechanisms that could potentially enhance infections, rather than attenuate or prevent them. The present section addresses empirical findings of low vaccine effectiveness, where mechanistic explanations may or may not have been offered. Because of similarities between influenza and COVID-19, and space limitations, the focus will be on the influenza vaccination experience. Significant

influenza VE could not be demonstrated for any season, age, or setting after adjusting for county, sex, insurance, chronic conditions recommended for influenza vaccination, and timing of influenza vaccination. [127]. We found a threefold increased risk of hospitalization in subjects who did get the TIV vaccine [128].

Using data from traditional control subjects, VE for those seasons was estimated to be 5 % (95 % CI, −52 % to 40 %), 11 % (95 % CI, −96 % to 59 %), and 37 % (95 % CI, −10 % to 64 %), respectively; confidence intervals included 0 [129]. Participants reporting pH1N1-related ILI during the period 1 April through 5 June 2009 were more than twice as likely to report having previously received seasonal influenza vaccine [130]. Unvaccinated children had more flu-specific CTLs than vaccinated children with CF [131].

Following 2009 H1N1 vaccination, subjects previously given a seasonal influenza virus vaccination exhibited significantly lower antibody responses, as determined by hemagglutination inhibition assay, than subjects who had not received the seasonal influenza virus vaccination [132]. Our study confirms the results from our previous interim report, and other studies, that failed to demonstrate benefit or harm from receipt of seasonal influenza vaccine in patients with confirmed infection with pandemic influenza H1N1 2009. [133]. Influenza vaccination seemed to be associated with an increased risk of non-influenza respiratory virus infections, which is consistent with temporary nonspecific immunity. [134]. A potential doubling of pandemic infection risk among prior seasonal vaccine recipients could be disastrous in the event of a more severe pandemic involving a higher per-case fatality risk" [135].

3.6. Potential short- and long-term diseases resulting from vaccines

- Tracking Deficiencies for Vaccine Adverse Effects

While the efficacy issues for a COVID-19 vaccine have been enumerated extensively in recent reviews [49,54], more emphasis needs to be placed on ensuring mid- and long-term safety are achieved. Vaccines do not appear to have the same safety requirements as many drugs. For example, consider the following excerpts from selected vaccine inserts relative to safety [136]:

- MMR Vaccine: M-M-R II has not been evaluated for carcinogenic or mutagenic potential, or potential to impair fertility. Animal reproduction studies have not been conducted with M-M-R II.;
- Influenza Vaccine FLUARIX QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential or male infertility in animals.
- DTAP Vaccine INFANRIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of fertility.
- HPV Vaccine [137] GARDASIL 9 has not been evaluated for the potential to cause carcinogenicity, genotoxicity or impairment of male fertility.

Long-term safety studies of vaccines are rare. The typical vaccine study is aimed at efficacy. Such studies tend to be a few months long, and the main evaluation criterion is titers of antibody in the serum.

Vaccines, especially childhood vaccines, are administered according to a schedule, which now comprises about seventy + doses covering about sixteen vaccines. The schedule-based combination effects of these seventy + vaccine doses have not been tested, and, therefore, adverse effects due to real-life vaccine synergies are unknown. Such vaccine combination experiments cannot be limited to the pristine environment of the laboratory, but require testing in humans who are exposed to myriad toxic stimuli that could impact vaccine combination synergies.

Much of the published data for vaccine adverse events (at least in the USA) originates from the Vaccine Adverse Event Reporting System (VAERS) database. VAERS is a passive monitoring system, and, like all similar systems, suffers from substantial under-reporting of adverse events [138]. A groundbreaking study [139], performed by Harvard Pilgrim Healthcare, Inc, reported that fewer than 1% of vaccine adverse events are reported. In other words, the actual numbers of adverse reactions to vaccines are one to two orders of magnitude higher than those reported in VAERS!

The methodology used by Harvard Pilgrim Healthcare. Inc, for obtaining this result was as follows: Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions are evaluated for values suggestive of an adverse vaccine event. When a possible adverse event was detected, it was recorded, and the appropriate clinician was to be notified electronically.

Thus, these adverse events that were identified are single-visit short-term adverse events (within thirty days of the vaccination). They do not reflect the results of vaccination combinations administered over a longer period than thirty days, and they do not reflect results of vaccinations of any type in the mid-or long-term [139].

If fewer than 1% of vaccine adverse events are reported, how well does this sample reflect the total number of adverse events actually experienced? This is not a randomly-selected sample, as would be required for a statistically-valid result. Thus, even analyses of short-term adverse effects based on VAERS data are severely flawed. And, if fewer than 1% of these short-term adverse events are reported, what fraction of longer-term adverse events (where the connection between the adverse event and the vaccination becomes more tenuous as time proceeds) would be reported? One can only conclude that a negligible fraction of long-term adverse events is reported in a passive monitoring system like VAERS.

3.7. Diseases triggered by vaccines

A brief analysis was performed of the vaccine biomedical literature to identify diseases potentially triggered by vaccination, especially in the long-term. It should be noted the biomedical literature is very sparse with studies on long-term vaccine effects, especially long-term adverse effects. Large numbers of people and long periods of time are required to identify such adverse events, and draw statistically-valid connections between vaccinations and disease. These efforts would be very resource-intensive, and there appears to be little motivation among the vaccine producers and regulators to make these resources available for such studies. Thus, the following examples reflect the extremely small tip of an extremely large iceberg of long-term adverse vaccine effects.

The two main categories of diseases reported in the biomedical literature triggered by vaccinations are Autoimmune (e.g., Systemic Lupus Erythematosus, Psoriasis, Arthritis, Multiple Sclerosis, Hepatitis, Uveitis, Pseudolymphoma, Guillain-Barre Syndrome, Thrombocytopenic Purpura, etc.) and Neurological (e.g., Central Demyelinating Diseases, Developmental Disability, Febrile seizures, Narcolepsy, Encephalomyelitis, Autonomic Dysfunction, etc.). Others include Diabetes, Gastrointestinal, Joint-related, Necrobiotic Granuloma, Neutropenia, Pulmonary Fibrosis, etc.

Main syndromes associated with systemic toxicity of adjuvanted vaccine: acute phase response (APR), hypersensitivity reactions, induction or worsening of autoimmune diseases, modification of drug hepatic metabolism, vascular leak syndrome (VLS), oral immunosuppression or tolerance post vaccination [140].

Vaccinations may also contribute to the mosaic of autoimmunity. Evidence for the association of vaccinations and the development of these diseases is presented in this review. Infrequently reported post-vaccination autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies, multiple sclerosis, Guillain-Barre syndrome, and vasculitis [141].

Toplak et al. reported the production of autoantibodies (such as antinuclear and antiphospholipid antibodies) in 92 healthy medical workers up to 6 months after influenza vaccination. Other studies have demonstrated a latency period of years between HiB vaccination and diabetes mellitus, and between HBV vaccination and demyelinating

events. In conclusion, latency periods can range from days to years for postinfection and postvaccination autoimmunity [142].

Adults receiving HBV had significantly increased odds ratios (OR) for multiple sclerosis (OR = 5.2, p < 0.0003, 95 % Confidence Interval (CI) = 1.9–20), optic neuritis (OR = 14, p < 0.0002, 95 % CI = 2.3–560), vasculitis (OR = 2.6, p < 0.04, 95 % CI = 1.03–8.7), arthritis (OR = 2.01, p < 0.0003, 95 % CI = 1.3–3.1), alopecia (OR = 7.2, p < 0.0001, 95 % CI − 3.2–20), lupus erythematosus (OR − 9.1, p < 0.0001, 95 % CI − 2.3–76), rheumatoid arthritis (OR = 18, p < 0.0001, 95 % CI = 3.1–740), and thrombocytopenia (OR = 2.3, p < 0.04, 95 % CI = 1.02–6.2) in comparison to the TCV group. Minimal confounding or systematic error was observed [143].

The difference in cumulative incidence between those receiving 4 doses and those receiving 0 doses is 54 cases of IDDM/100,000 (P = 0.026) at 7 years, (relative risk = 1.26). Most of the extra cases of IDDM appeared in statistically significant clusters that occurred in periods starting approximately 38 months after immunization and lasting approximately 6–8 months. Immunization with pediatric vaccines increased the risk of insulin diabetes in NOD mice.....Exposure to HiB immunization is associated with an increased risk of IDDM. NOD mice can be used as an animal model of vaccine induced diabetes [144].

3.8. Time required for credible COVID-19 vaccine safety studies

As the above results have shown, vaccines can have long-term impacts on the immune system (positive and negative), and short and long-term effects on other diseases. The effects of vaccines can vary according to route of infection, prior history of vaccinations, and, as stated by Benn et al. above, administration "with other vaccines, drugs, or micronutrients and in different sequences [78]. To accelerate the time required to demonstrate long-term safety, laboratory experiments are usually done using animals with relatively short lifespans whose responses to myriad toxic stimuli are similar to that of human beings.

One major difference between these animal experiments and the human model is that the laboratory experiments are usually performed with the administration of a single toxic stimulant, or maybe two, while the human model lives in a sea of toxic stimuli. Also, it is not always clear which animal model simulates the human model best for response to vaccination.

There are many examples in the biomedical literature where combined exposures to toxic stimuli showed adverse effects whereas exposures to the same stimuli in isolation (at the same dosages) showed no adverse effects [83,84]. Thus, unless these laboratory experiments are performed with a range of combinations of associated immunomodulators, they would not be credible for safety assessment purposes. Such experiments would require enormous amounts of financial and time resources.

The other alternative is to perform these safety studies with human beings. For long-term safety studies (e.g., potential vaccine effects on initiating cancer or Alzheimer's Disease), decades could be required for credible results. Thus, there is a major disconnect between the time required for credible safety studies of a COVID-19 vaccine and the one-year or less vaccine commercialization being propounded by decision-makers and the media today.

3.9. Molecular mimicry and the invalid genetic basis of vaccine pre-clinical tests: the new vaccinology scenario for designing safe and effective vaccines

The above analyzed COVID-19 vaccine safety considerations become even more cogent in light of the fact that cross-reactivity might represent the mechanism underlying the immunopathology and the disease multitude associated with the coronavirus infection [145]. The rationale is that the sharing of peptides between SARS-CoV-2 and human proteins might trigger immune responses hitting not only the virus but also the human proteins, with consequent autoimmune pathologies in the human host [146]. Hence, the massive viral vs. human peptide commonalities described since 2000 [147,148] clearly explain how the protective anti-viral antibody immune response can become a pathogenic autoimmune attack against the human organism, thereby addressing the issue of why SARS-CoV-2 attacks the respiratory system so heavily [149]. The scientific cross-reactivity context and the clinical data showing that immunization with SARS-CoV antigens causes severe pneumonia [150] suggest a prominent pathogenic role of anti-SARS-CoV antibodies in COVID-19. In fact, emerging reports show that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection precedes the appearance of various autoimmune and autoinflammatory diseases, including pediatric inflammatory multisystemic syndrome or multisystem inflammatory syndrome in children [151,152]. Simply put, the current race for obtaining a highly immunogenic anti-SARS-CoV-2 vaccine might actually equate to a race for producing a highly lethal vaccine also in light of the fact that adjuvanted anti-SARS-CoV-2 would have a higher immunogenicity and autoimmune pathogenicity when compared to SARS-CoV-2 infection.

This risk of cross-reactivity further increases when considering that it cannot be estimated with the current vaccine pre-clinical tests [153, 154]. Indeed, the level of peptide sharing is highest between pathogens and human, murine, and rat proteomes, and is lowest (or absent) with proteomes from nonhuman primates such as gorilla, chimpanzee, and rhesus macaque. That is, from the genetic point of view, primates are unreliable animal models for revealing potential autoimmune cross-reactions in preclinical testing of immunotherapies since, obviously, no cross-reactions can occur in primates in absentia of shared sequences.

On the whole, the data exposed above open new scenarios in vaccinology by confirming the basic concept first stated in 2000 [147] and then illustrated repeatedly [155–158], according to which only peptide sequences derived from pathogens and absent in the human proteome, i.e., 'non-self' peptides', can lead to safe and efficacious immunotherapies.

3.10. Macro-level considerations

The issues discussed previously can be viewed as micro-level issues. The focus is at the cellular-virus-antibody level. These micro-level issues need to be understood within the larger context of macro-level issues. Two of these macro issues will be discussed in the present section.

The first issue examines the role of vaccines from a larger systemic perspective, especially whether the vaccine target is based on local or global optimization. The second issue relates to the objectivity of the published vaccine effectiveness studies, and how the results are open to bias due to conflicts of interest with research sponsoring agencies.

3.11. Local vs global optimization

In the evolution of COVID-19, the impact of real-life exposures to multiple toxic stressors degrading the immune system is followed by the SARS-CoV-2 virus exploiting the degraded immune system to trigger a chain of events ultimately leading to the disease. A person with such a degraded immune system is more vulnerable to infections and other health assaults. Assume that person is given a vaccine to 'protect' against a specific virus. How does it perturb the immune system?

The vaccination is designed to provide a local optimization; it is not designed to provide a global optimization. A successful vaccine may offer some increased protection against the specific viral strain in the vaccine, ranging from one season to a lifetime. What protection does it offer against other strains of the same virus, or other viruses? Does it enhance or decrease protection against other challenges, such as the rapid cell increases characteristic of cancer?

One of the mechanisms examined previously is vaccine-Associated Virus Enhancement (where vaccinated individuals may be at increased risk for other respiratory viruses because they do not receive the non-

specific immunity associated with natural infection). This phenomenon doesn't always happen, but it can happen. Its occurrence would strengthen the argument for local optimization, where protection is increased against one viral strain at the expense of reduced protection against another viral strain. Given that (on average) measures are not being taken to reverse the immune system degradation in parallel with administering a vaccine, adding a vaccine may improve one type of protection but degrade another type. The immune system remains degraded, and it will express its limitations against other challenges. Considering the military logistics analogy of the immune system, forces/supplies are being withdrawn from one Front to increase defensive capabilities on another Front. Since overall forces or supplies have not been increased, net overall capabilities have not increased, and, to first order, the vacated Front becomes more vulnerable.

As mentioned previously, many (if not all) vaccine inserts state that the vaccine has not been tested for carcinogenicity (and mutagenicity and fertility) impacts. Such carcinogenicity testing would require long-term tracking and establishing a credible link between the vaccine administered long ago and the onset of cancer. There are few incentives for the developers and regulatory agencies (who tend to be vaccine promoters, especially for a COVID-19 vaccine) to conduct such safety tests; finding e.g. a vaccine-cancer or vaccine-fertility or vaccine-AD link would present major problems. But, if the local/global optimization concept is correct, such a link may be possible, or even greater than possible. Unless the immune system is intrinsically strengthened, it is difficult to see how its operations can be improved in one sector without reducing performance in another sector. Otherwise, vaccines could be developed against every conceivable challenge, and compensate for the degraded immune system.

There is a more fundamental problem with vaccines and other members of the vast armamentarium employed by most of modern-day medicine to treat chronic and infectious diseases. Assume that one of the main operational functions of the body (not subjected to hereditary-based dysfunctions or autoimmune dysfunctionality) is to heal itself continuously. One of the healing mechanisms is signaling when the body is being exposed to harmful substances or harmful behaviors, in order to motivate the elimination of these harmful inputs. Since the body can't speak vocally, it communicates through the language of symptoms. Rather than listen to the symptoms and take steps to eliminate the offending substances/behaviors, modern medicine uses the approaches of drugs/radiation/surgery and other therapies to attenuate the symptoms. Since the fundamental problem has not been eliminated by the symptom-suppression treatment(s), the body is forced to increase signaling through stronger symptoms, which may emerge short-term or long-term, and could range from modest to lethal. It is inconceivable how such an approach can lead to true healing/disease reversal.

As an example, the first author's group did a study to develop a protocol that would prevent and reverse Alzheimer's disease (AD) [159]. As part of the study, Dr. Dale Bredesen, a neurologist who had developed an AD reversal approach, was referenced and quoted as follows: "In the case of Alzheimer's disease, there is not a single therapeutic that exerts anything beyond a marginal, un-sustained symptomatic effect, with little or no effect on disease progression. Furthermore, in the past decade alone, hundreds of clinical trials have been conducted for AD, at an aggregate cost of billions of dollars, without success. This has led some to question whether the approach taken to drug development for AD is an optimal one [160]." That statement could apply in different degrees to myriad chronic diseases. In fact, it is challenging to identify a chronic disease to which that statement does not apply!

The situation may even be worse with vaccines. Most drugs and other therapies are required to undergo modest short-, mid-, and long-term testing for myriad adverse health effects. One motivator for this range of testing is that the manufacturers/vendors of these therapies can be held liable for damage suffered as a result of their products. Vaccine manufacturers today have waivers from these liabilities (at least in the USA) because of the National Childhood Vaccine Injury Act (NCVIA) of 1986 passed by Congress. So, the financial motivation for thorough testing does not exist for vaccines. Additionally, vaccines are not tested for enhancement or stimulation of serious diseases, as stated previously.

Literature surveys show that vaccines are rarely tested for mid-term adverse effects, and certainly not tested for long-term adverse effects. They are not tested for combinations as administered over time on a prescribed schedule, and they are not laboratory-tested in combination with other toxic substances. There appears to be little interest from the manufacturers or researchers in discovering/identifying these adverse effects. This disinterest is most pronounced in the present efforts to have a COVID-19 vaccine on the market, perhaps made mandatory, within a year after starting development. There cannot be any credible safety testing under such a schedule [161]. There are many potential adverse health effects that can result from vaccine-induced mechanisms, as our present study has shown, and these effects could emerge in the near-term or the long-term. To require the young people (who are not at risk from the most serious consequences of COVID-19) to take such vaccines with potential serious long-term consequences is unjustifiable.

3.12. Objectivity of vaccine effectiveness studies

The following is focused on the USA experience, and probably is relevant to most other countries. Most of the vaccine research and development studies published in the biomedical literature (especially the journals with reasonable Impact Factor threshold values) are sponsored by the pharmaceutical industry and the Federal government, with some funds coming from Foundations. In the USA, the government promotes vaccinations for myriad diseases. It provides funds to the CDC for distributing vaccines, while at the time gives the CDC responsibility for safety monitoring of vaccines. In essence, the Federal government (through different branches) that promotes vaccines also sponsors vaccine research, approves vaccines, distributes vaccines, and monitors the safety of vaccines. These intertwining responsibilities open the door for conflicts-of-interest.

It is in the interests of the Federal government that approved vaccines have high Vaccine Effectiveness (VE), and a reading of the VE literature for the VE section contained in this paper shows clearly the emphasis by the sponsored research community (at least in the High Impact Factor journals) to emphasize high VE for the vaccines examined. For the COVID-19 vaccines under development, and the COVID-19 emergency measures being taken by Federal, State, and Local governments, dissenting voices have to make themselves heard through venues other than peer-reviewed publications in mainline journals. This is a perversion of the scientific process, which requires that all knowledgeable voices be heard, and results in a published literature of questionable credibility.

4. Conclusions

Four types of treatments are being used in different degrees to help counter the COVID-19 pandemic: reducing viral transmission (quarantine, face masks, social distancing, use of sanitizers, etc); treatments (mainly repurposed anti-viral drugs); vaccines (under development); immune system strengthening (eliminating immune degrading toxic stimuli; adding immune enhancing behaviors/substances). The first three types can be viewed as immune-augmenting; the last type is immune-strengthening.

Reducing viral transmission may offer some benefit, but has proven to be damaging psychologically and economically. Anti-viral treatments have had mixed results, and none have achieved consensus within the medical community. Vaccines are under accelerated development. Lifestyle and regulatory changes to strengthen the immune system have been minimal

Vaccines are being promoted by the healthcare industry, politicians, decision-makers, and the mainstream media as the best hope for containing the COVID-19 pandemic, and this is reflected in the funding their

accelerated development is receiving (Moderna, a leading COVID-19 vaccine contender, has "scored $2.48 billion in R&D and supply funding from the U.S. government for its program" [161]). The goal appears to be initial vaccine distribution by around end of 2020, although it is questionable whether such an accelerated vaccine development program would include adequate mid-term and long-term safety testing [161].

This optimistic outlook for early vaccine dissemination to the public contradicts vaccine development history, especially for coronavirus vaccines. Vaccine development, including limited safety testing, has taken an average of 12–15 years. Vaccines for the coronaviruses most closely associated with the SARS pandemic/outbreak of 2002 and the MERS pandemic/outbreak of 2012 have yet to be developed successfully, even after one-two decades of research.

The present study examined many viral mechanisms that could lead to vaccines exacerbating rather than attenuating viral infection, based on findings from [162]. Generically, the main problem is that prior viral exposure (vaccine-induced or wild/natural) could impact future viral exposure (vaccine-induced or wild/natural) positively or negatively. Years could be required to determine which outcome would result, both in the short-term and in the long-term. Additionally, many chronic diseases have been shown to result from viral exposure, and years of tracking in human trials could be required to determine which, if any, of these diseases would result from a COVID-19 vaccine. Possibly safer (non-autoimmunity-inducing) vaccines could result using peptide sequences derived from pathogens and absent in the human proteome, although the degree of safety enhancement might require years of tracking to determine.

Authors' contributions

All authors contributed equally, read and approved the final manuscript.

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Conceptualization: RNK; Data curation: DK, ALP, YS, MBB; Validation, Writing - all authors, Supervision, writing - review & editing: RNK, YS, DC, DAS, AT. All authors contributed equally, read and approved the final manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Exhibit "Y"

This is **Exhibit "Y"** referred to in the
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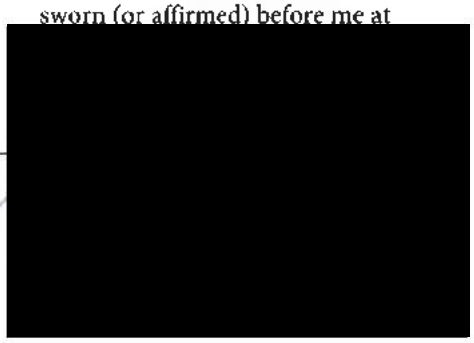
sworn (or affirmed) before me at



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Microglial activation and tau propagate jointly across Braak stages

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Compelling experimental evidence suggests that microglial activation is involved in the spread of tau tangles over the neocortex in Alzheimer's disease (AD). We tested the hypothesis that the spatial propagation of microglial activation and tau accumulation colocalize in a Braak-like pattern in the living human brain. We studied 130 individuals across the aging and AD clinical spectrum with positron emission tomography brain imaging for microglial activation ([^{11}C]PBR28), amyloid-β (Aβ) ([^{18}F]AZD4694) and tau ([^{18}F]MK-6240) pathologies. We further assessed microglial triggering receptor expressed on myeloid cells 2 (*TREM2*) cerebrospinal fluid (CSF) concentrations and brain gene expression patterns. We found that [^{11}C]PBR28 correlated with CSF soluble TREM2 and showed regional distribution resembling *TREM2* gene expression. Network analysis revealed that microglial activation and tau correlated hierarchically with each other following Braak-like stages. Regression analysis revealed that the longitudinal tau propagation pathways depended on the baseline microglia network rather than the tau network circuits. The co-occurrence of Aβ, tau and microglia abnormalities was the strongest predictor of cognitive impairment in our study population. Our findings support a model where an interaction between Aβ and activated microglia sets the pace for tau spread across Braak stages.

Microglial activation is part of the repertoire of immune responses in the human brain and is a key element associated with the development of AD[1,2]. Microglia coexist in the brain with the hallmark pathological features of AD, Aβ plaques and tau neurofibrillary tangles[3,4]. Rather than merely being an inflammatory epiphenomenon, recent studies in animal models suggested that microglial activation drives tau pathology[5,6]. These animal studies suggested microglial activation as a key player in the progression of tau pathology in the living human brain leading to dementia.

Postmortem studies suggested that the accumulation of tau tangles in AD follows a stereotypical pattern known as Braak stages[3,7]. The deposition of tau tangles in early Braak stages in the mesial temporal cortex is a typical finding in cognitively healthy populations[8,9], whereas the presence of neocortical tau is normally associated with cognitive impairment. In fact, the propagation of tau over the neocortex has been postulated as the culprit of dementia symptoms[3]. Although it has been suggested that Aβ triggers the spread of tau from transentorhinal/entorhinal structures to the neocortex[10], the low concentration of Aβ plaques in mesial temporal regions challenges this framework[6,7,11]. Thus, understanding the mechanisms by which tau tangles spread spatially, leading to dementia, is still a pressing issue in AD.

Although animal studies have supported the role of microglial activation in the propagation of tau pathology and human studies have suggested that tau pathology propagates in Braak stages, no previous study has tested whether microglial activation plays a role in the stereotyped spread of tau in Braak stages in patients with AD. Positron emission tomography (PET) imaging allows the quantification of microglial activation, Aβ deposition and tau propagation over time in vivo and provides topographical information across the entire brain. In this study, using a PET agent selective for microglial activation[12,13], applying recent advances in brain molecular network analysis[14], using a large cohort of genetically screened individuals

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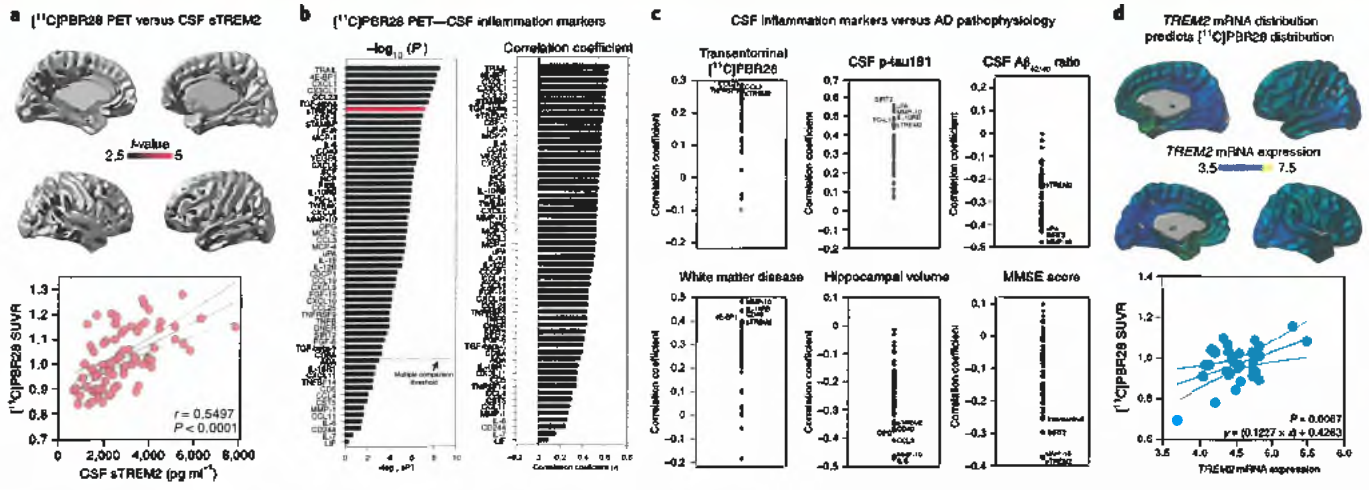


Fig. 1 | [^{11}C]PBR28 is associated with TREM2 microglial activation. a, *T*-statistical parametric map (FDR-corrected for multiple comparisons at $P < 0.05$) overlaid on a structural template shows the regions where linear regression indicated a significant positive association between [^{11}C]PBR28 SUVR and CSF sTREM2 in our population (top). No brain region showed a significant negative association between these markers after correction for multiple comparisons. The scatter plot (bottom) shows the results of a two-sided Pearson correlation between [^{11}C]PBR28 SUVR in the significant brain regions and CSF sTREM2. The error bands denote the 95% confidence intervals (CIs). **b**, The graphs show *P* values ($-\log_{10}$) (left) of two-sided Pearson correlation coefficients (right) testing the association between [^{11}C]PBR28 SUVR and CSF sTREM2 and other 55 inflammation-related markers. The dashed line indicates the Bonferroni correction threshold determining significance at $-\log_{10}(P) > 3.1$. **c**, The plots show the two-sided Pearson coefficient between CSF inflammation-related markers and transentorhinal [^{11}C]PBR28 SUVR, CSF p-tau181, CSF $A\beta_{42/40}$ ratio, white matter hyperintensities load, hippocampal volume and MMSE score. The aforementioned results support that CSF sTREM2 microglial activation is a marker highly associated with [^{11}C]PBR28 uptake in AD. The entire list of CSF protein abbreviations is found in Supplementary Table 4. $n = 75$; 48 CU ($n = 15$ males, mean age = 53 (25)); 18 individuals with MCI ($n = 12$ males, mean age = 72 (6)); and 9 individuals with AD dementia ($n = 3$ males, mean age = 68 (7)). uPA, urokinase-type plasminogen activator. **d**, Brain map showing the distribution of *TREM2* mRNA expression in six CU individuals ($n = 4$ males, mean age = 42.5 (13.4)) obtained from the Allen Human Brain Atlas, overlaid on a structural MRI template. Linear regression (bottom) analysis between mean Allen *TREM2* mRNA expression and mean [^{11}C]PBR28 SUVR from our CU aged population in the respective brain regions suggested that *TREM2* gene expression partially predicted [^{11}C]PBR28 uptake topographical distribution. The error bands denote the 95% CIs.

to optimize the signal of microglia activation imaging[14–16] and a tau imaging agent capable of capturing early and late Braak stages[17,18], we studied the association between microglial activation and tau propagation. Inspired by experimental literature showing that microglial activation precedes tau pathology in a similarly topographical fashion[3–5,19], we hypothesized that microglial activation sets the stage for tau propagation in a Braak-like pattern in patients with AD pathophysiology. Additionally, we also tested the hypothesis that the coexistence of widespread Aβ, tau and activated microglia drives dementia symptoms. The characterization of microglial activation as the basis of tau spread in Braak stages would provide a critical understanding of an elementary mechanism associated with the development of AD dementia and could lead to new therapeutic strategies aiming to contain disease progression.

Results

We genotyped 503 individuals for the *TSPO* polymorphism (rs6971); 263 (52.3%) were identified as *TSPO* high-affinity binders. Out of those, we studied 130 individuals with *TSPO* high-affinity binding across the aging and AD clinical spectrum (22 cognitively unimpaired (CU) young, 64 CU aged, 28 with mild cognitive impairment (MCI), 16 with AD) who had complete cognition, magnetic resonance imaging (MRI) and PET data at baseline (Extended Data Fig. 1). Aged individuals had an additional 1-year (mean = 1.2 years (0.3)) follow-up tau PET scan (Extended Data Fig. 2). Extended Data Fig. 3 shows the demographic characteristics of the population.

[^{11}C]PBR28 standardized uptake value ratio as a proxy of microglial activation. We found that the [^{11}C]PBR28 standardized uptake value ratio (SUVR) correlated with the CSF microglial activation marker soluble triggering receptor expressed on myeloid cells 2 (TREM2) in regions typically affected by AD (Fig. 1a). This association survived correction for age, sex, Aβ and cognitive status (Supplementary Table 1) and was better represented by a linear than nonlinear function (Supplementary Table 2). We also found that several other inflammation-related proteins were positively correlated with [^{11}C]PBR28 SUVR and AD pathology, supporting [^{11}C]PBR28 as a robust marker of neuroinflammation in AD (Fig. 1b). CSF sTREM2 was highly associated with tau pathology, atrophy, vascular white matter pathology and cognitive impairment (Fig. 1c), supporting the link between sTREM2 and AD pathophysiology. The regional patterns of postmortem *TREM2* messenger RNA expression in six CU individuals obtained from the Allen Human Brain Atlas was associated with the regional patterns of [^{11}C]PBR28 SUVR uptake in our CU aged population (Fig. 1d and Supplementary Fig. 1).

[^{11}C]PBR28 microglial activation and AD pathophysiology. [^{11}C]PBR28 SUVR was progressively higher from CU young to CU aged, as well as individuals with MCI and individuals with AD dementia in a typical AD-related brain region in the posterior cingulate/precuneus, inferior parietal and lateral temporal cortices (Extended Data Fig. 4). Voxel-wise receiver operating characteristic curves revealed that [^{11}C]PBR28 uptake differentiated CU young from CU aged with the highest accuracy in the posterior cingulate, precuneus, lateral temporal and inferior parietal cortices. [^{11}C]PBR28 uptake differentiated CU aged from individuals with MCI and individuals with MCI from individuals with AD dementia with the highest accuracy in posterior cingulate/precuneus, inferior parietal and occipital cortices (Extended Data Fig. 4). Microglial activation measured with [^{11}C]PBR28 positively correlated with Aβ [^{18}F]AZD4694 ($r = 0.3696$, $P < 0.0001$) and tau [^{18}F]MK-6240 ($r = 0.3415$,

$P<0.0001$). Microglial activation measured with CSF sTREM2 correlated with CSF $A\beta_{42/40}$ ($r=-0.2967$, $P=0.0278$) and CSF p-tau181 ($r=0.4749$, $P=0.0002$) (Extended Data Fig. 5). We did not find a significant sex effect on the associations between microglial activation and Aβ or tau pathology.

Microglial activation network drives tau propagation in Braak stages. Partial correlation matrices revealed that microglial activation levels within regions comprising Braak histopathological stages were hierarchically correlated with each other from Braak I through to Braak VI (Fig. 2a and Extended Data Fig. 6). Similarly, partial correlation matrices revealed that regional tau tangle levels correlated with each other according to the hierarchical stages proposed by Braak (Fig. 2b and Extended Data Fig. 6). Notably, the elements of the microglia–microglia and tau–tau matrices were highly correlated with one another ($r=0.8269$, $P<0.0001$), supporting a strong link between the patterns of microglial activation and tau accumulation in the human brain (Fig. 2c). Partial correlation matrices revealed that the rates of longitudinal tau propagation within PET Braak-like regions hierarchically correlated with each other from Braak I through to Braak VI (Fig. 3a and Extended Data Fig. 7). Regression analysis covarying for the two baseline networks (tau and microglia) suggested that the longitudinal tau propagation pathways depended on the microglial network rather than the tau network circuits (Fig. 3b and Supplementary Figs. 2 and 3). In addition, we found that baseline [^{11}C]PBR28 SUVR values in the transentorhinal cortex (Braak I) were correlated with longitudinal changes in tau PET uptake within brain regions comprising PET Braak-like stages II–VI, accounting for age, sex, *APOE ε4* status and Aβ (Extended Data Fig. 8). Baseline [^{11}C]PBR28 SUVR values in other Braak regions were not significantly associated with changes in tau PET. CSF sTREM2 levels in CU individuals and individuals with MCI were associated with [^{18}F]MK-6240 tau load in early (Braak I and II) and late (Braak III–VI) Braak regions, respectively (Extended Data Fig. 9). No significant association between CSF sTREM2 and [^{18}F]MK-6240 SUVR was found in patients with AD dementia after correction for multiple comparisons.

Aβ potentiates the effect of microglial activation on tau spreading. Voxel-wise interaction models showed that high levels of global Aβ burden and high levels of microglial activation in the transentorhinal cortex were synergistically associated with tau load predominantly in allocortical regions (Fig. 4a). Similarly, high levels of global Aβ burden and microglial activation in the Braak IV region synergistically determined widespread neocortical tau (Fig. 4b), supporting the effect of Aβ on the hierarchical propagation of tau. Also, we found a synergistic interaction between global Aβ deposition and microglial activation on the topographical spread of tau pathology (percentage of the brain with tau pathology) (Fig. 4c).

Aβ, tau and activated microglia are associated with dementia. We tested the associations between cross-sectional biomarkers and cross-sectional dementia symptoms. We found that a synergistic interaction between Aβ, tau and microglial activation PET concentrations rather than independent or grouped by two effects was highly associated with a worse Mini-Mental State Examination (MMSE) score ($P=0.0003$). Analysis of variance supported that the model with the triple interaction best described this relationship than the possible reduced models ($P<0.0001$). In addition, we found a progressive increase in the percentage of individuals with concomitant Aβ, tau and microglial activation abnormalities from CU young (0%) to CU aged (4.5%), individuals with MCI (27%) and individuals with AD dementia (67%) (Extended Data Fig. 10). On the other hand, the prevalence of microglial activation abnormality alone or together with only Aβ or only tau abnormalities was not different between clinical diagnostic groups, further suggesting that the presence of both Aβ and tau is necessary for the association of microglial activation with cognitive impairment. The concomitant presence of Aβ, tau and microglial activation abnormalities was the factor most highly associated with cognitive impairment in our population (Fig. 5).

Discussion

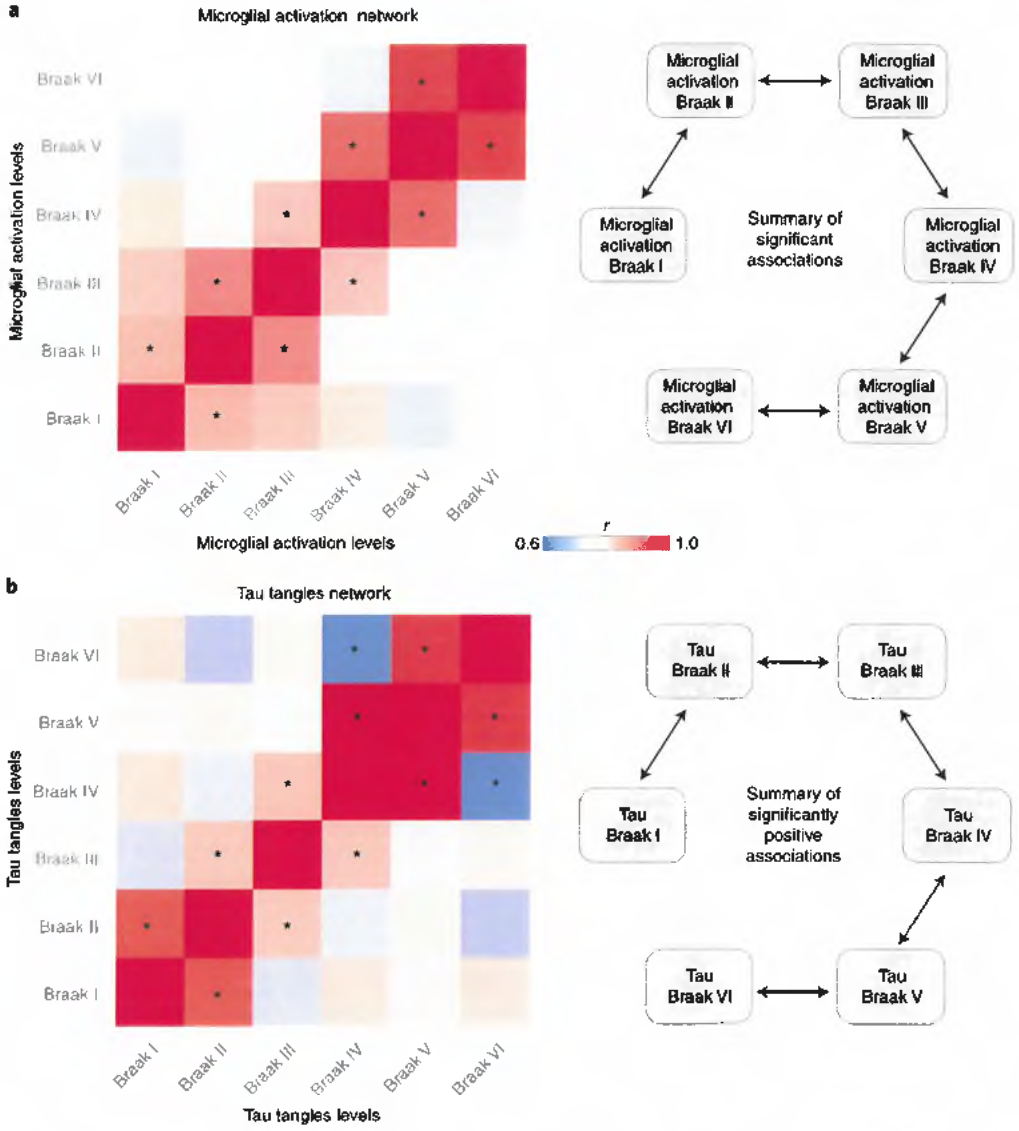
Our results suggest that microglial activation and tau accumulation spatially propagate in parallel following brain circuits predicted by postmortem series from the transentorhinal/entorhinal to sensorimotor cortices. Our findings also support that Aβ potentiates the effects of microglial activation on tau spreading (Fig. 6). Furthermore, we found evidence suggesting that when these three pathologies are present in the human brain concomitantly, they synergistically interact with each other to determine dementia.

We found that [^{11}C]PBR28 uptake was related to TREM2, which is exclusively expressed by microglia[20]. Thus, our findings further support [^{11}C]PBR28 uptake as a proxy of activated microglia in the living human brain[21,22]. In our analyses, [^{11}C]PBR28 SUVR correlated with CSF sTREM2 concentration and at least partially overlapped with the distribution of *TREM2* gene expression obtained from the Allen Human Brain Atlas[23]. Similarly, a previous publication suggested a link between TREM2 expression and TSPO PET tracer uptake in a mouse model of AD[24]. Interestingly, several studies suggested an association between the microglial marker TREM2 and AD[20]. sTREM2 has been shown to have a positive correlation with tau biomarkers[25,26]. Silencing *TREM2* in a model presenting tau pathology in the absence of Aβ exacerbates tau pathology[27], which supports that Aβ is crucial for the deleterious association between microglia and tau. We also found that sTREM2 was strongly associated with [^{11}C]PBR28 in key AD-related brain regions, cognitive impairment, hippocampal atrophy, tau pathology and white matter disease. On the other hand, sTREM2 showed a modest association with Aβ pathology. Altogether, these results support a link between [^{11}C]PBR28 and TREM2 microglial activation pathways and the notion that Aβ and microglial activation may be two partially independent processes that, when they converge, synergistically potentiate tau pathology.

We also measured 55 other inflammation-related proteins in CSF. We found that several cytokines previously associated with AD,

Fig. 2 | Microglial and tau networks spatially converge to Braak-like stages. a, Microglial activation network analysis between regions comprising Braak histopathological stages (Braak I–VI) demonstrates that microglial activation regions correlated hierarchically with each other, where a PET Braak-like stage region only correlated with the previous and subsequent stages. **b**, Similarly, tau tangle network analysis between Braak regions demonstrated that tau regions correlated hierarchically with each other, where a Braak stage only correlated with the previous and subsequent stages. In the matrices, we presented Pearson partial correlation coefficients (r) controlled for age, sex, education and *APOE ε4* status. We also considered in each partial correlation the tracer values in the remaining Braak-like regions. The matrix correlations that survived Bonferroni correction at $P<0.05$ are indicated with a single asterisk. $n=108$; 64 CU aged ($n=14$ males, mean age = 72 (6)); 28 individuals with MCI ($n=17$ males, mean age = 73 (9)); and 16 individuals with AD dementia ($n=6$ males, mean age = 70 (8)). The scatter plots with the correlations used in the matrices are presented in Extended Data Fig. 6. **c**, Remarkably, two-sided Pearson correlation shows that the elements of the microglial activation–microglial activation matrix and tau–tau matrix were highly positively correlated with each other, reinforcing the notion of a joint topographic propagation of microglial activation and tau tangles through Braak stages. The error bands denote the 95% CIs.

such as TNF-related apoptosis-inducing ligand (TRAIL)[28], C-X-C motif chemokine 1 (CXCL1)[29], fractalkine (CX3CL1)[30], transforming growth factor alpha (TGF-alpha)[31], C-C motif chemokine 3 (CCL3)[32] and C-C motif chemokine 23 (CCL23)[33] were highly correlated with [^{11}C]PBR28 SUVR, further supporting [^{11}C]PBR28 as a proxy of brain inflammation in AD. Interestingly, CSF SIR2-like protein 2 (SIRT2) and matrix metalloproteinase-10 (MMP-10) were strongly associated with tau and Aβ but showed a modest correlation with [^{11}C]PBR28, suggesting that other pathways are possibly linked to inflammation[34,35] and AD may not be strongly represented by [^{11}C]PBR28 uptake. Overexpression of SIRT2 and matrix metalloproteinases have already been associated with tau pathology[36]. Also, in line with previous literature, we found that interleukin-8 was highly associated with reduced cortical volume[37], whereas



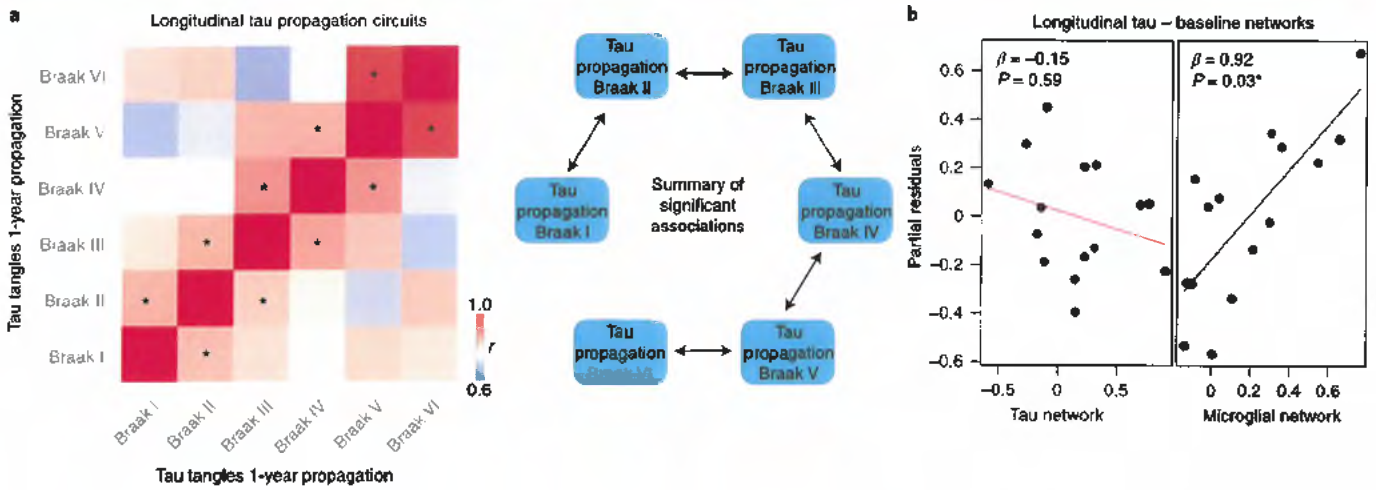


Fig. 3 | The patterns of longitudinal tau propagation depend on baseline microglial network circuits. a, The matrix revealed that the rates of longitudinal tau propagation correlated hierarchically with each other according to the Braak stages. In the matrix, we presented Pearson partial correlation coefficients (*r*) controlled for age, sex, education, *APOE ε4* status and remaining Braak-like regions. The correlations that survived Bonferroni correction at $P < 0.05$ are highlighted with a single asterisk. $n = 56$; 34 CU aged ($n = 6$ males, mean age = 73 (6)); 13 individuals with MCI ($n = 9$ males, mean age = 74 (6)); and 9 individuals with AD dementia ($n = 4$ males, mean age = 70 (7)). The scatter plots with the correlations used in the matrix are presented in Extended Data Fig. 7. **b**, The partial residuals plots show the results of a linear regression between the longitudinal tau propagation network and both baseline topographic networks, microglial activation–microglial activation and tau–tau covariate in the same model. The model suggests that the pathways of longitudinal tau propagation depended on the underlying microglia, rather than tau circuits.

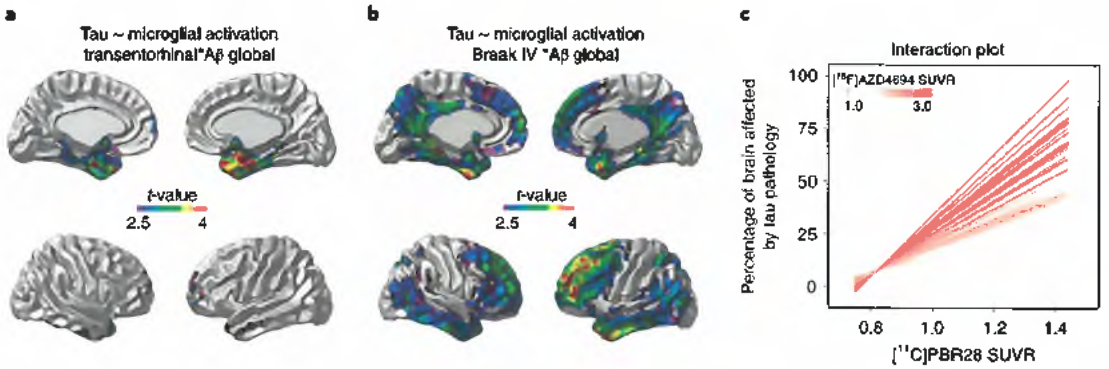


Fig. 4 | Aβ load potentiates microglial activation effects on tau spreading. a,**b**, The parametric maps show the brain regions with a significant interactive effect (FDR-corrected for multiple comparisons at $P < 0.05$) between microglial activation ([^{11}C]PBR28 SUVR) in the transentorhinal cortex (**a**) and Braak IV cortex (**b**) with global Aβ ([^{18}F]AZD4694 SUVR) on voxel-wise tau tangles deposition ([^{18}F]MK-6240 SUVR) across our aged population. Here, '*' (transentorhinal*Aβ global; Braak*Aβ global) indicates an interaction between pathologies. $n = 108$; 64 CU aged ($n = 14$ males, mean age = 72 (6)); 28 individuals with MCI ($n = 17$ males, mean age = 73 (9)); and 16 individuals with AD dementia ($n = 6$ males, mean age = 70 (8)). **c**, The plot shows the graphical representation of the interaction between global Aβ and microglial activation on the spatial spreading of tau (percentage of abnormal voxels) across the brain cortex. The model suggests that high levels of both brain Aβ pathology and microglial activation are required to determine widespread tau pathology across Braak stages.

MMP-10 was strongly correlated with vascular pathology[38]. It is important to emphasize that sTREMs and the panel with the 55 proteins were measured using the Meso-Scale Discovery and multiplex immunoassay platforms, respectively. The fact that different platforms may have inherently different sensitivity for protein quantification precludes a direct comparison between the results obtained with sTREMs and with the other proteins. Altogether, the aforementioned results supported [^{11}C]PBR28 SUVR as a proxy of neuroinflammation in AD and the idea that other inflammation-related pathways, unrelated to TSPO tracer uptake, also play a role in AD progression.

The brain spatial distribution of microglial activation and tau tangles accumulation followed a similar stereotyped pattern of progression, which were previously described for tau tangles pathology by Braak and colleagues in postmortem brain tissue[6,7,39]. These findings support the in vitro literature suggesting that microglial activation occurs in a similarly topographic fashion as tau pathology[1–5,19]. Although it has been demonstrated that microglia can degrade pathological tau[40], one may speculate that the mechanism by which this joint propagation occurs rests on the fact that microglia may phagocytize and release exosomes containing inefficiently degraded tau, which would 'contaminate' surrounding cells with tau pathology[41,42]. Indeed, recent studies confirm that microglia release tau seeds, which is the tau conformation capable of inducing tau aggregation in affected cells[41,42], as well as increased levels of exosomes containing tau in AD[43,44]. Expanding on these in vitro studies,

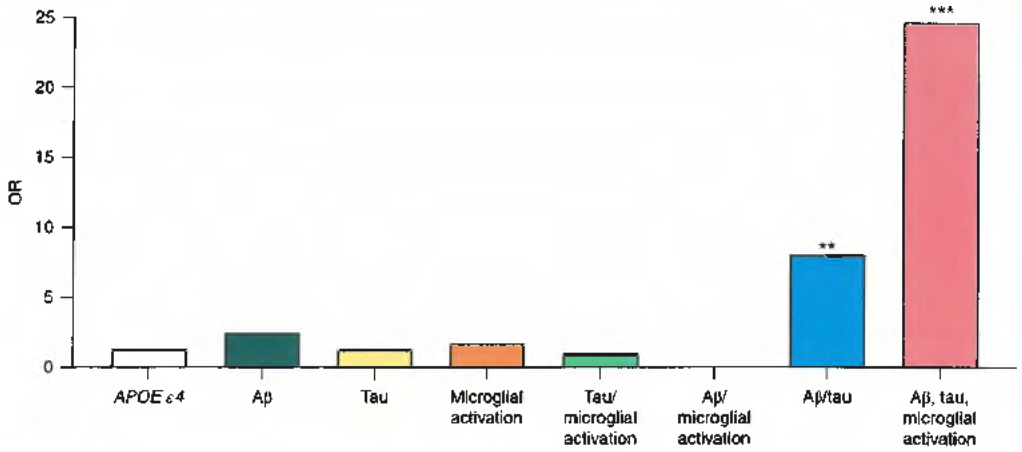


Fig. 5 | The concomitant presence of Aβ, tau and microglial activation abnormalities is associated with cognitive symptoms. The figure shows the odds ratios (OR) from the multivariate logistic regression analysis on the diagnostic of cognitive impairment (groups coded as CU aged = 0 and aged with cognitive impairment = 1) in our aged population. $n = 108$; 64 CU ($n = 14$ males, mean age = 72 (6)); 44 individuals with cognitive impairment ($n = 23$ males, mean age = 72 (8)). The co-occurrence of Aβ, tau and microglial activation abnormalities was the strongest predictor of cognitive impairment in our population. *APOE ε4* (OR = 1.2827, $P = 0.6321$), Aβ (OR = 2.4596, $P = 0.5229$), tau (OR = 1.2552, $P = 0.7887$), microglial activation (OR = 1.7066, $P = 0.6115$), tau/microglial activation (OR = 0.9702, $P = 0.9807$), Aβ/microglial activation (OR = 0.0000, $P = 0.9914$), Aβ/tau (OR = 8.0521) (95% CI = 1.9163–43.7123; $^{**}P = 0.0075$), Aβ, tau and microglial activation (OR = 24.670 (95% CI = 5.2797–156.8821; $^{***}P = 0.0002$).

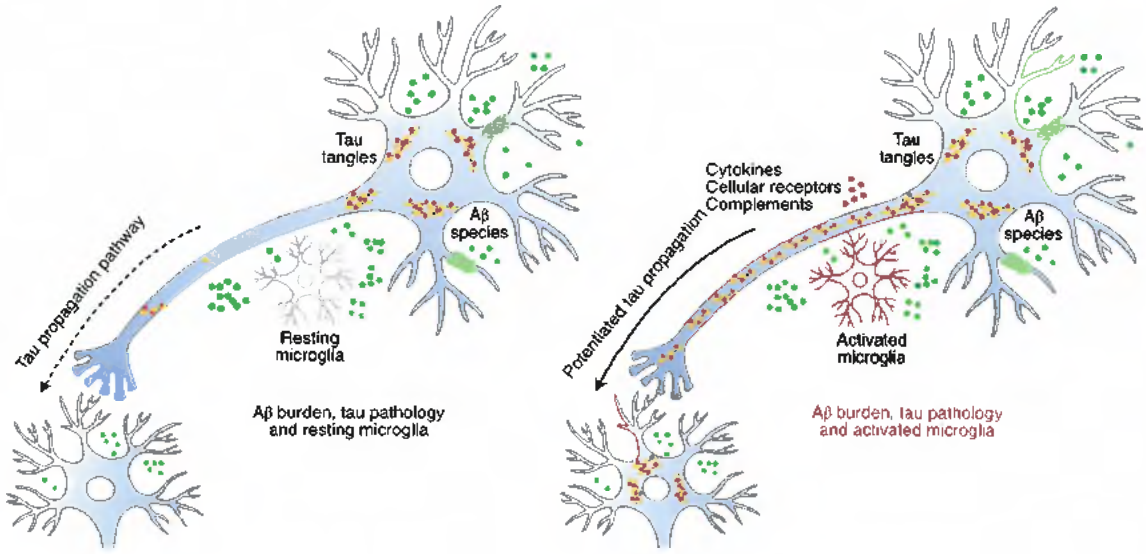


Fig. 6 | Schematic representation of the effect of microglial activation on tau propagation in the presence of Aβ pathology. The brain colocalization of Aβ, tau and activated microglia potentiates tau propagation (right). This model suggests Aβ and microglial activation as two partially independent processes that, when they converge, lead to neocortical tau pathology.

we showed in this study that this joint propagation of microglia and tau hierarchically follows Braak-like stages in the human brain. Our results bring together two important concepts in AD, Braak-like tau propagation[6,7] and the emerging concept of the effects of microglial activation on tau spread[9]. Furthermore, the fact that the patterns of longitudinal tau propagation depended on underlying microglial circuits supports the pathogenic protein spread hypothesis[18], with the addition that the physical propagation of tau protein through neurons may be catalyzed by activated microglia. Understanding the relationship between microglia and the spatial spread of tau in vivo may prove crucial to designing new therapies targeting the interplay between these pathologies.

Although there is compelling evidence supporting that Aβ pathology is associated with widespread tau tangle pathology in AD[3], the mechanisms by which Aβ triggers neocortical tau are poorly understood. Indeed, the lack of a strong anatomical correlation between Aβ plaques and tau tangle pathology and the relatively low concentrations of Aβ in the allocortex challenge a direct link between Aβ and tau spreading in Braak stages from allocortical to neocortical regions[4,5,10]. In this study, we found that Aβ pathology leads to tau propagation over the neocortex by potentiating the effects of microglial activation on tau spreading. Our models also suggested that Aβ pathology in the absence of activated microglia was associated with modest levels of neocortical tau. These results are in line with recent experimental literature, supporting that Aβ potentiates tau propagation in the presence of activated microglia[1]. Together, our findings support the notion that microglial activation links the deleterious effects of Aβ with tau propagation. Interestingly, we found that *TREM2* gene expression was particularly elevated in the transentorhinal cortex of CU adults, the brain

region where tau spread is postulated to begin[46]. Importantly, similar to previous postmortem literature[47], we also showed neocortical tau in the absence of activated microglia in a small number of individuals with AD, suggesting that other pathways may also potentiate tau propagation.

We found that the co-occurrence of Aβ, tau and microglia activation abnormalities was synergistically associated with the development of cognitive impairment and dementia. These results support previous literature suggesting a double hit of Aβ pathology on AD progression[13], first potentiating the effect of microglial activation on tau spread and then interacting with these pathologies to determine dementia. Additionally, our results suggest a close link between microglial activation, AD hallmark biomarkers and dementia symptoms, supporting that microglial activation may be a key element associated with AD progression with the potential to be incorporated in the biological definitions of the disease[48].

Our results may have implications for clinical trials. Since we found results suggesting that microglial activation in the transentorhinal/entorhinal cortex is potentially involved in 'unleashing' mesial temporal tau over the neocortex, we may predict that individuals with tau deposition confined to medial temporal regions would benefit from preventive therapies targeting neuroinflammation. Supporting this notion, previous studies have already associated microglial dysfunction within the entorhinal cortex to AD progression[49,50]. In addition, our results also suggest that these clinical trials could have advantages from using well-validated fluid or brain imaging markers of microglial activation to assess drugs' target engagement and efficacy.

Strengths of the present study include the use of only high-affinity binders for the [^{11}C]PBR28 tracer, avoiding the need for corrections associated with mixed-affinity cases. The use of only high-affinity binders allowed us to perform methods highly sensitive to noise associated with artificial uptake variations, such as network analyses. All PET scans were performed using a single high-resolution brain-dedicated camera to avoid scanner-related variabilities. However, this study has methodological limitations. It would be highly desirable to replicate our results in cohorts using long-term sequential biomarker measurements at multiple time points to better evaluate the longitudinal progression of the pathological processes and better understand their temporal interrelationships. However, due to the limited availability of large-scale studies including microglial activation PET imaging, we will probably have to wait for our cohort to mature before being able to make such observations. Although it is desirable to replicate our findings in larger populations, it is important to emphasize that, to the best of our knowledge, this is the largest study available using microglial activation PET imaging across a disease spectrum. *TSPO* expression indirectly captures microglia[11]. Still, there is no technology available that has been better validated for assessing cerebral microglial activation than *TSPO* PET ligands[21,22,51]. Previous studies suggested that microglial activation assumes different functional phenotypes in the course of AD, which may lead to protective or deleterious effects depending on the disease stage[52,53]. TSPO PET tracers are not capable of differentiating between these distinct microglial activation phenotypes, which limits the interpretability of results generated with these tracers. Thus, we could not examine whether different microglial activation phenotypes were associated differently with tau propagation in our population. One challenge for *TSPO* and numerous other PET tracers targeting physiological processes is the lack of an appropriate reference region for measuring non-displaceable binding because no brain area is devoid of specific binding for those molecules. However, previous studies suggested that the cerebellum may be a useful reference region that can substitute, with possible advantages, absolute [^{11}C]PBR28 quantitation performed with complex setups for arterial blood sampling and high-performance liquid chromatography measurements[54]. In fact, the use of a reference region as opposed to arterial sampling allows the design of large-scale studies, such as the one presented in this article. Biomarkers naturally provide a continuous spectrum of measurements; therefore, thresholds are invariably subject to conceptual and analytical idiosyncrasies and may change depending on the method used in the analysis. Thus, the use of more conservative or liberal thresholds could change some of our results. Although we found that sTREM2 was a biomarker closely related to [^{11}C]PBR28 uptake, it is important to emphasize that it is possible that several other neuroinflammation pathways not assessed in this study have a stronger link with [^{11}C]PBR28 uptake than sTREM2. Previous studies suggested a U-shaped association between TREM2 and AD[55]. Although our models suggested that a linear function best described the association between CSF sTREM2 and PET [^{11}C]PBR28 techniques, we cannot entirely exclude that a nonlinear association may better represent the biological link between these markers. Finally, due to self-selection bias, our participants may not represent the general population.

To conclude, our results support that microglial activation is a key element linking the effects of Aβ to tau spread and ultimately dementia.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01456-w.

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Methods

Genotype and characteristics of participants. Study participants were genotyped for the Ala147Thr polymorphism of the *TSPO* gene (rs6971, https://www.ncbi.nlm.nih.gov/snp/rs6971), which is a predictor of the binding affinity of the tracer [^{11}C]PBR28 to the 18-kDa translocator protein (*TSPO*). *TSPO* is overexpressed in the outer membrane of the mitochondria in activated microglia. Based on this genotype, individuals can either be high-, mixed- or low-affinity binders[54]. Since the [^{11}C]PBR28 signal in low-affinity binders is negligible and mixed and high-affinity binders show inherently different tracer signals, we used only high-affinity binders in our study to increase the reliability of the results. All participants underwent clinical and cognitive assessments, including clinical dementia rating (CDR) and MMSE. CU individuals had no objective cognitive impairment and a CDR score of 0. Individuals with MCI had subjective/objective cognitive impairment, a CDR score of 0.5 and relatively preserved activities of daily living. Individuals with mild-to-moderate sporadic AD dementia met the National Institute on Aging and Alzheimer's Association criteria for probable AD as determined by a physician and had a CDR score between 0.5 and 2. We excluded participants with inadequately treated systemic conditions, active substance abuse, recent head trauma, major surgery or presenting with MRI/PET safety contraindications. The study was approved by the Montreal Neurological Institute PET Working Committee and the Douglas Mental Health University Institute Research Ethics Board; written informed consent was obtained from all participants.

Brain imaging methodology. Study participants had a three-dimensional MRI (Siemens), as well as Aβ [^{18}F]AZD4694, tau [^{18}F]MK-6240 and microglial activation *TSPO* [^{11}C]PBR28 PET imaging in the same brain-dedicated scanner (Siemens high-resolution research tomograph). [^{18}F]AZD4694 images were acquired at 40–70 min after the intravenous bolus injection of the tracer and reconstructed with an ordered subset expectation maximization (OSEM) algorithm on a four-dimensional (4D) volume with 3 frames (3 × 600 s). [^{18}F]MK-6240 images were acquired at 90–110 min after the intravenous bolus injection of the tracer and reconstructed using the same OSEM algorithm on a 4D volume with 4 frames (4 × 300 s)[17]. [^{18}F]MK-6240 is a new tau PET imaging agent with subnanomolar affinity to tau tangles. Previous studies have shown that this tracer recapitulates early and late Braak stages with high sensitivity[18]. Finally, [^{11}C]PBR28 images were acquired at 60–90 min after the intravenous bolus injection of the tracer (mean injected dose = 384 (17) megabecquerel, mean molar activity = 193 (99) gigabecquerel μmol^{-1}) (Supplementary Table 3) and reconstructed using the OSEM algorithm on a 4D volume with 6 frames (6 × 300 s)[54]. A 6-min transmission scan with a rotating ^{137}Cs point source was performed at the end of each PET emission acquisition for attenuation correction. PET images were also corrected for motion, dead time, decay and scattered and random coincidences. Briefly, PET images were automatically registered to the native T1-weighted MRI and MRIs were linearly and nonlinearly registered to the Montreal Neurological Institute (MNI) space. Then, PET images were registered to the MNI space using transformations from PET to native MRI and from native MRI to the MNI space. [^{18}F]MK-6240 SUVRs used the Crus I gray matter[56], whereas [^{18}F]AZD4694 SUVRs and [^{11}C]PBR28 SUVRs used the whole cerebellum gray matter as the reference region[17]. Previous studies suggested that simplified methods using the cerebellum as reference offer reliable estimates for [^{11}C]PBR28 binding, in fact, with a possibly higher sensitivity than using full quantitation[54]. Finally, PET images were spatially smoothed to an 8-mm full-width at half maximum resolution. The ideal determination of PET thresholds would be anchored in postmortem data, which were not available in our cohort. Thus, Aβ [^{18}F]AZD4694 positivity was statically defined as described in detail elsewhere[17]. Abnormal tau [^{18}F]MK-6240 and microglial activation [^{11}C]PBR28 were defined as SUVR values 2.5 s.d. above the mean of the SUVR of CU young, similar to what has been proposed previously[18]. To separate individuals into Aβ PET positive and negative groups, we used mean SUVR in a composite region in the frontal, parietal, temporal and cingulate cortices[58]. To separate individuals into tau PET positive and negative groups, we considered as tau negative individuals negative in all Braak regions[18]. To separate individuals into microglial activation PET positive and negative groups, we used the brain region with the greatest statistical difference between CU aged and individuals with AD dementia groups (posterior cingulate cortex). The Desikan-Killiany-Tourville atlas was used to define the regions of interest[59]. PET Braak-like stages were determined based on results from previous autopsy studies[6,7]. The transentorhinal cortex was defined based on an established segmentation procedure on a 1-mm isotropic voxel matrix in stereotaxic space[18]. Braak I (transentorhinal), II (entorhinal and hippocampus), III (amygdala, parahippocampal gyrus, fusiform gyrus, lingual gyrus), IV (insula, inferior temporal, lateral temporal, posterior cingulate and inferior parietal), V (orbitofrontal, superior temporal, inferior frontal, cuneus, anterior cingulate, supramarginal gyrus, lateral occipital, precuneus, superior parietal, superior frontal, rostromedial frontal) and VI (paracentral, postcentral, precentral and pericalcarine). Hippocampal volume was measured with Freesurfer v.6.0 using the Desikan-Killiany-Tourville gray matter parcellation in a structural T1-weight MRI[59]. White matter hyperintensities were segmented in fluid-attenuated inversion recovery using the lesion prediction algorithm implemented in the LST toolbox v.2.0.15 (www.statistical-modelling.de/lst.html) for SPM. A *TREM2* gene expression distribution image was derived from microarray data obtained from the open-source Allen Human Brain Atlas (www.brain-map.org)[23], which is composed of mRNA expression intensity values measured on 3,702 samples from 6 healthy human brains (n = 4 males, mean age = 42.5 (13.4), postmortem delay = 20.6 (7) h). The *TREM2* mRNA expression derived from a Gaussian process[60] was downloaded from www.meduniwien.ac.at/neuroimaging/mRNA.html.

CSF measurement. CSF sTREM2 concentration was measured using a Meso-Scale Discovery assay, as described previously[61]. LUMIPULSE G1200 (Fujirebio) was used to measure CSF p-tau181 and Aβ$_{42/40}$[62]. Additionally, CSF samples were analyzed for 92 proteins using multiplex immunoassay analysis (inflammation panel; Olink; https://www.olink.com/products/inflammation/). We excluded from the analysis protein values below the lower limit of quantification for each biomarker; 37 out of 92 protein biomarkers that had >15% of measures below the lower limit of quantification were entirely excluded from subsequent analyses. Fifty-five out of 92 remaining protein biomarkers used in our study can be found in Supplementary Table 4 and Supplementary Fig. 4.

Statistical methods. Statistical models were generated using R v.3.1.2 (http://www.r-project.org/); voxel-wise statistics were carried out with MATLAB v.9.2 (http://www.mathworks.com) and VoxelStats package[63]. The associations between biomarkers were tested using Pearson correlations and linear regressions Bonferroni-corrected for multiple comparisons at $P < 0.05$ when appropriate. The Akaike information criterion was used to compare models. Voxel-wise associations between biomarkers were tested using linear regressions accounting for age, sex, education and *APOE ε4* status and multiple comparisons using a false discovery rate (FDR) threshold of $P < 0.05$. Voxel-wise receiver operating characteristic curves contrasting groups provided the area under the curve for the PET tracer uptake of different clinical groups. The network analysis for tau tangles ([^{18}F]MK-6240) and microglia ([^{11}C]PBR28) was performed using uptake values from composite brain regions corresponding to Braak histopathological stages (PET Braak-like stages I–VI)[18]. The edge values used as matrix elements were the correlation coefficients between regions of interest. The interregional correlation coefficients were calculated using Pearson partial correlation accounting for age, sex, education, *APOE ε4* status and remaining Braak regions. We verified the adequacy of these associations by verifying that the residuals of the same aforementioned associations—tested using linear regression—had a normal distribution, as described previously[18]. Networks were evaluated with a symmetric matrix, showing the strength of the correlations between regions. Tau and microglial matrix elements were further correlated with each other using Pearson correlation. Partial residuals of covaried regressions were generated with the R package termplot function. We measured the annual rate of progression in [^{18}F]MK-6240 uptake across brain regions as the difference between follow-up and baseline uptakes divided by baseline uptake and time between scans. A voxel-wise, two-sided, paired t-test assessed the presence of significant longitudinal changes in [^{18}F]MK-6240 SUVR. A voxel-wise interaction model was built to test the interactive and main effects of Aβ and microglial activation on tau. To assess individuals' percentage of abnormal voxels on tau PET scans, we created voxel-wise maps indicating the frequency of tau positivity. First, we built z-score parametric maps for each individual, anchored on the normative data of CU young. Then, we generated binary maps of tau positivity for each individual, yielding all voxels with 2.5 s.d. higher than the mean SUVR of CU young and averaged these maps. Finally, we calculated the percentage of abnormal voxels for each individual.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All requests for raw and analyzed data and materials will be promptly reviewed by McGill University to verify if the request is subject to any intellectual property or confidentiality obligations. Anonymized data will be shared upon request from a qualified academic investigator for the purpose of replicating the procedures and results presented in this article. Any data and materials that can be shared will be released via a material transfer agreement. Data are not publicly available due to information that could compromise the privacy of research participants.

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Author contributions

T.A.P., S.G. and P.R.-N. conceptualized the work. T.A.P., A.L.B., N.J.A. and P.R.-N. contributed to the design of the analyses. T.A.P., A.L.B., M.S.K., J.T., M.C., M. Savard, F.Z.L., C.T., T.K.K., J.O., S.M., J.S., G.M., J.-P.S., M.J.L., P.E. and P.R.-N. contributed to the acquisition, processing and analysis of the neuroimaging data. N.J.A., T.K.K., M. Schöll, K.B. and H.Z. contributed to the analysis of the fluid biomarkers. T.A.P. and P.R.-N. drafted the manuscript. All authors interpreted the data and contributed to revising the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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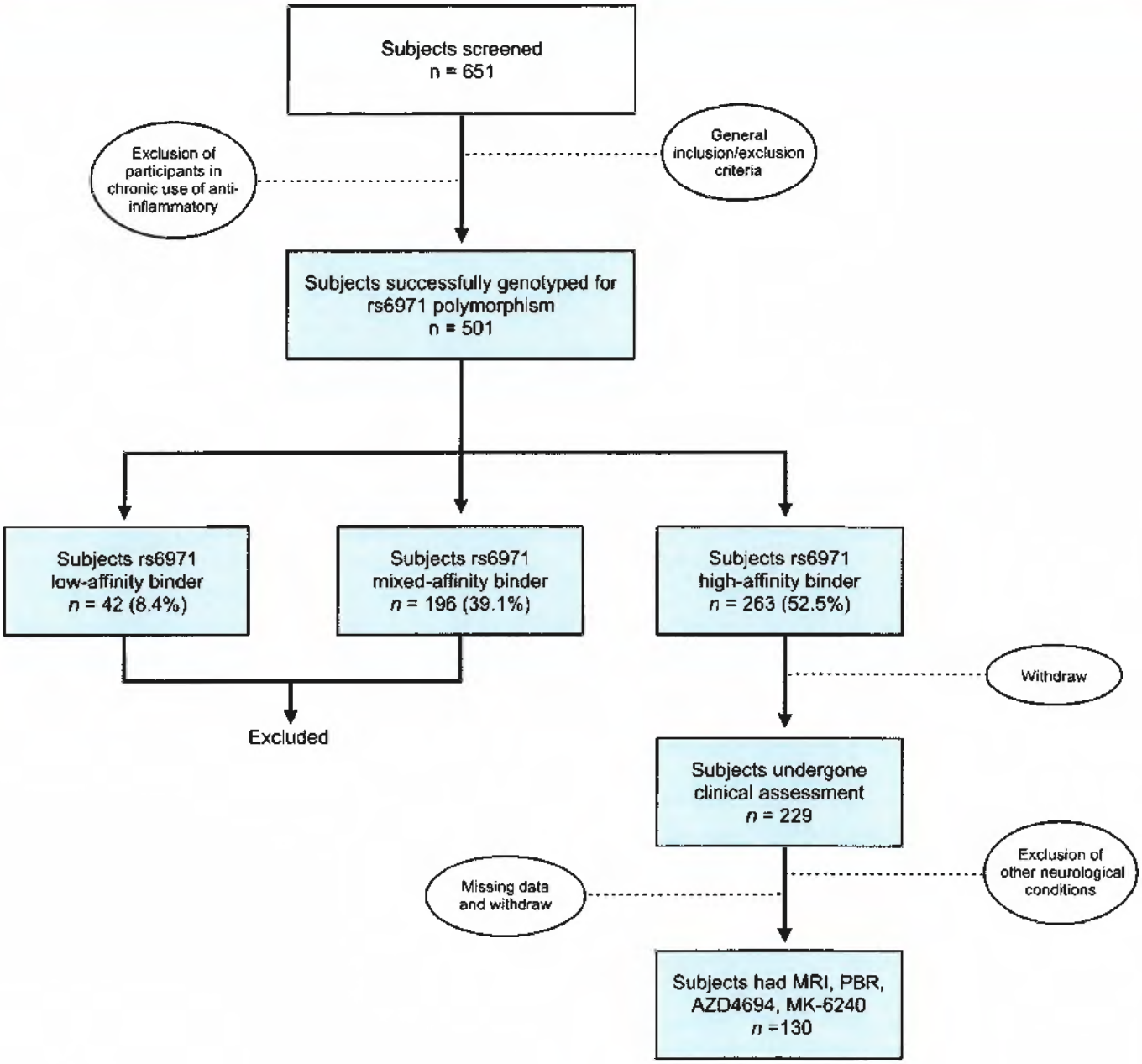
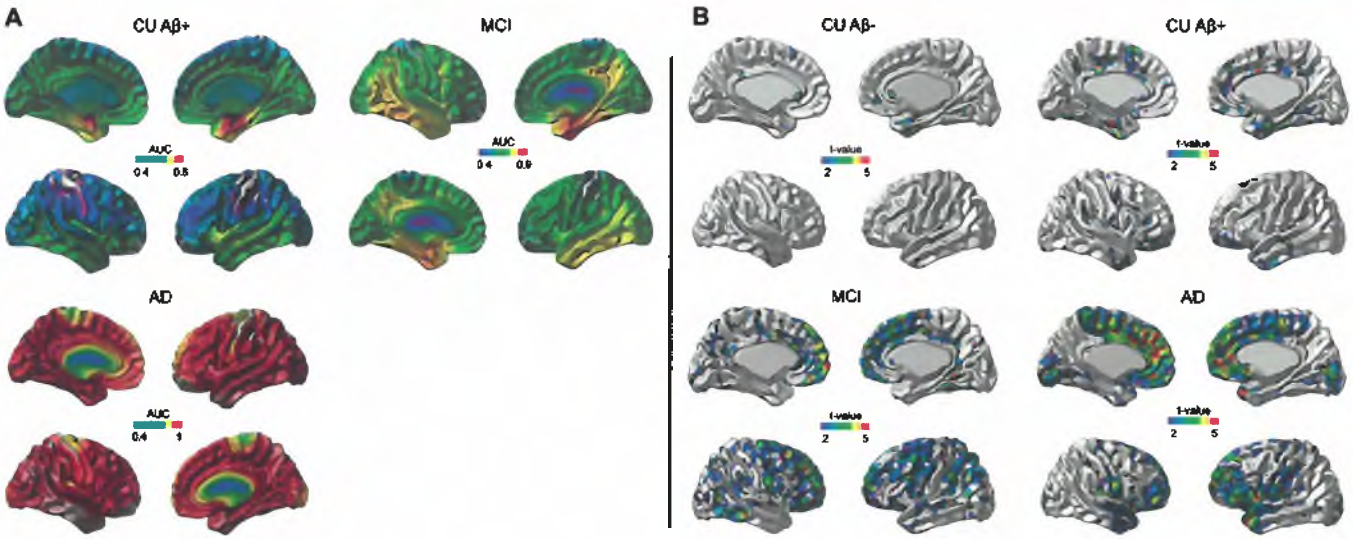


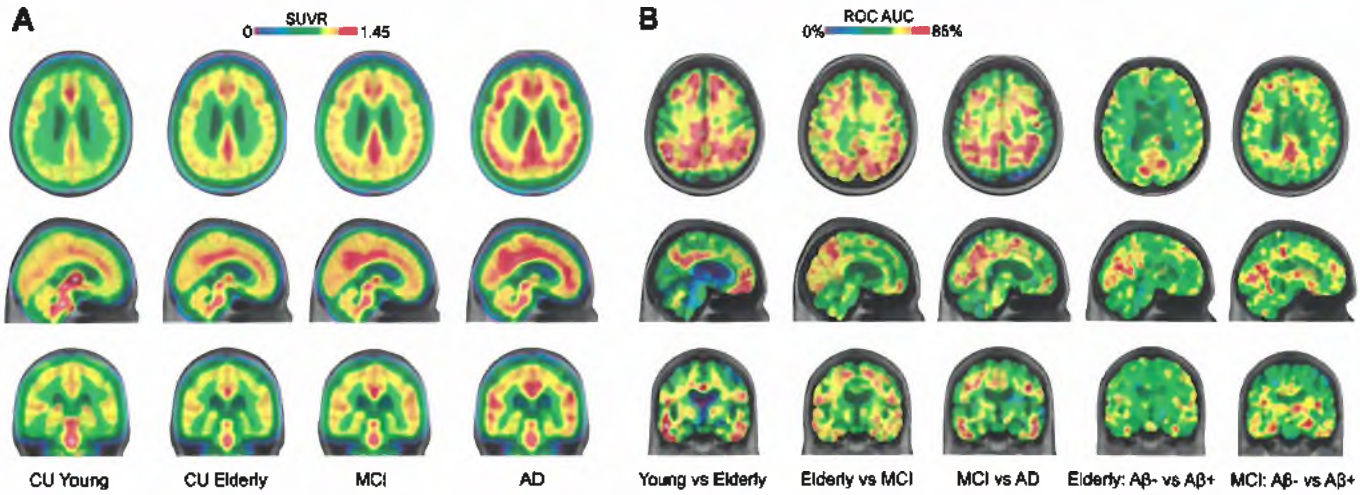
Extended Data Fig. 1 | Study flowchart.



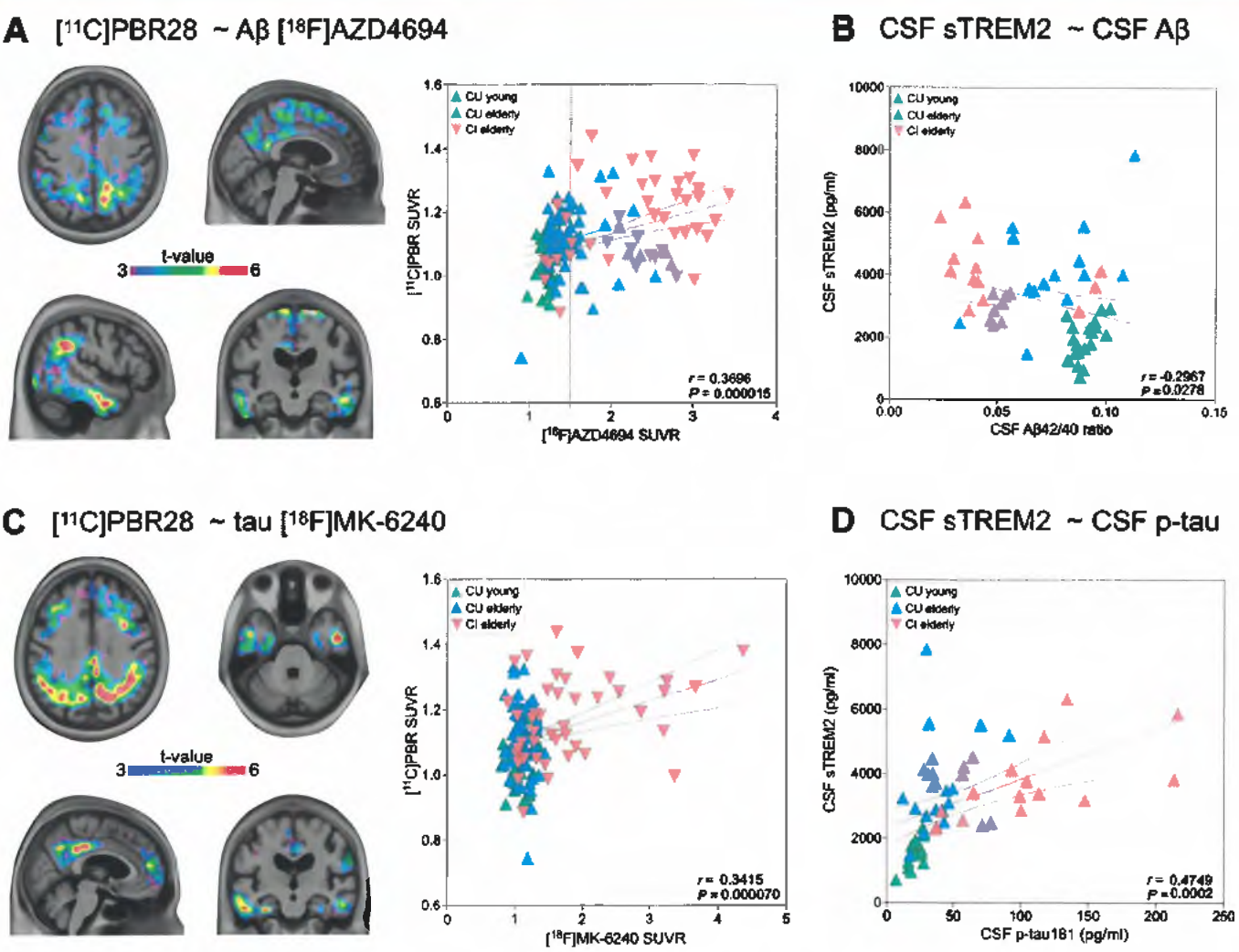
Extended Data Fig. 2 | Cross-sectional and longitudinal abnormalities in [^{18}F]MK-6240 across clinical groups. (A) The brain images (cross-sectional analyses) show voxel-wise AUC results obtained from ROC curves between [^{18}F]MK-6240 SUVR values of CU Aβ- versus CU Aβ+, MCI, or AD dementia. [^{18}F]MK-6240 SUVR was increased in CU Aβ+, MCI, and AD dementia individuals in regions comprising early PET Braak-like stages, intermediary Braak stages, and across the whole brain cortex, respectively. Cross-sectional analysis was performed in 64 CU elderly (14 males, mean age = 72 (6)), 28 MCI (17 males, mean age = 73 (9)), and 16 AD dementia (6 males, mean age = 70 (8)). (B) The brain images (longitudinal analyses) show the results of voxel-wise paired t-test comparison between the baseline and follow-up [^{18}F]MK-6240 SUVR images. CU, MCI, and AD dementia showed a more preeminent longitudinal [^{18}F]MK-6240 SUVR increase in early, intermediary, and late Braak regions, respectively. Longitudinal analysis was performed in 34 CU elderly (6 males, mean age = 73 (6)), 13 MCI (9 males, mean age = 74 (6)), and 9 AD dementia (4 males, mean age = 70 (7)). Results survived to false discovery rate correction for multiple comparisons at $P < 0.05$.

Characteristic	CU Young	CU Elderly	MCI	AD dementia
Number	22	64	28	16
Age, years, mean (SD)	23 (2.4)	72 (5.5)	73 (8.6)	70 (7.7)
Male, number (%)	8 (36)	14 (22)	17 (61)	6 (38)
Education, years, mean (SD)	16.6 (1.7)	15.5 (3.7)	14.9 (3.4)	14.4 (3.3)
MMSE score, mean (SD)	29.8 (0.5)	29.2 (1)	27 (4)	21 (6.3)
APOE ε4, number (%)	3 (14)	18 (28)	13 (46)	7 (44)
Aβ PET + (%)	0 (0)	18 (28)	11 (61)	16 (100)

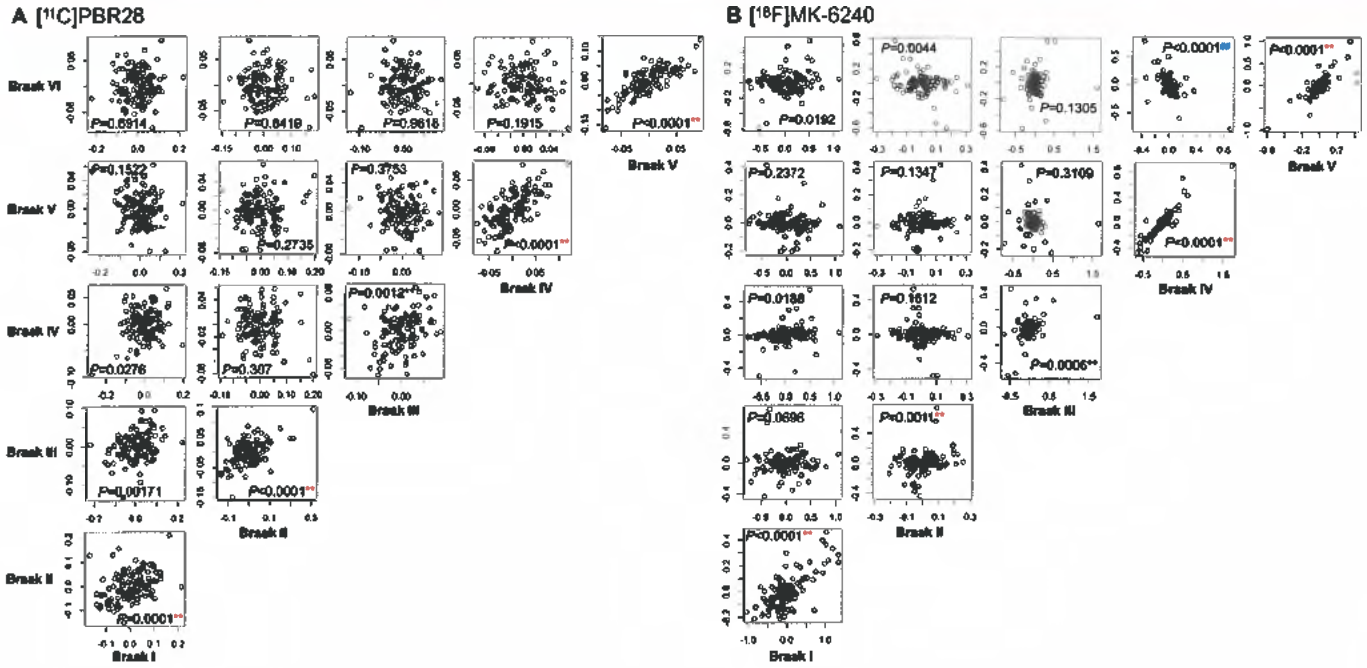
Extended Data Fig. 3 | Demographics and key characteristics of the population.



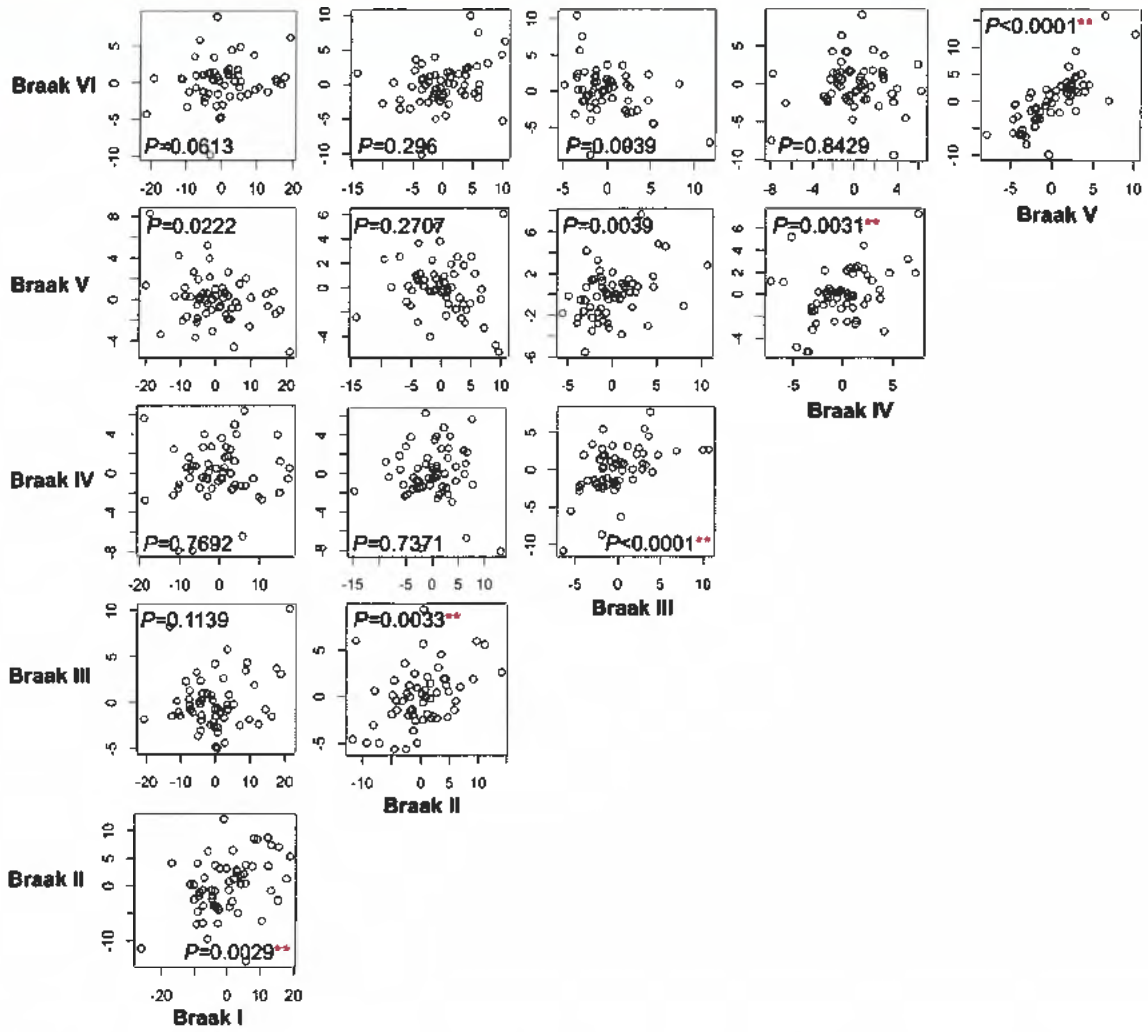
Extended Data Fig. 4 | Groups difference in [^{11}C]PBR28 SUVR. (a) Averaged [^{11}C]PBR28 SUVR maps, overlaid on a structural MRI template, suggest a progressively higher uptake in typical AD-related region in the posterior cingulate/precuneus, inferior parietal, and lateral temporal cortices from CU young (n=22 (8 males, mean age = 23 (2)) to CU elderly (n=64 (14 males, mean age = 72 (6)), MCI (n=28, 17 males, mean age = 73 (9)), and AD dementia (n=16, 6 males, mean age = 70 (8)) individuals. (b) Voxel-wise AUC maps obtained from ROC curves supported the above-mentioned differences between groups. Voxel-wise AUC also revealed the regions with higher [^{11}C]PBR28 SUVR uptake in CU elderly Aβ+ than CU elderly Aβ - and higher uptake in MCI Aβ+ than MCI Aβ - (for example, medial temporal, posterior cingulate, and precuneus cortices). Young = cognitively unimpaired young; Elderly = cognitively unimpaired elderly; AD = AD dementia.



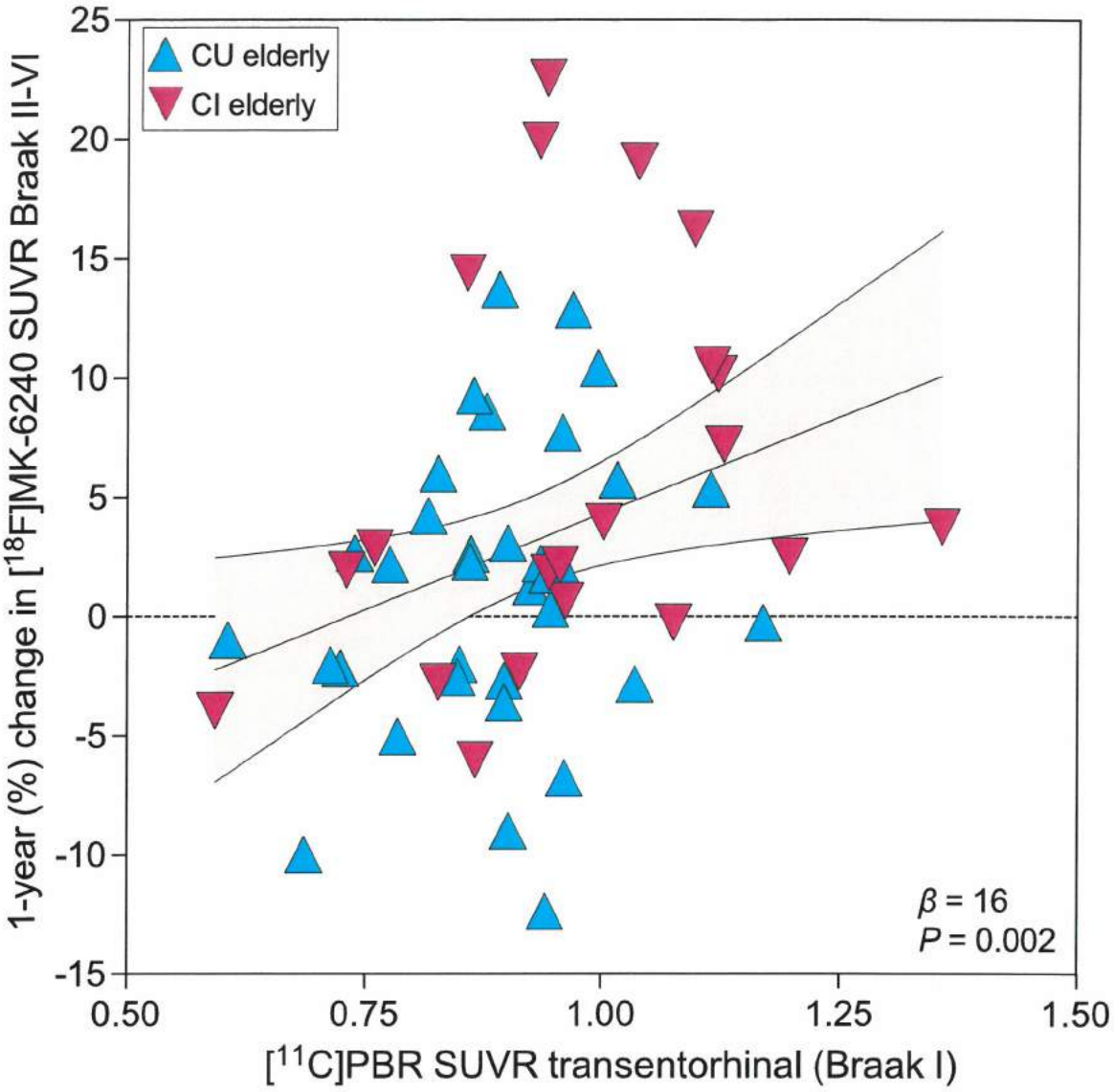
Extended Data Fig. 5 | Microglial activation positively associates with brain Aβ and tau. *T*-statistical parametric maps (false discovery rate corrected for multiple comparison at $P < 0.05$) overlaid on an MRI template show the results of voxel-wise linear regressions analysis between [^{11}C]PBR28 SUVR and (A) Aβ [^{18}F]AZD4694 SUVR and (B) tau [^{18}F]MK-6240 SUVR. This analysis was performed in CU young ($n = 22$ (8 males, mean age = 23 (2)), CU elderly ($n = 64$ (14 males, mean age = 72 (6)), and 44 CI elderly (23 males, mean age = 72 (8)). The scatter plots show the results of two-side Pearson correlations between CSF sTREM2 and (C) CSF Aβ42/40 ratio and (D) CSF p-tau181 levels (CU young ($n = 19$ (9 males, mean age = 23 (2)), CU elderly ($n = 29$ (7 males, mean age = 73 (5)), and CI elderly ($n = 27$, 15 males, mean age = 71 (7)). The error bands denote 95% confidence intervals.



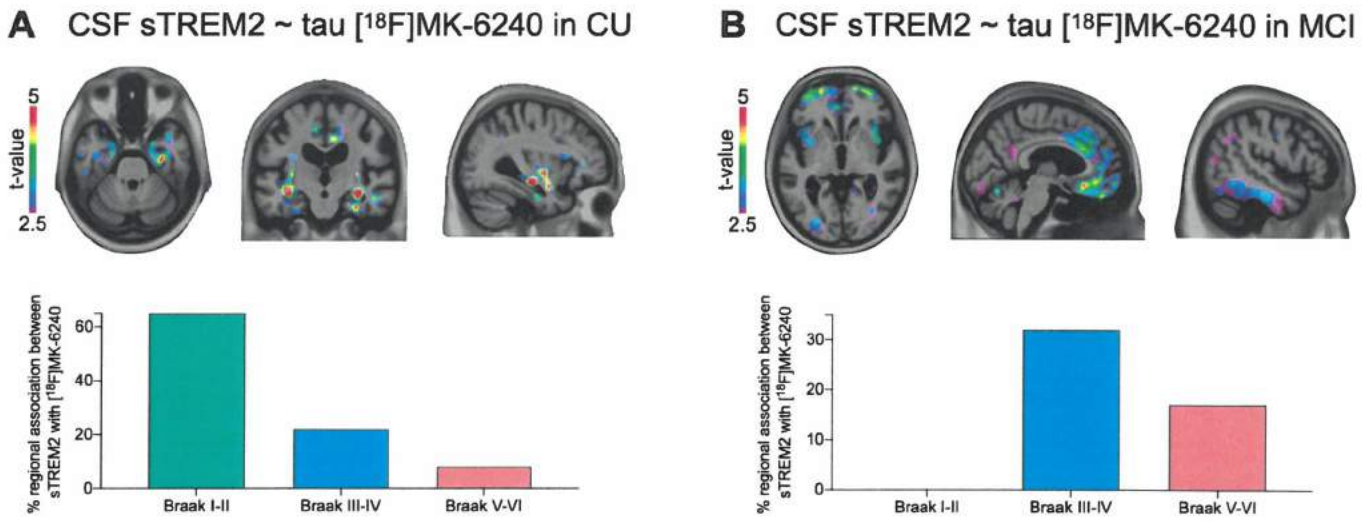
Extended Data Fig. 6 | Microglia and tau networks correlations. The plots show the correlations across PET Braak-like regions used in the network analyses presented in Fig. 2 for (**a**) [^{11}C]PBR28 and (**b**) [^{18}F]MK-6240. *P* values reflect the results of two-sided Pearson's correlation between PET SUVR values corrected for age, sex, education, APOE ε4 status, and the remaining Braak regions not used in the given correlation. A correlation was interpreted as significant if it survived Bonferroni correction for multiple comparisons (30 tests, $P < 0.0017$). ** indicates a significant positive correlation; ## indicates a significant negative correlation. This analysis was performed in the elderly population (n=108, 64 CU elderly (14 males, mean age = 72 (6)), 28 MCI (17 males, mean age = 73 (9)), and 16 AD dementia (6 males, mean age = 70 (8)).



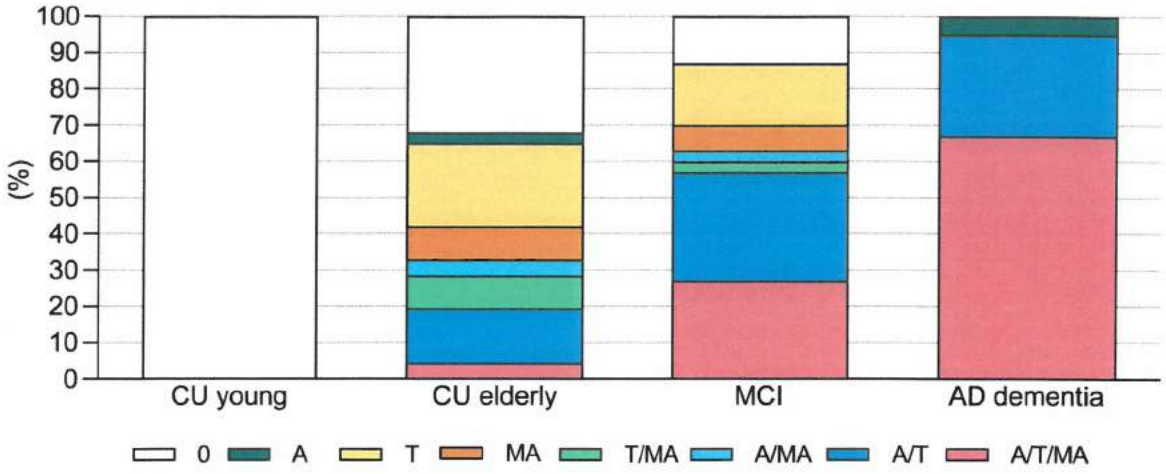
Extended Data Fig. 7 | Longitudinal tau propagation network correlations. The plots show the correlations across PET Braak-like regions used in the longitudinal tau network analysis presented in Fig. 3A. *P* values reflect the results of two-sided Pearson's correlation between changes in [^{18}F]MK-6240 SUVR corrected for age, sex, education, APOE ε4 status, and the changes in the remaining Braak regions not used in the given correlation. A correlation was interpreted as significant if it survived Bonferroni correction for multiple comparisons (15 tests, $P < 0.0034$). ** indicates a significant positive correlation ($n = 56$, 34 CU (6 males, mean age = 73 (6)), 13 MCI (9 males, mean age = 74 (6)), and 9 AD dementia (4 males, mean age = 70 (7)).



Extended Data Fig. 8 | Baseline microglial activation in Braak I region was associated with longitudinal tau accumulation over the neocortex. The linear regression analysis shows that [^{11}C]PBR28 SUVR value in the transentorhinal cortex (PET Braak-like stage I) was positively associated with 1-year change in tau PET uptake in brain regions comprising PET Braak-like stages II-VI, accounting for age, sex, *APOE ε4* carriage status, and global Aβ load. The analysis was performed in 34 CU elderly (6 males, mean age = 73 (6)) and 22 CI elderly (13 males, mean age = 72 (7)). The error bands denote 95% confidence intervals.



Extended Data Fig. 9 | The association between CSF sTREM2 and tau PET load recapitulates Braak stages. The figure shows the results of voxel-wise regressions (false discovery rate corrected for multiple comparisons at $P < 0.05$) overlaid in a structure template (top) between CSF sTREM2 and [^{18}F]MK-6240 SUVR in (A) CU ($n = 48$, 15 males, mean age = 53 (25)) and (B) MCI ($n = 18$, MCI (12 males, mean age = 72 (6)) participants. The bar plots (bottom) represent the percentage of the area showing a significant association between CSF sTREM2 and [^{18}F]MK-6240 across Braak-like stage regions. The bars show that in CU individuals, CSF sTREM2 associates with tau pathology in early Braak stages, whereas in MCI individuals, CSF sTREM2 associates with tau pathology in late Braak stages, supporting a role for microglial activation in the spatial spread of tau in the human brain. No significant association was found in AD dementia patients after correction for multiple comparisons.



Extended Data Fig. 10 | Distribution of Aβ, tau, and microglia activation abnormalities across diagnostic groups. The figure shows the distribution of Aβ [^{18}F]AZD4694, tau [^{18}F]MK-6240, and microglia activation [^{11}C]PBR28 abnormalities (+/-) in clinical groups. The co-occurrence of Aβ (A), tau (T), and microglia activation (MA) abnormalities (A/T/MA) was more prevalent in AD dementia (67%), followed by MCI (27%), CU elderly (4.5%), and CU young (0%). CU young (n=22 (8 males, mean age = 23 (2)), CU elderly (n=64 (14 males, mean age = 72 (6)), MCI (n=28, 17 males, mean age = 73 (9)), and AD dementia (n=16, 6 males, mean age = 70 (8)).

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☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	MATLAB software version 9.2 (http://www.mathworks.com); R statistical software version 3.1.2 (http://www.r-project.org/); SPM12 with the LST toolbox version 2.0.15 (www.statistical-modelling.de/lst.html), Freesurfer version 6.0 , and VoxelStats package version 1.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Our initial preliminary data suggested a Cohen's d of 1.2 to differentiate CU and CI groups. Thus, assuming 80% of power and 5% significance level, we would require as little as 12 individuals per group to test a difference (two-tailed t-test). We genotype 503 individuals we had assess to for the TSPO polymorphism (rs6971), whereof 263 (52.5%) were identified as TSPO high-affinity binders. Out of those, we studied 130 TSPO high-affinity binding individuals across the aging and AD clinical spectrum (22 CU young, 64 CU elderly, 28 MCI, 16 AD) who had complete cognition, MRI, and PET data at baseline. To the best of our knowledge, this is the largest study available using microglia activation PET imaging across a disease spectrum.
Data exclusions	We genotype 503 individuals for the TSPO polymorphism (rs6971) and excluded 42 low affinity binders and 196 mixed-affinity bindings. In addition, we excluded participants with inadequately treated systemic conditions, active substance abuse, recent head trauma, major surgery, or presenting with magnetic resonance imaging (MRI) / PET safety contraindications.
Replication	We used a single cohort to generate the results of our study. Most of the analyses obtained with PET biomarkers were replicated using CSF biomarkers.
Randomization	TRIAD is a longitudinal observational cohort and no allocation in experimental groups is performed or required for studies such as the one proposed here. Therefore, randomization is not relevant to this study.
Blinding	All biomarker (PET and CSF) collection and analysis performed in this study were performed blinded to the clinical diagnosis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Cognitively unimpaired young (mean age = 23 (2)) and elderly (mean age = 72 (5)) individuals had no objective cognitive impairment and a CDR score of 0. Mild cognitive impairment (mean age = 73 (9)) subjects had subjective/objective cognitive impairment, a CDR score of 0.5, and relatively preserved activities of daily living. Mild-to-moderate sporadic AD dementia (mean age = 70 (8)) met the National Institute on Aging and the Alzheimer's Association criteria for probable AD as determined by a physician and had a CDR score between 0.5 and 2.
Recruitment	The study participants were from the community or outpatients of memory clinic. Thus, due to self-selection bias, our participants may not represent the general population. These participants were further genotyped for the Ala147Thr polymorphism of the TSPO gene (rs6971, https://www.ncbi.nlm.nih.gov/snp/rs6971). Based on this genotype, individuals can either be high-, mixed-, or low-affinity binders. For this study, we enrolled only high-affinity binders to increase the reliability of the results. We recruited 130 TSPO high-affinity binding individuals across the aging and AD clinical spectrum (22 CU young, 64 CU elderly, 28 MCI, 16 AD).
Ethics oversight	The study was approved by the Montreal Neurological Instituted PET Working Committee and the Douglas Mental Health

Ethics oversight University Institute Research Ethics Board, and written informed consent was obtained from all participants.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Exhibit "Z"

Alberta Health Services

POLICY

TITLE
CONSENT TO TREATMENT/PROCEDURE(S)

SCOPE
Provincial

DOCUMENT #
PRR-01

APPROVAL AUTHORITY
Executive Leadership Team

INITIAL EFFECTIVE DATE
October 31, 2010

SPONSOR
Vice President, Health Professions & Practice;
Associate Chief Medical Officer, Quality & Medical Affairs

REVISION EFFECTIVE DATE
January 16, 2020

PARENT DOCUMENT TITLE, TYPE AND NUMBER
Not applicable

SCHEDULED REVIEW DATE
January 16, 2023

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OBJECTIVES

- To facilitate an **informed consent process** within Alberta Health Services (AHS) that reflects good practice, contributes to **patient** safety, and enhances the patient experience.
- To facilitate a fair, respectful process for **informed consent** that is achieved consistently across all care areas within AHS.
- To facilitate compliance with applicable law.

PRINCIPLES

The principle of respect for persons is foundational within this policy and demonstrated by patients being supported in determining what happens to their own bodies, in keeping with their own values and beliefs. Where patients cannot make their own decisions, respect for persons is upheld by recognizing the decision-making role of an appropriate **alternate decision-maker**.

Informed consent:

- requires **capacity**;
- shall be informed;
- shall be specific;
- shall be voluntary;
- requires understanding; and

This is **Exhibit "Z"** referred to in the Affidavit of Nadr Jomha sworn (or affirmed) before me at

[redacted]

- shall be documented.

On an exceptional basis, patient-informed consent decisions can be overridden in accordance with legislation such as the *Mental Health Act* and the *Public Health Act*.

The **most responsible health practitioner (MRHP)** providing the **treatment/procedure(s)** to a patient has a duty to obtain informed consent.

AHS is committed to providing continuing education for all personnel to implement this policy and the subsequent procedures.

APPLICABILITY

Compliance with this document is required by all Alberta Health Services employees, members of the medical and midwifery staffs, Students, Volunteers, and other persons acting on behalf of Alberta Health Services (including contracted service providers as necessary).

ELEMENTS

1. Informed Consent is Required

1.1 Before providing a specific treatment/procedure(s) or plan of treatment/procedure(s), the MRHP shall obtain **express informed consent** or **implied informed consent** from the patient, unless a valid exception to informed consent applies (see Section 5 below).

1.2 The MRHP is responsible for determining the most appropriate method of obtaining informed consent (express or implied).

1.3 All consent, whether express or implied, shall be informed.

1.4 Implied informed consent may be presumed in (but is not limited to) circumstances where the patient presents voluntarily for an examination, investigation, or minor or less invasive treatment/procedure(s) which the MRHP determines does not require express informed consent.

a) The MRHP shall be satisfied that the circumstances or the actions of the patient imply permission for the examinations, investigations, and treatment/procedure(s) proposed.

b) If there is any doubt that there is implied informed consent, the MRHP shall obtain express informed consent from the patient.

c) Implied informed consent is encouraged to be documented by the MRHP in the patient's **health record**.

1.5 When the MRHP determines that express informed consent is required to evidence the patient's informed consent to the treatment/procedure(s):

a) verbal consent shall be documented by the MRHP in the patient's health record; or

b) written (signed) consent shall be documented by the MRHP through obtaining the signature of the patient on the applicable **consent form** (see Section 1.7 below), which shall then be attached to the patient's health record. Where a consent form is used, documentation in the patient's health record regarding the informed consent discussion is also recommended.

1.6 Notwithstanding Section 1.2 above, written (signed) consent shall be obtained for:

a) the transfusion of blood and blood products;

b) surgery;

c) invasive investigative procedures;

d) human tissue and organ donation; and

e) medical assistance in dying, consistent with the AHS *Medical Assistance in Dying* Policy.

1.7 The following consent forms shall be used in the following situations or any other treatment/procedure(s) for which the MRHP deems written (signed) consent to be appropriate:

a) The AHS *Consent to Surgery or Invasive Procedure* Form should be used for all surgical or invasive procedures including endoscopy or cardiac catheterization. This form includes sections about possible transfusion and testing for blood-borne viruses in the event of needle-stick injuries or body fluid splashes as well as for the retention of tissue and the involvement of trainees.

b) The AHS *Consent to Treatment Plan/Intervention or Procedure* Form should be used for lesser or non-invasive procedures or treatment plans and interventions that are deemed to reach the threshold of requiring written (signed) consent such as a bedside procedure or blood product transfusion.

c) The AHS *Emergency Health Care: Documentation of Exception to Consent* Form should be used in situations where it is deemed that a procedure, which would otherwise require written (signed) consent, is occurring in an emergency situation where it is not possible to do so.

1.8 Informed consent may be obtained in the MRHP's community office rather than at the applicable **Alberta Health Services (AHS) setting** where the patient will be receiving the treatment/procedure(s). Any completed consent forms shall then

be forwarded to the applicable AHS setting where the patient will be receiving the treatment/procedure(s).

2. Accountability

2.1 The accountability to obtain informed consent shall rest with the MRHP who is providing the specific treatment/procedure(s).

2.2 The MRHP remains accountable for the informed consent process when one (1) or more than one (1) **health care provider** is involved in providing the treatment/procedure(s).

2.3 The MRHP is responsible for confirming the validity of informed consent prior to the delivery of the treatment/procedure(s).

2.4 For programs that offer multiple treatment/procedure(s), each MRHP is accountable for the informed consent process related to the treatment/procedure(s) they are providing.

3. Required Components of Informed Consent

3.1 Capacity:

a) The MRHP is responsible to conduct initial assessment of the patient for determination of capacity to make treatment and care decisions.

(i) Where the MRHP cannot complete an assessment of the patient for the determination of capacity to make treatment decisions, the MRHP shall ensure assessment of the patient's capacity by an appropriate clinical expert (refer to list of approved capacity assessors).

b) An **adult** patient is presumed to have capacity to make treatment/procedure(s) decisions unless the patient is determined to lack capacity.

(i) When an adult patient lacks capacity to consent to a treatment/procedure(s), the authority of a co-decision-maker or an alternate decision-maker shall be recognized in accordance with the AHS *Consent to Treatment/Procedure(s): Adults with Impaired Capacity and Adults who Lack Capacity* Procedure.

(ii) Capacity for a **minor** patient shall be determined in accordance with the AHS *Consent to Treatment/Procedure(s): Minors / Mature Minors* Procedure.

c) The MRHP shall be satisfied that the patient has the capacity to make each treatment/procedure(s) decision.

(i) If a patient is considered to have capacity and consents to the proposed treatment/procedure(s), they may be treated.

(ii) A patient's capacity can change depending on changes to their mental and physical health.

(iii) The determination of capacity shall relate to each specific treatment/procedure(s) or plan of treatment/procedure(s).

(iv) Informed consent shall be obtained prior to the administration of any medication that may significantly affect the patient's capacity to make an informed decision (i.e., analgesic, narcotic, or anaesthetic).

(v) A patient may have capacity even if they are unable to communicate verbally. Communication with the patient shall be facilitated by any means that enables understanding (see Section 3.5 below).

(vi) The patient's choice to make decisions based on their values and beliefs shall be supported, subject to exceptions (see Section 5 below).

3.2 Informed:

a) The MRHP shall ensure all necessary information has been provided to the patient so that the patient can make an informed decision about the treatment/procedure(s). Necessary information shall include but is not limited to:

(i) the condition for which the treatment/procedure(s) is proposed;

(ii) the treatment/procedure(s) plans/interventions and/or list of agreed upon treatment/procedure(s), that are clinically indicated and approved for the condition;

(iii) the potential risks and benefits of the proposed treatment/procedure(s);

(iv) information applicable to the patient's particular circumstances or as specifically requested by the patient;

- If the patient alerts the MRHP of particular circumstances that might affect the information the patient would want for their treatment/procedure(s), the MRHP shall be responsible for addressing those particular circumstances with further information as requested by the patient.

(v) alternatives to the proposed treatment/procedure(s);

(vi) the potential consequences of both providing consent or refusing to provide consent for the proposed treatment/procedure(s); and

(vii) who will perform the treatment/procedure(s) and who may provide assistance, including whether the treatment/procedure(s) will include health care providers in training (i.e., residents, students).

3.3 Specific:

a) The provision of informed consent shall relate to each specific treatment/procedure(s) or a plan of treatment/procedure(s).

b) Treatment/procedure(s) that:

(i) are in addition to the treatment/procedure(s) already consented to;

(ii) are different from the treatment/procedure(s) consented to;

(iii) were unanticipated at the time informed consent was obtained;

(iv) may be convenient to do; or

(v) may be beneficial to the patient,

shall not be performed without obtaining further informed consent, unless a valid exception to informed consent applies (see Section 5 below).

c) New informed consent shall be obtained when one (1) or more of the following occurs:

(i) the patient's condition has materially changed;

(ii) the medical knowledge about the patient's condition or the treatment/procedure(s) available has materially changed;

(iii) when the treatment/procedure(s) for the patient changes;

(iv) the previously given consent and/or any portion of the previously given consent has been withdrawn (see Section 4 below); and

(v) the patient has refused the involvement of particular individuals in their treatment/procedures(s) (i.e., medical students).

d) If the previous informed consent was evidenced using a consent form, then the new or subsequent informed consent should also be evidenced using a consent form.

3.4 Voluntary:

a) The patient shall have the opportunity, without undue influence, to accept or refuse a treatment/procedure(s).

b) As time permits in the clinical circumstance, informed consent discussions shall occur when the patient has a reasonable opportunity to reflect on the decision and ask questions.

c) When appropriate to do so, informed consent discussions should not take place in the operating room or the operating room environment.

d) The patient shall be given an opportunity to take the time required to reflect on the information and to consult with whom they choose prior to making a decision.

e) A patient's decision to accept or refuse a treatment/procedure(s) shall not prejudice their access to ongoing or future health care.

3.5 Understanding:

a) The MRHP accountable for the informed consent process shall:

(i) provide the patient with the opportunity to ask questions;

(ii) provide responses to the questions asked by the patient; and

(iii) ensure the patient has understood the information sufficiently to proceed with the informed consent discussion.

b) The informed consent discussion is a shared process between the patient and the MRHP, resulting in the patient's decision to accept or refuse the proposed treatment/procedure(s).

c) The MRHP shall communicate with the patient in a manner that supports the patient's ability to understand and shall address all communication barriers including, but not limited to:

(i) hearing;

(ii) sight;

(iii) language;

(iv) culture;

(v) literacy;

(vi) level of education;

(vii) level of anxiety and stress; and

(viii) environmental factors, including location of discussion.

d) If the patient is having difficulty understanding the discussion or reading and completing the consent form (if applicable), the discussion and contents of the consent form shall be read and explained to the patient in the presence of a witness and with the assistance of an interpreter, as necessary. Documentation of this process is recommended. The MRHP may allow, at the patient's request, their **family** to accompany the patient and offer their assistance to help the patient to understand or demonstrate an understanding of the information provided.

4. Refusal of Treatment/Procedure(s) and Withdrawal of Informed Consent

4.1 Subject to situations in which a treatment/procedure(s) is ordered in accordance with applicable legislation, an adult patient with capacity to consent to a treatment/procedure(s) may at any time:

a) refuse to consent to all or a portion of a proposed treatment/procedure(s); or

b) withdraw previously given informed consent to any or all of the treatment/procedure(s) at any time prior to or during the treatment/procedure(s).

4.2 Subject to situations in which a treatment/procedure(s) is ordered in accordance with applicable legislation, an adult patient with capacity may refuse to consent to a treatment/procedure(s) or withdraw informed consent on any grounds prior to the start of the treatment/procedure(s), even when it is clear that the treatment/procedure(s) is necessary to preserve their life or health. In such an instance, the treatment/procedure(s) shall not be carried out, even if failure to provide such a treatment/procedure(s) may result in the patient's death.

a) The alternate decision-maker for an adult patient lacking capacity may refuse a treatment/procedure(s) or withdraw previously given informed consent in accordance with the AHS *Consent to Treatment/Procedure(s): Adults with Impaired Capacity and Adults who Lack Capacity* Procedure.

b) A **mature minor** or a minor's **legal representative** may refuse a treatment/procedure(s) or withdraw previously given informed consent in accordance with the AHS *Consent to Treatment/Procedure(s): Minors / Mature Minors* Procedure.

4.3 After a treatment/procedure(s) has been commenced, the MRHP shall stop providing the treatment/procedure(s) immediately upon the withdrawal of the informed consent and shall revisit the informed consent process with new or additional information that should be shared with the patient.

a) If the termination of the treatment/procedure(s) will result in immediate and serious risk to the patient, the MRHP may be required to continue with the originally consented to treatment/procedure(s) to the extent required to limit the immediate and serious risk to the patient.

4.4 Where a patient refuses to consent to a treatment/procedure(s) or withdraws previously given informed consent, the MRHP shall explain the potential risks and consequences of the refusal or withdrawal of informed consent, without undue influence.

a) This explanation can be witnessed by a second **health care professional** to confirm patient identity and confirm the discussion occurred.

b) The MRHP shall document on the patient's health record:

(i) the refusal or withdrawal of informed consent;

(ii) the circumstances of the refusal, including the patient's reasons for withdrawing informed consent or refusing the treatment/procedure(s);

(iii) a summary of the discussion with the patient about the patient's clinical condition, the planned treatment/procedure(s) or interventions, the expected outcomes, material risks, and potential consequences of withdrawing informed consent or refusing the treatment/procedure(s);

(iv) the outcome of the discussion;

(v) the presence of witnesses, if any; and

(vi) where written (signed) informed consent was previously given, withdrawal of consent shall be documented in the 'withdrawal' section of the consent form.

4.5 The patient may provide informed consent again at any time following a subsequent informed consent discussion.

4.6 Adult patients who carry written and signed statements refusing the infusion of blood products shall have their wishes respected. This includes situations where the patient presents to an AHS setting for emergency health care.

5. Exceptions to Informed Consent

5.1 Emergency Health Care Exception:

a) For adult patients:

(i) If an adult patient requires emergency treatment/procedure(s) but the adult lacks the capacity to provide informed consent or refuses informed consent due to altered consciousness from trauma, drugs, alcohol, or any other cause, or where informed consent cannot be immediately obtained from the adult's alternate decision-maker, emergency health care may be provided by a MRHP:

- only where it is immediately necessary to preserve the patient's life, prevent serious physical or mental harm to the patient, or to alleviate serious pain; and
- where there is no knowledge that the patient would have objected to the treatment/procedure(s).
- If a Physician is not available, a Nurse Practitioner or Registered Nurse may initiate emergency health care as per their scope of practice.

(ii) The MRHP shall document that an emergency situation exists by completing the relevant section of the AHS *Emergency Health Care: Documentation of Exception to Consent* Form. In all possible situations, a second Physician or MRHP shall confirm the existence of the emergency situation, although it is recognized that in rural settings there may not always be a second Physician available.

- If a second Physician is not available, a Nurse Practitioner or Registered Nurse may confirm the existence of the emergency situation and document the same on the AHS *Emergency Health Care: Documentation of Exception to Consent* Form.
- Resident Physicians are not permitted to provide a written opinion to confirm the criteria for emergency health care.

(iii) The details of the emergency situation and all treatment/procedure(s) decisions shall be documented in the patient's health record. All reasonable efforts shall be made to contact the patient's alternate decision-maker or next of kin, as appropriate, to advise that emergency treatment/procedure(s) was provided.

(iv) The Emergency Health Care Exception is only valid during the emergency situation. All future treatment/procedure(s) provided outside of the emergency situation shall require informed consent.

b) For minor patients:

(i) The applicability of the Emergency Health Care Exception for a minor patient shall be determined in accordance with the AHS *Consent to Treatment/Procedure(s): Minors / Mature Minors* Procedure.

5.2 Exceptional Circumstances:

a) The requirement for informed consent may be overridden by a warrant, subpoena, court order, or applicable legislation (e.g., a review panel's treatment order under the *Mental Health Act*, orders under the *Public Health Act*, orders under the *Mandatory Testing and Disclosure Act*, etc.).

6. Documentation

6.1 The MRHP is responsible for ensuring appropriate documentation of the informed consent process and outcomes in the patient's health record. Specifically, the following outcomes shall be documented:

a) agreement with informed consent to the treatment/procedure(s);

b) refusal of the treatment/procedure(s) (refer to Section 4 above); and

c) withdrawal of consent previously given (refer to Section 4 above).

6.2 All relevant legal documents including, but not limited to, court orders, warrants, subpoenas, **personal directives**, capacity assessments, and evidence of the formal status of alternate decision-makers, shall be documented on the patient's health record.

6.3 While the requirements for documentation outlined in Section 6.1 above are met by appropriately filling in the applicable consent form where written (signed) consent has been deemed necessary, documentation in the patient's health record regarding the consent discussion is recommended.

6.4 Completed consent forms required for treatment/procedure(s) may be faxed or scanned (refer to the AHS *Transmission of Information by Facsimile or Electronic Mail* Policy). When possible, and at the earliest opportunity, the original consent form shall be obtained and placed on the patient's health record.

6.5 When an interpreter is used to assist in obtaining consent, the interpreter shall complete the relevant documentation on the consent form.

a) The MRHP shall follow up to ensure the consent form has been completed as required.

6.6 A blind or disabled person's 'mark' is recognized as a valid signature on the consent form.

6.7 Witness documentation of informed consent:

a) A written (signed) consent form should be witnessed.

b) Any person, other than a relative of the patient, the MRHP, or the interpreter for the patient, may witness the signing of a consent form.

(i) Before acting as a witness or signing the consent form as a witness, confirmation of the patient's identity by the witness shall be required.

(ii) If the signee is not the patient, the witness shall request to see a form of the signee's identification and confirm that the person making a mark on behalf of the patient has been asked to do so by the patient.

c) Witnessing a consent form indicates only that the witness observed the consent form being signed and is not evidence of the consent process.

d) In the event that the patient expresses doubt about the consent process and/or requests further explanation, the witness shall not sign the consent form and the MRHP shall be notified.

DEFINITIONS

Adult means a person aged 18 years and older.

Agent means the person(s) named in a personal directive who can make decisions on personal matters according to the wishes expressed by the patient.

Alberta Health Services (AHS) setting means any environment where treatment/procedures and other health services are delivered by, on behalf of or in conjunction with, Alberta Health Services.

Alternate decision-maker means a person who is authorized to make decisions with or on behalf of the patient. These may include, specific decision-maker, a minor's legal representative, a guardian, a 'nearest relative' in accordance with the *Mental Health Act* (Alberta) or an agent in accordance with a personal directive or a person designated in accordance with the *Human Tissue and Organ Donation Act* (Alberta). This also includes what was previously known as the substitute decision-maker.

Capacity means the ability for the patient to 1) understand the nature, risks, and benefits of the procedure and the consequences of consenting or refusing; and 2) understand that this explanation applies to them.

Consent form means an Alberta Health Services approved form of documentation that can be used to provide evidence of the outcome of the consent process, that is, agreement to or refusal of a treatment/procedure.

Express informed consent means direct, explicit agreement to undergo treatment/procedure(s), given either verbally or in writing (signed).

Family means one or more individuals identified by the patient as an important support, and who the patient wishes to be included in any encounters with the health care system, including, but not limited to, family members, legal guardians, friends, and informal caregivers.

Guardian means, where applicable:
For a minor:

a) A guardian as defined by the *Family Law Act* (Alberta), a divorced parent with custody of the minor, or a person appointed pursuant to a will, personal directive, court order, agreement or by authorization of legislation (e.g., *Child, Youth and Family Enhancement Act* [Alberta]).

For an adult:

a) An individual appointed by the Court in accordance with the *Adult Guardianship and Trusteeship Act* (Alberta) to make decisions on behalf of the adult patient when the adult patient lacks capacity.

Health care professional means an individual who is a member of a regulated health discipline, as defined by the *Health Disciplines Act* (Alberta) or the *Health Professions Act* (Alberta), and who practises within scope and role.

Health care provider means any person who provides goods or services to a patient, inclusive of health care professionals, staff, students, volunteers and other persons acting on behalf of or in conjunction with Alberta Health Services.

Health record means the collection of all records documenting individually identifying health information in relation to a single person.

Implied informed consent means consent inferred from the patient's or alternate decision-maker's (if applicable) actions and surrounding circumstances.

Informed consent means the patient's agreement (or alternate decision-maker) to undergo a treatment/procedure after being provided, in a manner the patient can understand, with the relevant information about the nature of the treatment/procedure(s), its benefits, potential risks and alternatives, and the potential consequences of refusal.

Informed consent process means a discussion or series of discussions and interactions that may occur over a period of time between the most responsible health practitioner and patient or their alternate decision-maker (if applicable) including: i) the determination of capacity, as necessary, ii) the provision of relevant information, iii) the verification of understanding, iv) the decision-making and v) documentation of the consent process and outcome.

Legal representative means the following in relation to a minor, as applicable:

a) guardian; or

b) nearest relative as defined in the *Mental Health Act* (Alberta), who has the authority to consent to treatment for a minor formal patient or minor who is subject to a Community Treatment Order.

Mature minor means a person aged less than 18 years, who has been assessed and determined as having the intelligence and maturity to appreciate the nature, risks, benefits, consequences, and alternatives of the proposed treatment/procedure(s), including the ethical, emotional, and physical aspects.

Minor means a person aged less than 18 years.

Most responsible health practitioner (MRHP) means the health practitioner who has responsibility and accountability for the specific treatment/procedure(s) provided to a patient and who is authorized by Alberta Health Services to perform the duties required to fulfill the delivery of such a treatment/procedure(s) within the scope of their practice.

Patient means all persons, inclusive of residents and clients, who receive or have requested health care or services from Alberta Health Services and its health care providers. Patient also means, where applicable:

a) a co-decision-maker with the person; or
b) an alternate decision-maker on behalf of the person.

Personal directive means a written document in accordance with the requirements of the *Personal Directives Act* (Alberta), in which an adult names an agent(s) or provides instruction regarding their personal decisions, including the provision, refusal, and/or withdrawal of consent to treatments/procedures. A personal directive (or part of) has effect with respect to a personal matter only when the maker lacks capacity with respect to that matter.

Physician means a person licensed in independent practice and in good standing with the College of Physicians and Surgeons of Alberta pursuant to the *Health Professions Act* (Alberta).

Specific Decision-Maker means a nearest relative who may be selected from a hierarchy of relatives to make a specific decision on behalf of the patient according to the *Adult Guardianship and Trusteeship Act.*

Treatment/procedure(s) means a specific assessment, treatment, investigative procedure(s), or series of treatments/procedures planned to manage a clinical condition; these can be presented as a treatment plan/intervention.

REFERENCES

- Alberta Health Services Governance Documents:
  - *Consent to Mental Health Treatment/Procedure(s): Formal Patients and Persons Subject to Community Treatment Orders Under the Mental Health Act* Policy (#PRR-01-04)
  - *Consent to Treatment/Procedure(s): Adults with Impaired Capacity and Adults who Lack Capacity* Procedure (#PRR-01-02)
  - *Consent to Treatment/Procedure(s): Deceased Donation of Human Organs and Tissues* Policy (#PRR-01-05)
  - *Consent to Treatment/Procedure(s): Minors / Mature Minors* Procedure (#PRR-01-03)
  - *Medical Assistance in Dying* Policy (#HCS-165-01)
  - *Transmission of Information by Facsimile or Electronic Mail* Policy (#1113)
- Alberta Health Services Forms:
  - *Consent and Declaration for Treatment/Procedure (on Behalf of a Formal Patient or Person Subject to a Community Treatment Order who lacks capacity)* Form (#09565)
  - *Tissue and/or Organ Donation Consent (Human Tissue and Organ Donation Act of Alberta)* Form (#09816)
  - *Consent to Surgery or Invasive Procedure* Form (#18628)
  - *Consent to Treatment Plan/Intervention or Procedure* Form (#09741)
  - *Emergency Health Care: Documentation of Exception to Consent* Form (#18629)
- Non-Alberta Health Services Documents:
  - *Adult Guardianship and Trusteeship Act* (Alberta)
  - *Child, Youth and Family Enhancement Act* (Alberta)
  - *College of Physicians and Surgeons of Alberta: Standards of Practice* (Alberta)
  - *Family Law Act* (Alberta)
  - *Health Information Act* (Alberta)
  - *Health Professions Act* (Alberta)
  - *Human Tissue and Organ Donation Act* (Alberta)
  - *Mandatory Testing and Disclosure Act* (Alberta)
  - *Mental Health Act* (Alberta)
  - *Personal Directives Act* (Alberta)
  - *Protection for Persons in Care Act* (Alberta)
  - *Protection of Children Abusing Drugs Act* (Alberta)
  - *Public Health Act* (Alberta)

VERSION HISTORY

Date	Action Taken
August 01, 2011	Revised
February 27, 2012	Non-substantive change
January 16, 2020	Revised

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COLLEGE OF PHYSICIANS
& SURGEONS OF ALBERTA

STANDARDS OF PRACTICE

Informed Consent

Under Review: No
Issued By: Council: January 1, 2010
Reissued by Council: June 1, 2016

cpsa

COLLEGE OF PHYSICIANS
& SURGEONS OF ALBERTA

The ***Standards of Practice*** of the College of Physicians & Surgeons of Alberta ("CPSA") are the **minimum** standards of professional behavior and ethical conduct expected of all regulated members registered in Alberta. Standards of Practice are enforceable under the *Health Professions Act* and will be referenced in the management of complaints and in discipline hearings. CPSA also provides **Advice to the Profession** to support the implementation of the Standards of Practice.

1. A regulated member **must** obtain a patient's informed consent[i] prior to an examination, assessment, treatment or procedure; such consent may be implied, expressed orally or in writing as appropriate.

2. If a patient is under the age of 18 years, a regulated member **must**:

   a. determine whether the patient is a mature minor with the capacity to give informed consent[1]; and

   b. if the patient is not a mature minor, seek informed consent from the patient's legal guardian, in accordance with legislation[1].

3. If an adult patient lacks capacity to give informed consent, a regulated member **must** seek informed consent from the patient's legal guardian or substitute decision maker, in accordance with legislation[1].

4. A regulated member who has reasonable grounds to believe an informed consent decision by a legal guardian or substitute decision maker is not in the best interests of the patient **must** seek legal advice, such as from the Canadian Medical Protective Association, or advice from CPSA.

5. A regulated member obtaining informed consent from a patient, or the patient's legal guardian or substitute decision maker, **must** ensure the decision maker:

   a. is aware of his/her right to withdraw consent at any time;

Terms used in the Standards of Practice:

- "Regulated member" means any person who is registered or who is required to be registered as a member of this College. The College regulates physicians, surgeons and osteopaths.
- "Must" refers to a mandatory requirement.
- "May" means that the physician may exercise reasonable discretion.
- "Patient" includes, where applicable, the patient's legal guardian or substitute decision maker.

b. is free of undue influence, duress or coercion in making the consent decision;

c. receives a proper explanation that includes, but is not limited to:

   i. diagnosis reached;

   ii. advised interventions and treatments;

   iii. exact nature and anticipated benefits of the proposed examination, assessment, treatment or procedure;

   iv. common risks and significant risks;

   v. reasonable alternative treatments available, and the associated common risks and significant risks;

   vi. natural history of the condition and the consequences of forgoing treatment; and

d. demonstrates a reasonable understanding of the information provided and the reasonably foreseeable consequences of both a decision and a failure to make a decision.

6. A regulated member who assesses the capacity of a patient to give informed consent **must**:

   a. use accepted capacity assessment processes;

   b. to the extent possible, conduct the capacity assessment at a time and under circumstances in which the patient is likely to be able to demonstrate full capacity; and

   c. inform the patient of the nature and consequences of the capacity assessment.

7. A regulated member obtaining informed consent for a patient to participate in health research **must** comply with CPSA's *Human Health Research* standard of practice.

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- "May" means that the physician may exercise reasonable discretion.
- "Patient" includes, where applicable, the patient's legal guardian or substitute decision maker.

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(8) A regulated member **may** delegate responsibility for obtaining informed consent to another healthcare professional only when confident the delegate has the appropriate knowledge, skill and judgment to meet the expectations of this standard.

RELATED STANDARDS OF PRACTICE

- *Code of Ethics & Professionalism*
- *Human Health Research*
- *Medical Assistance in Dying*
- *Responsibility for a Medical Practice*
- *Supervision of Restricted Activities*

COMPANION RESOURCES

- Advice to the Profession: Informed Consent for Adults
- Advice to the Profession: Informed Consent for Minors
- Advice to the Profession: Legislated Reporting & Release of Medical Information
- Office of the Public Guardian's Guide to Capacity Assessment under the Personal Directives Act
- Office of the Public Guardian's Resources for Capacity Assessors
- CMPA's Consent: A guide for Canadian Physicians
- CMPA's Informed consent: Overview and objectives
- CMPA's Informed consent: Why and when do we need consent?

[i] See CPSA's Advice to the Profession: Informed Consent for Adults and Informed Consent for Minors.

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- "Must" refers to a mandatory requirement.
- "May" means that the physician may exercise reasonable discretion.
- "Patient" includes, where applicable, the patient's legal guardian or substitute decision maker.