Article

Not peer-reviewed version

Methotrexate and Tumor Necrosis Factor Inhibitors Independently Decrease Neutralizing Antibodies after SARS-CoV-2 Vaccination: Updated Results from the SUCCEED Study

Carol A Hitchon , Dawn M. E. Bowdish , Gilles Boire , Paul R Fortin , Louis Flamand , Vinod Chandran , Roya M Dayam , Anne-Claude Gingras , Catherine M. Card , Inés Colmegna , Maggie J. Larché , Gilaad G. Kaplan , Luck Lukusa , Jennifer LF Lee , Sasha Bernatsky *

Posted Date: 28 August 2024

doi: 10.20944/preprints202408.1998.v1

Keywords: Covid; Vaccination; immune-mediated inflammatory disease; Methotrexate; Tumor Necrosis Factor Inhibitors; TNF-blocking Antibody; Autoimmune Diseases

 doi:10.20944/preprints202408.1998.v1

Article

Methotrexate and Tumor Necrosis Factor Inhibitors Independently Decrease Neutralizing antibodies after SARS-CoV-2 Vaccination: Updated Results from the SUCCEED study

Carol Hitchon [1], Dawn ME Bowdish [2], Gilles Boire [3], Paul R Fortin [4,5], Louis Flamand [5], Vinod Chandran [6], Roya M. Dayam [7], Anne-Claude Gingras [7], Catherine M Card [8,9], Ines Colmegna [10,11], Maggie J Larche [12], Gilaad G Kaplan [13], Luck Lukusa [11], Jennifer LF Lee [11], Sasha Bernatsky [10,11,*] and SUCCEED Investigative Team

[1] Department of Internal Medicine, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada
[2] McMaster Immunology Research Centre, McMaster University, Hamilton, Ontario, Canada
[3] Division of Rheumatology, Department of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada
[4] Centre de Recherche ARThrite—UL, Division of Rheumatology, Department of Medicine, CHU de Québec—Université Laval, Québec City, Québec, Canada
[5] Axe maladies infectieuses et immunitaires, Centre de recherche du CHU de Québec, Université Laval, Québec City, Québec, Canada
[6] Schroeder Arthritis Institute, Krembil Research Institute, University Health Network, Toronto, Ontario, Canada
[7] Lunenfeld-Tanenbaum Research Institute at Mount Sinai Hospital, Sinai Health, Toronto, Ontario, Canada
[8] Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada
[9] JC Wilt Infectious Diseases Research Centre, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada
[10] Department of Medicine, McGill University, Montreal, Quebec, Canada
[11] The Research Institute of the McGill University Health Centre, 5252 Boul. de Maisonneuve Ouest, 3F.51, Montreal, Quebec, H4A 3S5, Canada
[12] Division of Rheumatology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada
[13] Division of Gastroenterology and Hepatology, Departments of Medicine and Community Health Sciences, University of Calgary, Calgary, Alberta, Canada.
* Correspondence: sasha.bernatsky@mcgill.ca

Abstract: Objective: SARS-CoV-2 remains the third most common cause of death in North America. We studied methotrexate and tumor necrosis factor inhibitor (TNFi) effects on neutralization responses post-COVID vaccination, in immune-mediated inflammatory disease(IMID). **Methods:** Prospective data and sera on adults with inflammatory bowel disease (IBD), rheumatoid arthritis (RA), spondyloarthritis (SpA), psoriatic arthritis (PsA) and systemic lupus (SLE) were collected at 6 academic centres in Alberta, Manitoba, Ontario, and Quebec between 2022-2023. Sera from two time points were evaluated for each subject. Neutralization studies were divided between 5 laboratories, and each lab's results analyzed separately using multivariate generalized logit models (ordinal outcomes: absent, low, medium, and high neutralization). Odds ratios (ORs) for methotrexate and TNFi effects were adjusted for demographics, IMID, other biologics and immunosuppressives, prednisone, COVID vaccinations (number/type), and infections in the 6 months prior to sample. Adjusted ORs for methotrexate and TNFi were then pooled in random-effects meta-analyses (separately for ancestral, and Omicron BA1 and BA5 strains). **Results:** Of 479 individuals (958 samples), 292 (61%) were IBD, 141 (29.4%) RA, and the remainder PsA, SpA and SLE. Mean age was 57 (62.2 % female). For both individual labs and the meta-analyses, adjusted ORs suggested independent negative effects of TNFi and methotrexate on neutralization. The meta-analysis

 doi:10.20944/preprints202408.1998.v1

adjusted ORs for TNFi were 0.56 (95% confidence interval, CI 0.39, 0.81) for the ancestral strain and 0.56 (95% CI 0.39, 0.81) for BA5. The meta-analysis adjusted OR for methotrexate was 0.39 (95% CI 0.19, 0.76) for BA1. **Conclusions:** SARS-CoV-2 neutralization in vaccinated IMID was diminished independently by TNFi and methotrexate. As SARS-CoV-2 circulation continues, ongoing vigilance regarding optimized vaccination is required.

Keywords: Covid; Vaccination; immune-mediated inflammatory disease; Methotrexate; Tumor Necrosis Factor Inhibitors; Autoimmune Diseases; TNF-blocking Antibody

Introduction

In North America, COVID-19 continues to be the third most common cause of death, right after cancer and heart disease [1]. Given ongoing SARS-CoV-2 circulation, COVID-19 vaccination response in people with immune-mediated inflammatory disease (IMID) remains a key issue, particularly regarding effects of common immunosuppressives like methotrexate and tumor necrosis factor inhibitors (TNFi). To date, most studies have focussed on a single disease and/or a single centre, or assessed only ancestral strains and/or only the presence of antibodies and not viral neutralization ability. When combining data across centres to achieve a larger and diverse sample, sophisticated modeling must allow for differences across centres, while aiming to achieve a single estimate of effects. Our purpose was to overcome these challenges.

Methods

In this prospective observational multicenter cohort study, we recruited adults with inflammatory bowel disease (IBD), psoriatic arthritis (PsA), rheumatoid arthritis (RA), spondylarthritis (SpA), and systemic lupus erythematosus (SLE) from participating tertiary care rheumatology centres across Canada (from Calgary, Winnipeg, Quebec City, Sherbrooke, Toronto, and Hamilton). Ethics approval was obtained from the McGill University Health Centre and participating centres, and subjects provided written or oral consent. Initial recruitment began early in 2021, shortly after Canada began vaccinating against SARS-CoV-2 (mostly with mRNA formulations) and continued until the end of 2022. We required only a physician diagnosis of IMID, not specific diagnostic criteria. Exclusion criteria were individuals who could not provide consent in English or French, or whose last COVID vaccination was over 6 months prior to recruitment (unless they planned to be vaccinated in the near future). The current analyses focused on neutralizing antibodies detected in paired sera (mostly post 3rd or 4th vaccination).

Participants provided baseline and follow-up information on demographics (current age, sex at birth, and self-identified race/ethnicity according to categories), past COVID-19 infections (any confirmed event reported by subjects), COVID-19 vaccinations (including dates and type) and clinical history (type of IMID, date of diagnosis, medications). Neutralization studies are time and resource intensive. Since the capacity across the labs that supported multiple clinical and research studies was limited, the analysis was performed across multiple laboratories. The sera from Toronto, Sherbrooke and some Winnipeg samples were assessed in the Gingras laboratory for neutralization using a lentivirus-based spike pseudotype assay of three SARS-CoV-2 strains as described previously [2]. Hamilton, Quebec City, Calgary, and some samples from Winnipeg were processed locally, as per Table 1. All labs (Toronto, Hamilton [3], Quebec City [4], Calgary [5], Winnipeg [6]) performed neutralization for the ancestral strain, four of those labs (those in Winnipeg, Quebec City, Toronto and Hamilton) also assessed Omicron BA1, and three (in Winnipeg, Quebec City and Toronto) also assessed Omicron BA5.

 doi:10.20944/preprints202408.1998.v1

Table 1. Information on the laboratories conducting COVID neutralization assays.

Location N	Variants	Result				Units reported	Description
		Negative	Low Positive	Medium Positive	High Positive		
Calgary 246	Ancestral	<20	20-200	200-1620	>1620	50% neutralization titer (NT50).	Surrogate-vesicular stomatitis virus plaque reduction neutralisation test (PRNT)
Gingras Lab Toronto 116	Ancestral, Omicron, BA.1, BA.5	<1.5 (<32)	1.5-2 (32-100)	2-3 (100-1000)	>3 (>1000)	Log_{10} ID50 (ID50 is dilution at which 50% neutralization occurs)	Spike-pseudotyped lentivirus neutralization
Bowdish Lab Hamilton 50	Ancestral, Omicron BA.1	<= 5	10-160	329-640	1280	Highest dilution achieving geometric microneutralization of 50% (MNT50)	Cell culture assays with live SARS-CoV-2
Card Lab Winnipeg 35	Ancestral, Omicron, BA.1, BA.5	< 40 % inhibition	40-69.9%	70-89.9%	>90 % inhibition	% inhibition	Surrogate nAb analysis using the MSD Platform. Kit: V-PLEX SARS-CoV-2 Key Variant
Flamand Lab Quebec City 30	Wuhan, Omicron BA.1, BA.5	<20	20-200	200-1620	>1620	Highest serum dilution preventing infection (100% neutralization)	Live-virus SARS-CoV-2 neutralization

Two serum samples from each participant, collected between 2022-2023 were assessed. We required samples to be at least 30 days beyond the last vaccination. Each lab's results were analyzed separately using 3 multivariate generalized logit models for ancestral, Omicron BA1, and BA5 strains, with ordinal outcomes for no, low, medium, and high neutralization, as detailed in Table 1. Then, results were pooled across labs in random-effects meta-analyses (separately for ancestral, BA1 and BA5 strains). This approach allowed for differences of effect sizes since methods differed across labs. As an exploration/validation study, the Toronto (Gingras) lab also received and analyzed 10 random samples from each of the other centres.

In all our models for the primary analyses, potential predictors, effect-modifiers and/or confounders included demographics (age, biologic sex, self-reported race/ethnicity), COVID-19 vaccinations (timing and type), reports of SARS-CoV-2 infection (all lab-confirmed events reported by the subject, within 6 months of sample), and clinical factors such as IMID type and current (at our study enrolment) medication use. Besides methotrexate and TNFi, we adjusted for other disease-modifying agents (DMARDs, including azathioprine, sulfasalazine, leflunomide, janus kinase (-inhibitors (JAKi), 6-mercaptopurine), other biologics (ustekinumab, vedolizumab, abatacept, rituximab, tocilizumab, secukinumab) and prednisone. All medication variables in the model reflected current use at enrolment, yes or no, as per Table 2.

Table 2. SUCCEED participants contributing samples for neutralization assays.

Variables	N = 479
Province, N (%)	

 doi:10.20944/preprints202408.1998.v1

Variables	N = 479
Alberta (Calgary)	257 (53.7)
Manitoba	90 (18.8)
Ontario	73 (15.2)
Quebec	59 (12.3)
Mean days between samples, (standard deviation, SD)	97.2 (50.8)
Mean IMID duration at first/second sample, (SD) years	18.9 (14.4)
Baseline prednisone, N (%)	92 (19.2)
Baseline prednisone dose, N (%)	
1–10 mg	58 (12.1)
11–20 mg	9 (1.9)
20+ mg	24 (5.0)
Missing dose	1 (0.2)
Baseline biologic, N (%)	
Tumor Necrosis Factor inhibitor	186 (38.8)
Ustekinumab	80 (16.7)
Vedolizumab	47 (9.8)
Abatacept	15 (3.1)
Rituximab	8 (1.7)
Other biologics[b]	8 (1.7)
Baseline non-biologic drugs, N (%)	
Methotrexate	122 (25.5)
Azathioprine	24 (5.0)
Sulfasalazine	29 (6.1)
Leflunomide	20 (4.2)
JAK-inhibitor	18 (3.8)
6-mercaptopurine	1 (0.2)

[b]Other biologics included tocilizumab and secukinumab

Variables	First sample	Second sample
Vaccine doses before the sample N (%)		
Two	288 (60.1)	147 (30.8)
Three	110 (23.0)	216 (45.0)
Four	40 (8.4)	67 (14.0)
Five or more	41 (8.6)	49 (10.2)
Vaccine type		
BNT-162b2 monovalent only	313 (65.3)	302 (63.0)
Mixed bivalent	65 (13.6)	72 (15.1)
Mixed monovalent	61 (12.7)	69 (14.4)
mRNA1273 monovalent	33 (6.9)	30 (6.3)
Other	7 (1.5)	6 (1.3)
Mean days between last vaccine and sample (SD)	38.5 (33.7)	87.6 (57.3)
Calendar year ≥ 2022, N (%)b	109 (22.8)	161 (33.6)
Calendar period N (%)		
April to Sept	305 (63.6)	171 (35.7)
Oct to March	174 (36.3)	308 (64.1)

Vaccine history variables in our models included number of vaccines at time of sample (dichotomous variable: 2-3 vs 4+ doses) and type (categorical for BNT162b2 monovalent only, mRNA1273 monovalent only, any combination of these monovalent pre-Omicron vaccines, any bivalent formulations targeting Omicron or other), as per Table 2. We also controlled for days between the last vaccine and the sample (dichotomized at 120+ days post-vaccine versus earlier) and

 doi:10.20944/preprints202408.1998.v1

whether or not the individual reported a positive test for COVID-19 infection in the 6 months prior to the sample collection.

Statistical analyses were performed with R software.

Results

We studied 479 individuals; of these, 292 (61%) had IBD, 141 (29.4%) RA, 24 PsA (5%), 13 SpA (2.7%) and 9 SLE (1.9%). Most (447, 93.3%) individuals were white, 62.2 % were female, and the mean age was 56.8 (standard deviation 14.8) years. Other characteristics are shown in Table 2.

About 20% of individuals were on prednisone at study entry, most below 10 mg per day (Table 2). In terms of biologics, 186 (38.8% of the 479 individuals) were on a TNFi, 80 (16.7%) were on ustekinumab, 47 (9.8%) on vedolizumab, 15 (3.1%) on abatacept, and 8 (1.7%) on rituximab. Of the 186 TNFi users, 79 were adalimumab, 78 infliximab, 20 etanercept, 7 golimumab, and 2 certolizumab.

Regarding DMARDs, 122 (25.5% of the 479 individuals) were on methotrexate, 29 (6.1%) on sulfasalazine, 24 (5%) on azathioprine, 20 (4.2%) on leflunomide, and 18 (3.8%) on a JAKi. A number (82, 17.1%) were on hydroxychloroquine. Still, we did not include these in the DMARD category given that it does not have the same effects on suppressing antibody formation post-vaccination, that other DMARDs may have. [7]

At first sample, about 40% of subjects had more than two vaccinations while at second sample, this had increased to almost 70%. About two-thirds of subjects had received BNT162b2 monovalent only, prior to their providing a sample. The majority (about 85%) of samples were provided in individuals who had not received an Omicron-targeted vaccine formulation. Thirty-four (7.1%) of individuals providing samples reported clinically confirmed COVID-19 infection in 6 months prior to collection of both the first and second samples.

Table 3 shows the odds ratios (ORs) for neutralization related to methotrexate and to TNFi, adjusted for sex, age, race/ethnicity, IMID, DMARDs, biologics, prednisone, and details of past COVID vaccinations and infection. For both individual labs and the meta-analyses, adjusted ORs suggested that TNFi and methotrexate were independently associated with lower neutralization ability. The meta-analysis adjusted ORs for TNFi were 0.56, 95% confidence interval, CI 0.39, 0.81 for the ancestral strain and 0.56, 95% CI 0.39, 0.81 for Omicron BA5. The meta-analysis adjusted OR for methotrexate was 0.39, 95% CI 0.19, 0.76) for Omicron BA1.

Table 3. Multivariate ordered logit regression[a] and random-effects meta-analyses[b]: Adjusted odds ratios (aOR) for methotrexate and tumor-necrosis factor inhibitors (TNFi) effects on neutralization, with 95% confidence intervals (CIs).

SARS-Cov2 strain	Number subjects	Methotrexate		TNFi	
		aOR	95% CI	aOR	95% CI
Ancestral	N=116 Gingras	**0.25**	**(0.11, 0.56)**	0.74	(0.31, 1.79)
	N=30 Flamand	0.51	(0.01, 38.7)	0.79	(0.19, 3.11)
	N=35 Card	**0.04**	**(0.01, 0.22)**	0.29	(0.07, 1.15)
	N=50 Bowdish	2.55	(0.77, 8.99)	1.53	(0.40, 6.05)
	N= 248 Calgary	0.64	(0.36, 1.14)	**0.48**	**(0.30, 0.75)**
	Meta-analysis[b]	0.41	(0.10, 1.61)	**0.56**	**(0.39, 0.81)**
Omicron BA1	N=116 Gingras	**0.29**	**(0.14, 0.59)**	1.11	(0.54, 2.27)
	N=30 Flamand	0.41	(0.05, 3.14)	**0.04**	**(0.01, 0.27)**
	N=35 Card	0.11	(0.01, 1.44)	0.59	(0.03, 6.79)
	N=50 Bowdish	0.80	(0.26, 2.44)	0.29	(0.07, 1.08)
	Meta-analysis[b]	**0.39**	**(0.19, 0.76)**	0.35	(0.09, 1.39)
Omicron BA5	N=116 Gingras	**0.33**	**(0.16, 0.67)**	0.73	(0.35, 1.51)
	N=30 Flamand	2.02	(0.27, 17.1)	**0.06**	**(0.01, 0.29)**
	N=35 Card	0.50	(0.09, 2.67)	**0.08**	**(0.01, 0.39)**

 doi:10.20944/preprints202408.1998.v1

Meta-analysis[b]	**0.48**	**(0.20, 1.13)**	**0.18**	**(0.03, 0.95)**

[a] Repeated measures ordered logit generalized estimating equation regression adjusting for sex, age at sample, race/ethnicity, IMID type, other biologics and non-biologic immunosuppressives, prednisone, COVID infection in the 6 months prior to sample, and number/type of COVID vaccinations. The level of outcome was negative, low, medium or high neutralization. [b] Random effects meta-analyses of adjusted ORs.

The supplemental tables show the results for individual models related to each strain and lab. Across these models, the effects of demographics, medications, and vaccine history were largely similar.

Discussion

Neutralizing antibodies against the SARS-CoV-2 inhibit the virus' ability to enter human cells, and likely have a key role in protecting an individual from COVID-19 infection and severity. [8] Our results indicate that neutralization responses in immunosuppressed IMID hosts may be diminished by both TNFi and methotrexate in an independent manner.

Methotrexate is a 'cornerstone' DMARD in RA and also useful in SLE and other autoimmune diseases. It has adenosine-mediated immune suppressive effects and limits B-cell activation, thus having the potential to lower antibody production not only in rheumatic disease, but also post-vaccination. Given the importance of this drug, several advisory groups have sought to provide recommendations to patients and healthcare providers, such as holding methotrexate (and/or some biologics) around the time of COVID-19 vaccination. However, individuals may be concerned that this might trigger IMID symptoms. In a recent small meta-analysis, holding MTX for approximately 2 weeks following COVID-19 vaccination, though associated with significantly higher antibody titres, was also with a higher disease flare rate. [9] A potential limitation of our study is that we did not include in our analyses information on whether patients: continued or held their medications (and for how long)

TNF inhibitors have also been reported to lower concentrations of anti-receptor binding domain (and other anti-spike antibodies)greater decay over time in those antibodies, as well as an increased occurrence of breakthrough infections. [10–12] However, in most prior studies data were collected in a single disease (e.g., IBD or RA), and/or focussed on a single drug class (e.g., anti-TNFi), either excluding those with other concomitant medications (including prednisone) or not adjusting for them. In the real world, decisions have to be made across a broad range of IMID types and concomitant medications. Our data thus add to the existing literature, particularly it is the first detailed analyses of the independent effects of methotrexate and TNFi on neutralizing antibodies in a broad range of IMID individuals, and accounting for both past infection and detailed vaccination history.

In many countries, including Canada and the United States, though two mRNA vaccines represent a primary series for immunocompetent people [13], three doses are considered the primary series in individuals receiving immunosuppressives, according to Centre for Disease Control, CDC, Public Health Agency of Canada, and ACR guidelines, These guidelines additionally point out that, in individuals taking IMID medications that impair vaccine responsiveness, supplemental doses are recommended (e.g., ≥2 additional boosters, for a total of 5 doses, as per the ACR and CDC guidelines).

The strengths of our work are that we studied a large group of individuals from multiple centres, across several diseases, on a range of different therapies, which reflects how complicated IMID care is. We had multiple samples and detailed, longitudinal information on vaccine and infection history. The potential limitations include the complex nature of this real-world dataset, particularly with respect to the logistical challenges of de-centralized analysis that resulted in heterogeneity of approaches used by the different labs to assess neutralization. However, we were able to see similar effects of our demographic and clinical variables across the different methods, and to acknowledge the differences between laboratories, we modelled the data separately, then performed a meta-analysis to produce overall estimates for the effects of methotrexate and TNFi.

Another strength of our study is that we controlled all estimates for concomitant use of a variety of medications. However, our current approach did simplify medication exposures in that we

 doi:10.20944/preprints202408.1998.v1

modelled medications at cohort entry only. Fortunately, our data spanned a relatively short time period and medications taken were relatively stable over time, in the individuals that we studied. Even with almost 500 patients and almost 1000 samples however, we did have relatively small numbers of individuals on specific medications of potential interest (e.g., we only had 8 individuals on rituximab), allowing us to adjust for these medications but precluding precise estimates of all drug effects.

Since an objective of our study was to capture the breadth of the general IMID population, we aimed to use as few exclusion criteria as possible (i.e., individuals with IMID were clinically confirmed, but we did not require specific diagnostic criteria or medication exposures). It remains impossible to exclude some selection bias, and the age and sex characteristics of our sample are largely similar to what is expected. Race/ethnicity diversity was somewhat lacking, in that over 93% of subjects self-reported as white; however many of the centres from which people were recruited were white-predominant (including Quebec City and Sherbrooke, which are over 90% white). Non-white Canadians may be less likely to be vaccinated against preventable diseases, including COVID-19. [14]

In summary, our detailed analyses of neutralization ability post-COVID vaccination in IMID suggest independent effects of methotrexate and TNFi. This reminder is important given the stark reality of vaccine hesitancy, even among immunocompromised individuals [15]. Given ongoing waves of SARS-CoV2 circulation (including new variants), people with IMID, and their care givers, should remain aware of the need to optimize vaccine coverage against SARS-CoV-2.

Author Contributions: Conceptualization, Carol Hitchon, Dawn Bowdish, Gilles Boire, Paul Fortin, Louis Flamand, Vinod Chandran, Roya Dayam, Anne-Claude Gingras, Catherine M. Card, Inés Colmegna, Maggie Larché, Gilaad Kaplan, Luck Lukusa, Jennifer Lee and Sasha Bernatsky; **Data curation:** Luck Lukusa; Formal analysis, Louis Flamand, Luck Lukusa and Sasha Bernatsky; **Funding acquisition:** Carol Hitchon, Dawn Bowdish, Vinod Chandran and Sasha Bernatsky; **Investigation**: Carol Hitchon, Dawn Bowdish, Gilles Boire, Paul Fortin, Vinod Chandran, Inés Colmegna, Maggie Larché, Gilaad Kaplan, Jennifer Lee and Sasha Bernatsky; **Methodology**: Carol Hitchon, Dawn Bowdish, Gilles Boire, Paul Fortin, Louis Flamand, Vinod Chandran, Roya Dayam, Anne-Claude Gingras, Catherine M. Card, Inés Colmegna, Maggie Larché, Gilaad Kaplan, Luck Lukusa, Jennifer Lee and Sasha Bernatsky; **Validation**: Louis Flamand, Roya Dayam, Anne-Claude Gingras and Catherine M. Card; **Writing—original draft:** Carol Hitchon, Dawn Bowdish, Gilles Boire, Paul Fortin, Louis Flamand, Vinod Chandran, Roya Dayam, Anne-Claude Gingras, Catherine M. Card, Inés Colmegna, Maggie Larché, Gilaad Kaplan, Luck Lukusa, Jennifer Lee and Sasha Bernatsky; **Writing—review & editing:** Carol Hitchon, Dawn Bowdish, Gilles Boire, Paul Fortin, Louis Flamand, Vinod Chandran, Roya Dayam, Anne-Claude Gingras, Catherine M. Card, Inés Colmegna, Maggie Larché, Gilaad Kaplan, Luck Lukusa, Jennifer Lee and Sasha Bernatsky. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by funding from the Public Health Agency of Canada, through the Vaccine Surveillance Reference group and the COVID-19 Immunity Task Force. The views expressed here do not necessarily represent the views of the Public Health Agency of Canada. Dr. Fortin holds a Canada Research Chair on Systemic Autoimmune Rheumatic Diseases. Dr. Vinod Chandran is supported by a clinician scientist salary award from the Department of Medicine, University of Toronto.

Institutional Review Board Statement: The study was approved by the ethics board of the research institute of the McGill University Health Center (MP-37-2022-7763) and all participating centers.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article. The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: This research was made possible by funding from the PHAC, via the Vaccine Surveillance Reference Group and the CITF. The opinions presented here are solely those of the authors and do not reflect the official stance of the PHAC. We extend our gratitude to all research team members for their excellent collaboration, their dedication and their efforts on this project, and to the patients who participated in the study. The serology team at the Network Biology Collaborative Centre at the Lunenfeld-Tanenbaum Research Institute, and particularly Reuben Samson, Dr. W Rod Hardy and Dr. Q Hu for design and optimization of the variant-specific spike neutralization assays, as well as CoVaRR-Net for funding the development of the assays. Dr. Louis

 doi:10.20944/preprints202408.1998.v1

Flamand and Dr. Anne-Claude Gingras are CoVaRR-Net pillar leads. CoVaRR-Net, or Coronavirus Variants Rapid Response Network, is a network of interdisciplinary researchers from institutions across the country created to assist in the Government of Canada's overall strategy to address the potential threat of emerging SARS-CoV-2 variants.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. Dr. Boire has received honoraria (none relevant to this work) for speaking or consultancy from Abbvie, BMS, Lilly, Novartis, Pfizer, Samsung BioEpis, Viatris; multi-centric research grants (none relevant to this work) from Janssen and Pfizer; unrestricted grant support (none relevant to this work) for local initiatives from BMS, Lilly and Pfizer. Dr. Bowdish is the Canada Research Chair in Aging & Immunity. Dr. Chandran has received research grants (none relevant to this work) from AbbVie, Amgen, and Eli Lilly and has received honoraria none relevant to this work) for advisory board member roles from AbbVie, Amgen, BMS, Eli Lilly, Janssen, Novartis, Pfizer, and UCB. His spouse is an employee of AstraZeneca. Dr. Gingras has received research funds from a research contract with Providence Therapeutics Holdings, Inc., for other projects, participated in the COVID-19 Immunity Task Force (CITF) Immune Science and Testing working party, chaired the CIHR Institute of Genetics Advisory Board, and chairs the SAB of the National Research Council of Canada Human Health Therapeutics Board. Dr. Gingras is the Canada Research Chair, Tier 1, in Functional Proteomics. Dr Hitchon participated in advisory boards for Astra-Zeneca- not related to this work and has received research grants (unrelated to this work) for multi-centric research and from Pfizer (unrelated to this work).

References

1. Statistic Canada. Leading causes of death, total population, by age group. https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310039401. Accessed July 29, 2024.
2. Dayam, R. M. et al. Accelerated waning of immunity to SARS-CoV-2 mRNA vaccines in patients with immune mediated inflammatory diseases. *JCI Insight* **7**, e159721 (2022).
3. Zhang, A., Stacey, H.D., D'Agostino, M.R. *et al.* Beyond neutralization: Fc-dependent antibody effector functions in SARS-CoV-2 infection. *Nat Rev Immunol* **23**, 381–396
4. Colmegna I, Valerio V, Amiable N, et al. COVID-19 Vaccine in Immunosuppressed Adults with Autoimmune rheumatic Diseases (COVIAAD): safety, immunogenicity and antibody persistence at 12 months following Moderna Spikevax primary series. *RMD Open*. 2023;9(4):e003400.
5. Sievers BL, Gelbart T, Tan GS. A high-throughput SARS-CoV-2 pseudovirus multiplex neutralization assay. STAR Protoc. 2022 Dec 16;3(4):101835.
6. Hitchon CA, Mesa C, Bernstein CN, et al. Immunogenicity and safety of mixed COVID-19 vaccine regimens in patients with immune-mediated inflammatory diseases: a single-centre prospective cohort study. BMJ Open. 2023 May 30;13(5):e071397.
7. Zheng YQ, Li HJ, Chen L, Lin SP. Immunogenicity of inactivated COVID-19 vaccine in patients with autoimmune inflammatory rheumatic diseases. Sci Rep. 2022 Oct 26;12(1):17955
8. Morales-Núñez JJ, Muñoz-Valle JF, Torres-Hernández PC, Hernández-Bello J. Overview of Neutralizing Antibodies and Their Potential in COVID-19. Vaccines (Basel). 2021 Nov 23;9(12):1376. doi: 10.3390/vaccines9121376. PMID: 34960121; PMCID: PMC8706198.
9. Imran, M., Ali, S., Ibrahim, A.A. et al. Effect of methotrexate hold on COVID-19 vaccine response in the patients with autoimmune inflammatory disorders: a systematic review and meta-analysis. Clin Rheumatol 43, 2203–2214 (2024)
10. Edelman-Klapper, H. et al. Lower serologic response to COVID-19 mRNA vaccine in patients with inflammatory bowel diseases treated with Anti-TNFα. *Gastroenterology* **162**, 454–467 (2022).
11. Saad, C.G. et al. Interaction of TNFi and conventional synthetic DMARD in SARS-CoV-2 vaccine response in axial spondyloarthritis and psoriatic arthritis. *Jt. Bone Spine* **90**, 105464 (2023).
12. Lin S et al. Antibody decay, T cell immunity and breakthrough infections following two SARS-CoV-2 vaccine doses in inflammatory bowel disease patients treated with infliximab and vedolizumab. *Nat. Commun.* **13**, 1379 (2022)
13. Link-Gelles R, Levy ME, Gaglani M, et al. Effectiveness of 2, 3, and 4 COVID-19 mRNA Vaccine Doses Among Immunocompetent Adults During Periods when SARS-CoV-2 Omicron BA.1 and BA.2/BA.2.12.1 Sublineages Predominated — VISION Network, 10 States, December 2021–June 2022. MMWR Morb Mortal Wkly Rep 2022;71:931–939. DOI: http://dx.doi.org/10.15585/mmwr.mm7129e1. https://www.cdc.gov/mmwr/volumes/71/wr/mm7129e1.htm. Accessed July 29, 2024.
14. Cénat JM, Noorishad PG, Moshirian Farahi SMM, Darius WP, Mesbahi El Aouame A, Onesi O, Broussard C, Furyk SE, Yaya S, Caulley L, Chomienne MH, Etowa J, Labelle PR. Prevalence and factors related to COVID-19 vaccine hesitancy and unwillingness in Canada: A systematic review and meta-analysis. J Med Virol. 2023 Jan;95(1):e28156.

 doi:10.20944/preprints202408.1998.v1

15. Felten R, Dubois M, Ugarte-Gil MF, Chaudier A, Kawka L, Bergier H, Costecalde C, Pijnenburg L, Fort J, Chatelus E, Sordet C, Javier RM, Gottenberg JE, Sibilia J, Fuentes-Silva YJ, Arnaud L. Cluster analysis reveals three main patterns of beliefs and intention with respect to SARS-CoV-2 vaccination in patients with autoimmune and inflammatory diseases. Rheumatology (Oxford). 2021 Oct 9;60(SI):SI68-SI76.