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Cohort profile: Stop the Spread Ottawa (SSO)—a community-based prospective cohort study on antibody responses, antibody neutralisation efficiency and cellular immunity to SARS-CoV-2 infection and vaccination

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ABSTRACT

Purpose To investigate the robustness and longevity of SARS-CoV-2 immune responses conferred by natural infection and vaccination among priority populations such as immunocompromised individuals and people with post-acute sequelae of COVID-19 in a prospective cohort study (Stop the Spread Ottawa—SSO) in adults living in the Ottawa region. In this paper, we describe the study design, ongoing data collection and baseline characteristics of participants.

Participants Since October 2020, participants who tested positive for COVID-19 (convalescents) or at high risk of exposure to the virus (under surveillance) have provided monthly blood and saliva samples over a 10-month period. As of 2 November 2021, 1026 adults had completed the baseline survey and 976 had attended baseline bloodwork. 300 participants will continue to provide bimonthly blood samples for 24 additional months (ie, total follow-up of 34 months).

Findings to date The median age of the baseline sample was 44 (IQR 23, range: 18–79) and just over two-thirds (n=688; 67.1%) were female. 255 participants (24.9%) had a history of COVID-19 infection confirmed by PCR and/or serology. Over 600 participants (60.0%) work in high-risk occupations (eg, healthcare, teaching and transportation). 108 participants (10.5%) reported immunocompromising conditions or treatments at baseline (eg, cancer, HIV, other immune deficiency, and/or use of immunosuppressants).

Future plans SSO continues to yield rich research potential, given the collection of pre-vaccine baseline data and samples from the majority of participants, recruitment of diverse subgroups of interest, and a high level of participant retention and compliance with monthly sampling. The 24-month study extension will maximise opportunities to track SARS-CoV-2 immunity and vaccine efficacy, detect and characterise emerging variants, and compare subgroup humoral and cellular response robustness and persistence.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Stop the Spread Ottawa (SSO) is a large-scale longitudinal cohort study with frequent and comprehensive monitoring of SARS-CoV-2 immune response among diverse subgroups, including priority populations such as immunocompromised people and people with post-acute sequelae of COVID-19 (PASC).

⇒ Pre-vaccine baseline data and samples were collected from the majority of participants, made possible through a successful recruitment plan and rapid launch early on in the pandemic.

⇒ Study extension allows for up to 34 months of follow-up of SARS-CoV-2 immunity elicited from natural infection and/or vaccination; severity, duration and changes in PASC; and breakthrough infections by emerging variants.

⇒ The study population was not intended to be, and is not, representative of the general population of the Ottawa region in terms of age, sex, ethnicity and total household income, and there is poor representation of ethnic minorities and no adults ≥80 years of age.

⇒ There is a risk of misclassification of some variables as participants self-reported data through online questionnaires, including dates of positive PCR test, vaccination history and health conditions.

INTRODUCTION

A beta-coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),

continues to drive the COVID-19 pandemic.[1] Since December 2019, the virus has infected over 300 million people and caused more than 5.4 million deaths worldwide.[2] Efforts have been made by the international research community to describe the robustness and longevity of SARS-CoV-2 immune response conferred by natural infection and/or vaccination among different groups of people,[3–9] including immunocompromised individuals[10–15] and people with PASC (post-acute sequelae of COVID-19).[16–19] People with an immunocompromised state may not elicit sufficient humoral and cellular response to vaccination.[20–26] PASC continues to be a major public health concern, causing severe and pervasive impacts on physical and mental health four or more weeks post-infection.[27–29] Given ongoing COVID-19 vaccinations and emerging variants of concern (VOC), there is still a need for longitudinal analyses of SARS-CoV-2 immune response and COVID-19 impacts among diverse groups at risk of infection/reinfection, severe disease and/or persistent symptoms.[30–39]

Most persons recovering from SARS-CoV-2 develop IgM, IgG and IgA antibodies targeting the SARS-CoV-2 nucleocapsid (N) or spike (S) proteins between 7 and 14 days post-onset of symptoms.[40 41] Seroconversion is dependent on the virological and clinical profile over time.[42] The receptor binding domain (RBD) of the S protein is the primary target of neutralising antibodies.[43] During the pandemic, several SARS-CoV-2 variants have become dominant in many countries in different periods.[34 35 44] These variants harbour mutations of the spike protein that can restrict antibody neutralisation capacity and hinder vaccine efficacy.[45–47] Neutralising antibodies comprise a core function of adaptive humoral immune response, predictive of COVID-19 severity and survival.[48 49] Substantial correlations have been found between neutralising antibody profile and disease severity,[50] anti-S IgG and neutralising titres,[51 52] anti-S/-N levels and PASC,[53 54] and immunosuppression and anti-S IgG non-response.[26 55–58]

Research to date has focused on hospitalised patients, more likely to have severe COVID-19 disease than people in community settings, and on small cohorts of people with specific conditions. Reports on serology continue to dominate analyses of SARS-CoV-2 immune responses. Other human coronaviruses, which do not confer strong protection against SARS-CoV-2,[59 60] may confound interpretation of serological analyses. Factors that influence the detection of cross-reactive antibodies include choice of antigen, the antibody isotype being detected and the relative sensitivity of various detection methods.[61–64] In addition to serology, immunoassays of complementary T-cell responses are required to assess impacts of exposure to SARS-CoV-2 and endemic human coronaviruses on coordinated antibody-mediated and cell-mediated responses to vaccination.[65–67] As an example, B.1.1.7 and B.1.351 variants were found to partially escape SARS-CoV-2-induced humoral immunity, but there were no observed changes in $CD4^+$ T-cell activation.[68] Investigation as to protection conferred by heterologous or homologous vaccination, and by different time intervals between vaccine doses is ongoing.[69–71] Impacts of infection and vaccination on emerging viral variants continue to be of major public health concern.[32 34 35] Priority topics given emerging variants include the transmissibility, pathogenicity and vaccine resistance of VOC,[3 34 44] and the impacts of vaccination and VOC on post-infection symptoms.[71–74]

To characterise the nature, intensity and longevity of immune response to the SARS-CoV-2 virus, we established a large longitudinal prospective cohort study, Stop the Spread Ottawa, with the objectives of:

1. Assessing COVID-19 humoral immune response over time;
2. Increasing knowledge of protective SARS-CoV2-specific immune responses through virus neutralisation and T-cell activation studies on a surveillance cohort and COVID-19 convalescent patients;
3. Comparing the use of dried blood spot cards and serum for monitoring antibody responses;
4. Tracking participant protocol adherence and drop-out;
5. Understanding the psychological and socioeconomic impacts of testing positive for COVID-19;
6. Assessing the seroprevalence of other common community-acquired viral respiratory illnesses by risk group; and
7. Comparing COVID-19 specific immunity derived from natural infection and from immunisation.

All participants provide monthly collection of blood and saliva samples and complete extensive serial questionnaires, used to track health history (eg, vaccinations), COVID-19 disease severity, persistent SARS-CoV-2 symptoms, risk factors of exposure, and socioeconomic and psychosocial impacts of the pandemic. This article describes our study protocol and cohort composition.

COHORT DESCRIPTION

Study setting and participants

The Stop the Spread Ottawa (SSO) prospective cohort study on SARS-CoV-2 immune response recruited over 1000 adults in the Ottawa region from 14 September 2020 to 28 September 2021. Since 19 October 2020, participants testing positive for COVID-19 or at high risk of exposure have provided monthly blood and saliva samples over a 10-month period. Three hundred participants will continue to provide bimonthly blood samples for 24 months (ie, for up to 34 months overall). Individuals ≥18 years of age in the Ottawa region (1) at risk of SARS-CoV-2 exposure/infection due to occupation or health condition, or (2) with any history of COVID-19 natural infection, confirmed by positive PCR test and/or serology, were eligible to participate. Participants at risk of exposure, but without a history of SARS-CoV-2 infection, were enrolled into the Surveillance cohort (n=750). Individuals known to have current or past COVID-19 infection confirmed by positive SARS-CoV-2 quantitative reverse transcription PCR (RT-PCR) or serology test were

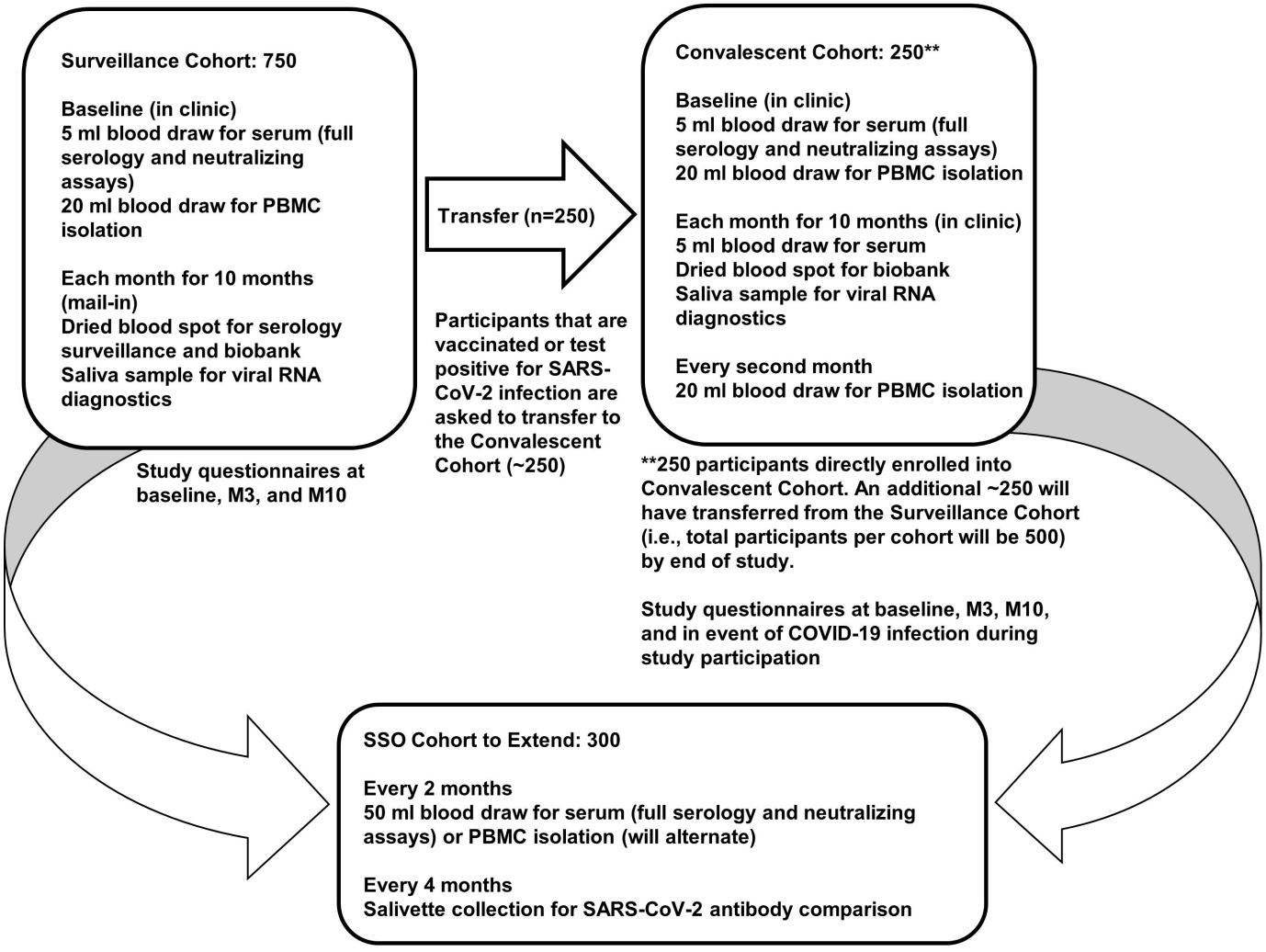

Figure 1 Procedures for Stop the Spread Ottawa (SSO) study participants, baseline to month 10 and extension to month 34. PBMC, peripheral blood mononuclear cell.

recruited into the Convalescent cohort (n=250). Beginning January 2021, vaccinated participants in the Surveillance cohort were given the option of transferring to the Convalescent protocol, to facilitate the collection of monthly post-vaccine whole blood samples (figure 1). To date, over 200 Surveillance participants have transferred. Approximately 500 adults will be participating in each cohort by end of study.

Multiple strategies were used to facilitate rapid recruitment early on in the pandemic, including a study website (https://omc.ohri.ca/SSO/) and SARS-CoV-2 antibody results portal; distribution of promotional materials to healthcare and dental staff, teachers and transportation workers; collaboration with organisations representing key target populations; and use of Eastern Ontario Regional Laboratory Association (EORLA) reports and The Ottawa Hospital COVID-19 Registry to identify SARS-CoV-2 positive cases for follow-up. Target populations for the Surveillance cohort included healthcare workers, long-term care facility staff, transportation workers and patients with HIV, chronic viral hepatitis and haematological malignancy. Other populations of interest include homeless shelter staff, dentists/allied dental care workers, elementary and secondary school teachers, elderly individuals living in high-density, long-term retirement homes, and daycare workers.

Enrolment closed 28 September 2021. Data collection is ongoing. The expected duration of the study with extension is 60 months. Primary results should be known approximately 6 months after the last participant has been recruited and completed testing procedures.

Data collection

All individuals who enrolled on the Stop the Spread Ottawa website (https://omc.ohri.ca/SSO) were sent a link to access an informed consent form. As of 2 November 2021, 1108 consented participants had been screened by the research coordinator (figure 2). One participant was ineligible as underaged (<18 years old) and approximately 30 participants resided outside of the Ottawa region. All eligible participants were sent a unique study identifier and links to book baseline bloodwork and complete a study questionnaire by secure email. By 2 November, 1026 participants had completed the baseline questionnaire

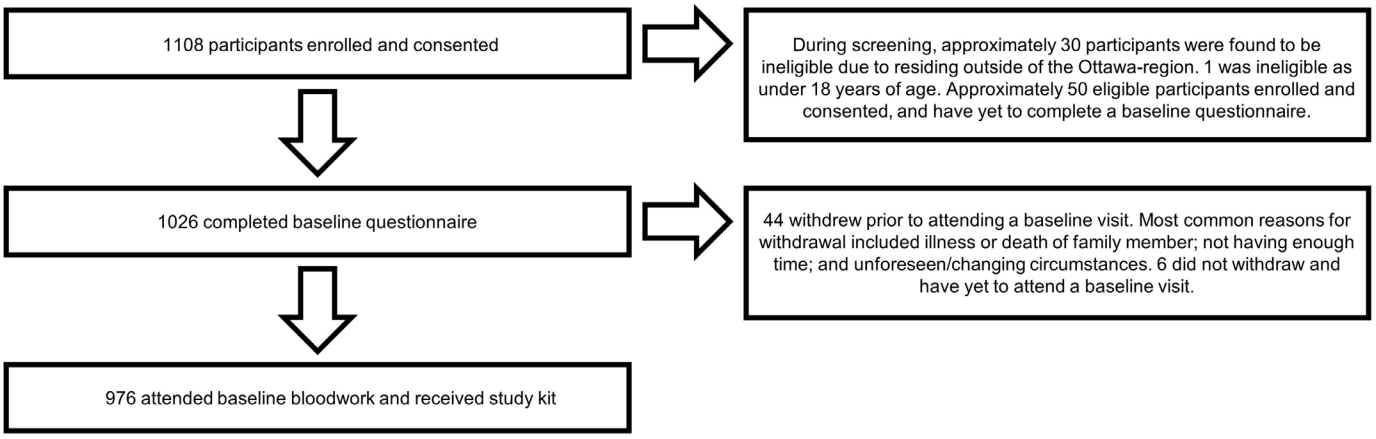

Figure 2 Flow diagram of enrolled participants, as of 2 November 2021.

and 976 had attended baseline visits. During the initial 10 months of this study, participants have a 7-day window to schedule bloodwork visits and send in saliva and/or sputum and dried blood spot samples. Thereafter (11–34 months post-baseline), a 21-day window to attend study visits is allotted.

Bloodwork

At baseline, for all participants, one (5 mL) tube with a separator gel with clot activator for serum and two (10 mL × 2) tubes with EDTA for lymphocyte isolation were drawn. During the first 10 months of the study, up to 500 participants with history of SARS-CoV-2 infection and/or vaccination in the Convalescent cohort attend monthly blood draws for serum and bimonthly plasma and peripheral mononuclear cells (PBMCs). After 10 months, participants who consent to study extension provide blood draws every 2 months over the next 24 months (figure 1). During this time, 10 (5 mL × 10) tubes with separator gel with clot activator will be collected every 4 months. Five (10 mL × 5) tubes with EDTA will be drawn every 4 months alternating.

Saliva/sputum and dried blood spot collection

Over the initial 10 study months, participants used home collection kits to submit monthly dried blood spots (DBS) for serology surveillance and saliva/sputum samples[75–77] (DNA Genotek: OMNIgene·ORAL OM-505) for viral RNA testing by mail to EORLA or drop-off at The Ottawa Hospital. Participants in the Convalescent cohort self-collect monthly DBS in addition to attending monthly blood draws for serum. We note that the sensitivity and specificity of DBS for detecting SARS-CoV-2 spike glycoprotein antibodies relative to serum have been documented previously.[78 79] However, as well as for quality control purposes, we compared serology results from DBS and serum to be able to report DBS results in international units, thus facilitating inter-study comparisons.[80 81]

Participants were provided with access to video demonstrations through the study website to aid self-collection. As per manufacturer instructions,[82] participants were asked to spit into the OM-505 kit first thing in the morning, prior to food or drink. While we acknowledge passive drool as the gold standard for saliva collection,[83] we opted to use the OM-505 kits given they are easy to use without professional assistance, thus encouraging monthly compliance, and contain a preservative and viricidal fluid, allowing for safe and stable storage and transport of samples.[82 84] Participants who were identified as SARS-CoV-2 PCR positive were contacted by the research coordinator, promptly linked to Public Health as needed, and advised to seek emergency medical care in the event of life-threatening symptoms. Disease transmission mitigation and self-isolation measures were explained over the phone. After 10 months, participants in the extension will collect and submit one salivette (Sarstedt, Numbrecht, Germany: 51.1534) for SARS-CoV-2 antibody testing every 4 months, starting month 16. Salivettes have been successfully used in other Canadian studies to detect IgM, IgG and IgA response to SARS-CoV-2 spike and RBD proteins.[85]

Questionnaires

Electronic study questionnaires are completed at baseline, and at 3 and 10 months post-baseline. Three hundred participants in extended follow-up complete questionnaires every 6 months (months 16, 22, 28 and 34). Participants who are infected or reinfected during the study are asked to complete an immediate follow-up questionnaire.

Study questionnaire categories include:

- Demographics (eg, age, ethnic group, gender)
- Health history (eg, vaccinations, medications)
- Severity of COVID-19 signs and symptoms
- Risks of SARS-CoV-2 exposure
- Socioeconomic impacts of the pandemic
- Psychosocial impacts of the pandemic

All participants are asked to notify the research coordinator if and when they test positive or receive a COVID-19 vaccine. The research coordinator collects and logs dates of infection/vaccination and vaccine type in a shared tracking file. All participants who report new infections/reinfections complete an immediate follow-up questionnaire, documenting positive test date and symptom type, severity, and duration.

LABORATORY INVESTIGATIONS

Full serology includes detection of the main antibody isotypes IgA, IgM, IgG and subtypes IgG1, IgG2, IgG3, IgG4 against the N, RBD and the full-length trimeric spike of SARS-CoV-2. Neutralisation efficiency against SARS-CoV-2 spike protein and antibodies against the full trimeric spike of all four seasonal human coronaviruses (229E, OC43, NL63, HKU-1) are also assessed. T-Cell characterisation studies include SARS-CoV-2-specific T-cell responses, cytokine production profiles and determination of immunodominant sequence domains on the S protein, the membrane glycoprotein (M) and N protein. Bimonthly sampling for plasma and PBMCs during the initial 10-month study will enable correlation of seroprevalence (anti-SARS-CoV-2 antibody titres and neutralising antibody profile) with $CD4^+$ and $CD8^+$ T-cell responses at five time points.

Serological testing of monthly blood samples submitted by Surveillance cohort participants will be performed using an automated high-throughput chemiluminescent direct ELISA[80] located within the University of Ottawa. This assay has been used in several studies across Canada[86–91] and has a reported sensitivity of 100% for the spike, RBD and N protein (IgG) and false-positive rates of 2% for spike, 1% for RBD and 6% for N.[80] All viral antigens required for serological assessment and anti-human IgG-HRP (horseradish peroxidase) fusion secondary antibody are provided by Yves Durocher at the National Research Council of Canada. Proteins are expressed in a CHO-DXB11-derived clone (CHOBRI/55E1) with yields estimated at 70–100 mg/L.[92 93] Briefly, 384-well plates are coated with the antigen of choice overnight at 4°C. Diluted patient sample is applied following a blocking step and incubated. Bound SARS-CoV-2 antibodies are then detected using an isotype-specific HRP-conjugated antibody. The plate is developed using a chemiluminescent substrate, which is compatible with automated instruments. Each assay plate contains commercially purified humanised antibodies (clones CR3022, CR3018 and HC2003), pooled positive and negative serum, and non-specific Ig control and blanks. A consistent layout and set of robust controls allow for quality control assessments and are key to raw data processing and subsequent analysis. To enable interplate comparison, background-corrected luminescence values are scaled in relation to the calibration curve. We used 123 serum samples and 320 DBS samples representative of pre-pandemic adults to generate thresholds to determine signal to cut-off ratios.[80] Samples with signal to cut-off values greater than 1.0 are considered positive. While positive and negative calls are interesting in the optics of seroprevalence surveys, quantification of antibody titres enables more robust analyses. As such, we have established a data analysis pipeline to report international antibody binding units (BAU) by correlating scaled luminescence values in linear range to the WHO-generated international standard (NIBSC 20/136).

We will investigate variabilities over time in the virus-neutralising properties and abundance of anti-SARS-CoV-2 antibodies and correlate these with individual case severity in the Convalescent cohort. In addition, we will analyse T cells to determine the proportion that are reactive to SARS-CoV-2 peptide antigens. Given the large number of samples from SSO and class three biocontainment restrictions on replicative SARS-CoV-2, we have implemented a high-throughput protein-based surrogate neutralisation assay, adapted from Abe *et al*.[94] The protein-based surrogate neutralisation was shown to correlate with lentiviral pseudotype-based neutralisation assay and with PRNT50.[94] In this assay, trimeric spike or RBD is coated in a 384-well plate and blocked. Diluted serum samples are applied and incubated to allow binding of antibodies to antigen. Unbound antibodies are washed off, and recombinant biotin-conjugated ACE2 is applied to compete with antibodies for binding to antigen. The presence of strongly neutralising antibodies will inhibit spike–ACE2 or RBD–ACE2 interaction. A streptavidin-HRP polymer is then applied to detect bound ACE2 and the plate developed using a chemiluminescent substrate. In this competitive binding assay[94], the signal is inversely correlated to the neutralisation efficiency. Results of this assay can be reported in titres using international units (IU/mL) as per WHO standards (NIBSC 20/136) or, alternatively, by reporting half maximal inhibitory dilution (ID50) or per cent inhibition as compared with maximum ACE2 binding.

To maximise the efficiency of high-quality sample analysis and data acquisition, we developed a Core Facility that has enabled massive upscaling of the output of the assays we have developed for (1) SARS-CoV-2 antibody measurements and neutralisation efficiency in blood and (2) viral diagnostics using reverse transcription droplet digital PCR technology (RT-ddPCR). Core architecture includes the following: (1) a robotic liquid handler (Hamilton MicroLab Star) dedicated to isolating serum or plasma from clinical bar-coded collection tubes and performing ELISAs using an integrated plate washer (Biotek 405 TS/LS LHC2) and plate reader (Biotek Synergy NEO2); (2) an instrument dedicated to isolating viral RNA from nasopharyngeal swabs (NPS) in viral transport media (VTM) or from human sputum in VTM and dispensing the purified RNA in a storage plate with barcode tracking (Hamilton MicroLab Star); (3) an automated ddPCR platform from Bio-Rad (AutoDG) for detecting and quantifying viral RNA. RT-ddPCR is a biotechnological refinement of RT-qPCR that provides absolute quantification of viral genomes in a sample and has demonstrated improved sensitivity and accuracy for SARS-CoV-2 detection, especially for tests involving samples with low viral load. Given this automation, the system can process >3200 blood samples and >2000 NPS/sputum samples per 5-day work week.

Power calculations and analyses

We have recruited over 1000 participants, of which more than 250 have current or past COVID-19 infection. Given limited knowledge of SARS-CoV-2 at the time of study conception (spring 2020) and the urgency to launch this study early on in the pandemic, no formal sample size calculations were performed to determine the number of required participants with history of COVID-19 infection (n=250) and the number of participants required overall (n=1000). These decisions were largely based on the funding and resources available to our team; we aimed to recruit the highest numbers feasible, to permit flexibility for a wide range of planned projects.

Primary and secondary outcomes were determined in advance of mass SARS-CoV-2 vaccination. At time of study conception, we had planned to (1) compare the proportion of IgG antibody in convalescent participants with and without comorbidities at month 6 post–COVID-19 infection, and (2) consider the influence of biological sex on the proportion of those with COVID-19 infection possessing IgG seropositivity at month 6 post–COVID-19 infection. Over the course of the pandemic, we have had to continuously adapt our plans for analyses, especially to account for SARS-CoV-2 vaccination history and circulating VOC at different sampling timepoints. Following March 2022, our team used the WHO International Standard[81] for anti-SARS-CoV-2 immunoglobulins to determine binding antigen units (BAU/mL) and neutralising antibodies (IU/mL) for collected serum. Plans to analyse these results are in progress and will be reported in future publications. As well as enabling the quantification of post-vaccine levels, as opposed to simply reporting a binary cut-off, the International Standard reduces inter-laboratory variation, thereby supporting combined analyses of results through ongoing collaborations with multiple teams.

Finally, the research team will undertake robust multivariate logistic regression analyses of predictors of PASC determined a priori based on clinical expertise and reviews selected using AMSTAR 2 guidelines. Purposeful selection of serological and non-serological predictors will be used to fit a multivariable logistic regression model. We will include a number of predictors to target a mean absolute prediction error <0.05 (Lasso).[95] As prevalence estimates of PASC continue to vacillate,[96 97] we will use Bayesian updating to estimate the prevalence of PASC using the most current data available.[98] Multiple imputation will be used to handle missing data, assumed to be MCAR or MAR. Potential overfitting of the final model will be determined through internal validation using bootstrap methods. Opportunities to collaborate with similar studies will allow for external validation of the model, as well as combined analyses with higher power. SAS V.9.4, GraphPad Prism V.9.3.1 and R V.3.6.1 will be used for all analyses.

Patient and public involvement

Our team is committed to engaging actively and meaningfully with key stakeholders and partners, especially people who have endured COVID-19 infection and post-COVID symptoms. We continue to embrace community input and work to ensure that our research plan addresses the needs and concerns of affected Canadians. A virtual presentation and discussion forum were hosted by SSO Principal Investigators on 18 October 2021, to address participant questions about the study and related research in depth. All participants are sent a letter from the research team thanking them for their commitment to COVID-19 research. Finally, due to multiple requests for access to SARS-CoV-2 antibody results, we created a secure antibody results portal, which participants can access throughout the study.

Findings to date

Of participants to complete a baseline questionnaire by 2 November 2021 (n=1026), 67.1% (n=688) are female, and the median age is 44 years (IQR 23, range 18–79, table 1).

In addition, 88.6% (n=909) are white and 85.3% (n=875) are born in Canada; 27% (n=277) are current or former smokers, 14% (n=144) are obese and 4.2% (n=43) have diabetes; 81.6% (n=837) are employed, and 38.2% (n=392) report an annual household income ≥$C120 000; 61.6% (n=632) have an undergraduate or graduate degree.

Furthermore, 24.9% (n=255) have COVID-19 infection history, by positive PCR test (n=231) or by positive serology result during the study without previous positive PCR test (n=24). Table 2 displays demographics by infection status. Members of the Convalescent cohort with history of laboratory-confirmed SARS-CoV-2 infection (n=255) had an older median age (47, IQR 26) than members without infection history (n=771, median age: 43, IQR 22). There were less females in the Convalescent cohort (61.2%) than in the Surveillance cohort (69.3%).

We enrolled priority populations with conditions of clinical significance, including members with self-report of immunocompromising conditions/treatments (eg, cancer, HIV, other immune deficiency and/or use of immunosuppressants, n=108). Table 3 lists baseline health conditions, 2.4% (n=25) report cancer, 3% (n=31) HIV, 7.5% (n=77) other immune deficiency and 6.5% (n=67) use of treatment that weakens the immune system.

Over 600 at-risk workers (60.0%), including healthcare workers, teachers and transportation workers, were recruited.

Also, 21.1% (n=216) of all study participants report having sought medical attention for SARS-CoV-2 symptoms at baseline. Of these, 29.2% were diagnosed with COVID-19 and 6.9% (n=15) were hospitalised for SARS-CoV-2 symptoms. In addition, 77.2% of all study participants report no impact of the pandemic on ability to meet essential/financial needs and a majority (69.9%)

Table 1 Baseline demographics of Stop the Spread Ottawa participants, recruited 14 September 2020 to 28 September 2021

	Stop the Spread Ottawa cohort (n=1026)*
Age, median (IQR)	44 (23)
Sex, female (%)†	688 (67.1)
Ethnicity (%)	
Aboriginal (Inuit, Métis, North American Indian)	10 (1.0)
Arab/West Asian (eg, Armenian, Egyptian, Iranian)	20 (1.9)
Black (eg, African, Haitian, Jamaican, Somali)	9 (0.9)
Chinese	7 (0.7)
Filipino	7 (0.7)
Korean	3 (0.3)
Latin American	9 (0.9)
South Asian	15 (1.5)
South East Asian	9 (0.9)
White	909 (88.6)
Other	26 (2.5)
Born in Canada (%)†	875 (85.3)
Smoking (%)	
Never	744 (72.5)
Former	231 (22.5)
Current	46 (4.5)
Currently employed (%)†	837 (81.6)
Annual household income (%)	
<$C60 000	139 (13.5)
$C60 000 to $C89 999	179 (17.4)
$C90 000 to $C119 999	197 (19.2)
$C120 000 to $C149 999	110 (10.7)
$C150 000 or more	282 (27.5)
Prefer not to answer	81 (7.9)
Do not know	11 (1.1)
Education level (%)	
Less than high school	2 (0.2)
High school	70 (6.8)
College/some university	281 (27.4)
Undergraduate degree	405 (39.5)
Graduate degree	227 (22.1)
Prefer not to answer	18 (1.8)
SARS-CoV-2 vaccination status (%)	
Participants to receive ≥1 SARS-CoV-2 vaccine prior to baseline visit (%)	316 (30.8)
1 dose received prior to baseline	74 (7.2)
2 doses received prior to baseline	242 (23.6)
SARS-CoV-2 vaccine types received prior to baseline visit (%)‡	
≥1 dose BNT162b2 (Pfizer–BioNTech)	204 (19.9)
≥1 dose mRNA-1273 (Moderna)	57 (5.6)
≥1 dose AZD1222 (Oxford–AstraZeneca)	34 (3.3)

*Number to complete baseline questionnaire as of 2 November 2021. Number missing for each variable: ethnicity 2, born in Canada 21, smoking 5, employed 23, income 27, education 23. Number of participants to receive ≥1 SARS-CoV-2 vaccine before baseline: 51. Vaccine types received before baseline: 49. Missing data for any single variable is <5%.
†Binary response.
‡Participants to receive 2 doses of SARS-CoV-2 vaccine prior to baseline may have received different vaccine types.

report no change in employment status in relation to the pandemic.

Strengths and limitations

SSO continues to generate rich research potential, given a majority of participants with pre-vaccine baselines, recruitment of priority populations, and a high level of participant retention and compliance with monthly sampling, driven by active research team communications,

Table 2 Baseline demographics of Surveillance and Convalescent cohorts in the Stop the Spread Ottawa study, recruited 14 September 2020 to 28 September 2021

	Convalescent cohort (n=255)†§	Surveillance cohort (n=771)‡
Age, median (IQR)	47 (26)*	43 (22)
Sex, female (%)¶	156 (61.2)*	534 (69.3)
Ethnicity, white (%)	222 (87.1)	687 (89.1)
Smoking (%)		
Never	189 (74.1)	555 (72.0)
Former	56 (22.0)	175 (22.7)
Current	9 (3.5)	37 (4.8)
Currently employed (%)¶	201 (78.8)	636 (82.5)

*p<0.05 compared with Surveillance cohort by χ^2/Fisher's test (categorical variables) or t-test (continuous variables).
†Number missing for each variable, Convalescent cohort: employed 5, smoking 1.
‡Number missing for each variable, Surveillance cohort: ethnicity 2, smoking 5, employed 18.
§Convalescent: history of SARS-CoV-2 infection by positive PCR test and/or serology.
¶Binary response.

Table 3 Baseline health conditions of Stop the Spread Ottawa participants

Health conditions, frequency (%)†	Participants (n=1026)*
Pregnancy	
Yes	12 (1.2)
No	762 (74.3)
Unknown	237 (23.1)
Not applicable	8 (0.8)
Cancer	25 (2.4)
Diabetes	43 (4.2)
HIV	31 (3.0)
Other immune deficiency	77 (7.5)
Obesity	144 (14.0)
Heart disease	42 (4.1)
Asthma	112 (10.9)
Chronic lung disease	23 (2.2)
Chronic liver disease	14 (1.4)
Chronic kidney disease	12 (1.2)
Chronic haematological disorder	18 (1.8)
Chronic neurological impairment/disease	27 (2.6)
Organ or bone recipient	21 (2.0)
Other health condition(s)	292 (28.5)
Treatment that weakens immune system	67 (6.5)

*Number missing for each variable: pregnancy 7, cancer 14, diabetes 10, HIV 10, other immune deficiency 11, obesity 11, heart disease 11, asthma 17, chronic lung disease 10, chronic liver disease 5, chronic kidney disease 14, chronic haematological disorder 16, chronic neurological impairment/disease 26, organ or bone recipient 20, other health condition(s) 24, treatment that weakens immune system 9. Missing data for any single variable is <5%.
†Binary response, unless stated otherwise.

automated e-reminders, an interactive study website and an innovative antibody results portal. Frequent and comprehensive sampling since October 2020 has yielded tens of thousands of blood and saliva specimens for use in SARS-CoV-2 immune analyses. The extension of follow-up for a subgroup of participants will maximise opportunities to track SARS-CoV-2 immune and vaccine efficacy, detect and characterise emerging variants, and compare subgroup humoral response robustness and persistence.

Demographics of the cohort have limitations in regards to diversity in age, race and income status. The sampling strategy of SSO involved the enrolment of multiple at-risk groups for SARS-CoV-2 exposure (eg, healthcare workers, transportation workers, teachers, immunocompromised patients, residents in retirement homes, elderly). Recruiting a high number of healthcare workers contributed to a larger proportion of females in the study than observed in the total Ottawa population. Participants also tend to be well educated with high total household income which will limit any inferences made in relation to pandemic economic impacts. The study population was not intended to be, and is not, representative of the general population of the Ottawa region in terms of age, sex, and total household income.

Another limitation is vulnerability of clinical data to response bias as self-reported through online study questionnaires. However, participants have frequent opportunities to add free text and explain responses throughout study questionnaires. In this way, study team members can more accurately assess answers to questions which may be broad or subjective. For example, participants are asked to report any history of immune deficiency or use of immunosuppressants. Participants may perceive themselves to have a deficiency which has minimal impact on their immune response. Ongoing data curation procedures include comparisons of selected health conditions with free-text entries on health history and documentation of rationale for any revisions based on the same. We anticipate that all data curation for the 10-month study will be completed 6 months after the last participants have attended the tenth study visit.

We have recruited over 100 participants with immunocompromising health conditions. This group is highly diverse; we acknowledge small numbers (n<50) of participants with specific conditions relative to other international cohorts.[14 15 22 25 26] We will compare serology trends among all participants to report immunocompromising conditions or treatments at baseline and healthy controls without these conditions. To investigate immune response for people with specific immunocompromising health conditions, we will pursue combined analyses with other studies.

Finally, lags in laboratory results are ongoing given the immensity of this project, staffing shortages and a high number of ongoing COVID-19 studies

FUTURE PLANS

Extended follow-up of a subset of participants for SSO launched 30 September 2021. The primary aims of study extension are to (1) evaluate and compare subgroup durability of SARS-CoV-2 immune responses over a lengthened time period, (2) advance ongoing investigations of VOC immunity and vaccine effectiveness, (3) maximise serial blood specimens for biobanking from participants with pre-immune baselines and (4) supply controls for multiple ongoing studies on SARS-CoV-2 vaccine immunogenicity in special populations, including 'PLAN-V: Pregnant and Lactating Individuals & Newborn COVID-19 Vaccination Study' (CIHR), 'Immunogenicity outcomes in people living with HIV following vaccination for COVID-19' (CITF)[99] and 'A prospective multi-site observational study of SARS-CoV-2 vaccination immunogenicity in patients with hematologic malignancies' (CITF, https://omc.ohri.ca/vip), all with planned 6-month and 12-month post-vaccine blood collections. Finally, the extension will augment ongoing efforts to

identify correlates of protection through 'Fine analysis of longitudinal immune responses to SARS-CoV-2 in vaccination: Harnessing the power of 'Stop the Spread Ottawa' to understand immune protection in COVID-19' (CITF).

COLLABORATION

Initial data analyses and publications will be generated by study investigators. The research team is open to potential research collaborations. Researchers interested in collaboration should contact the corresponding author. Access to data and analytical files can only be granted with permission from the approving research ethics committees and data custodians. Analysis of linked data is currently authorised to occur at one location, given ethical considerations. The Ottawa Methods Centre, the University of Ottawa, and the Coronavirus Variants Rapid Response Network (CoVaRR-Net) Biobank are the custodians of SSO biological materials and data.

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Contributors The authors confirm contribution to the paper as per ICMJE criteria: (1) substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; (2) drafting the work or revising it critically for important intellectual content; (3) final approval of the version to be published; (4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. CLC, M-AL, EC, RB, CAB, AMC, JL, MM and RS were involved in the conception and design of the study. CLC and EC drafted the manuscript. EC performed analyses. JL provided statistical support. YG, CA and KN significantly contributed to serological assay development, implementation, planning and analyses. CB, FS, KS, LT, AV and LCW planned and led PBMC and plasma processing efforts. AK and AH significantly contributed to database development and maintenance. LT oversees all CoVaRR-Net biobanking procedures. AMC and M-AL coordinate all laboratory processing of cohort biological specimens. M-AL is responsible for the overall content as the guarantor. All authors critically reviewed and approved the final manuscript.

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REFERENCES

1 Hu B, Guo H, Zhou P, *et al*. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 2021;19:141–54.
2 WHO. Coronavirus (COVID-19) Dashboard, 2022. Available: https://covid19.who.int/ [Accessed January 3, 2022].
3 Khan NA, Al-Thani H, El-Menyar A. The emergence of new SARS-CoV-2 variant (omicron) and increasing calls for COVID-19 vaccine boosters-the debate continues. *Travel Med Infect Dis* 2022;45:102246.
4 Boyton RJ, Altmann DM. The immunology of asymptomatic SARS-CoV-2 infection: what are the key questions? *Nat Rev Immunol* 2021;21:762–8.
5 Mangge H, Kneihsl M, Schnedl W, *et al*. Immune responses against SARS-CoV-2-Questions and experiences. *Biomedicines* 2021;9:1342.
6 Townsend JP, Hassler HB, Wang Z, *et al*. The durability of immunity against reinfection by SARS-CoV-2: a comparative evolutionary study. *Lancet Microbe* 2021;2:e666–75.

7 Shrotri M, van Schalkwyk MCI, Post N, *et al*. T cell response to SARS-CoV-2 infection in humans: a systematic review. *PLoS One* 2021;16:e0245532.
8 Bajaj V, Gadi N, Spihlman AP, *et al*. Aging, immunity, and COVID-19: how age influences the host immune response to coronavirus infections? *Front Physiol* 2020;11:571416.
9 PrabhuDas M, Fuldner R, Farber D, *et al*. Research and resource needs for understanding host immune responses to SARS-CoV-2 and COVID-19 vaccines during aging. *Nat Aging* 2021;1:1073–7.
10 Sakuraba A, Luna A, Micic D. Serologic response to coronavirus disease 2019 (COVID-19) vaccination in patients with immune-mediated inflammatory diseases: a systematic review and meta-analysis. *Gastroenterology* 2022;162:88–108.
11 Kinoshita H, Durkee-Shock J, Jensen-Wachspress M, *et al*. Robust antibody and T cell responses to SARS-CoV-2 in patients with antibody deficiency. *J Clin Immunol* 2021;41:1146–53.
12 Mahil SK, Bechman K, Raharja A, *et al*. The effect of methotrexate and targeted immunosuppression on humoral and cellular immune responses to the COVID-19 vaccine BNT162b2: a cohort study. *Lancet Rheumatol* 2021;3:e627–37.
13 Galmiche S, Luong Nguyen LB, Tartour E, *et al*. Immunological and clinical efficacy of COVID-19 vaccines in immunocompromised populations: a systematic review. *Clin Microbiol Infect* 2022;28:163–77.
14 Banham GD, Godlee A, Faustini SE, *et al*. Hemodialysis patients make long-lived antibodies against SARS-CoV-2 that may be associated with reduced reinfection. *J Am Soc Nephrol* 2021;32:2140–2.
15 Shields AM, Faustini SE, Hill HJ, *et al*. Increased seroprevalence and improved antibody responses following third primary SARS-CoV-2 immunisation: an update from the COV-AD study. *Front Immunol* 2022;13:912571.
16 Yelin D, Wirtheim E, Vetter P, et al. Long-Term consequences of COVID-19: research needs. *Lancet Infect Dis* 2020;20:1115–7 https://doi.org/10.1016/S1473-3099(20)30701-5
17 del Rio C, Collins LF, Malani P. Long-Term health consequences of COVID-19. *JAMA* 2020;324:1723–4 doi:10.1001/jama.2020.19719
18 Carvalho T, Krammer F, Iwasaki A. The first 12 months of COVID-19: a timeline of immunological insights. *Nat Rev Immunol* 2021;21:245–56.
19 Sivan M, Rayner C, Delaney B. Fresh evidence of the scale and scope of long covid. *BMJ* 2021;373:n853.
20 Jena A, Mishra S, Deepak P, *et al*. Response to SARS-CoV-2 vaccination in immune mediated inflammatory diseases: systematic review and meta-analysis. *Autoimmun Rev* 2022;21:102927.
21 Mehrabi Nejad MM, Shobeiri P, Dehghanbanadaki H, et al. Seroconversion following the first, second, and third dose of SARS-CoV-2 vaccines in immunocompromised population; a systematic review and meta-analysis. *Virol J* 2022;19:132 doi:10.1186/s12985-022-01858-3, PMID: 35941646
22 Linardou H, Spanakis N, Koliou G-A, *et al*. Responses to SARS-CoV-2 vaccination in patients with cancer (recover study): a prospective cohort study of the Hellenic Cooperative Oncology Group. *Cancers* 2021;13:4621.
23 Giannella M, Pierrotti LC, Helanterä I, *et al*. SARS-CoV-2 vaccination in solid-organ transplant recipients: what the clinician needs to know. *Transpl Int* 2021;34:1776–88.
24 Rincon-Arevalo H, Choi M, Stefanski A-L, et al. Impaired humoral immunity to SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients and dialysis patients. *Sci Immunol* 2021;6 doi:10.1126/sciimmunol.abj1031, PMID: 34131023
25 Parry H, McIlroy G, Bruton R, *et al*. Impaired neutralisation of SARS-CoV-2 delta variant in vaccinated patients with B cell chronic lymphocytic leukaemia. *J Hematol Oncol* 2022;15:1–12.
26 Shields AM, Venkatachalam S, Shafeek S, et al. SARS-CoV-2 vaccine responses following CD20-depletion treatment in patients with haematological and rheumatological disease: a West Midlands Rresearch Consortium study. *Clin Exp Immunol* 2022;207:3–10 doi:10.1093/cei/uxab018, PMID: 35020852
27 Greenhalgh T, Knight M, A'Court C, *et al*. Management of post-acute covid-19 in primary care. *BMJ* 2020;370:m3026.
28 Skyrud K, Hernæs KH, Telle K, et al. Impacts of COVID-19 on long-term health and health care use. *medRxiv* 2021.
29 Nalbandian A, Sehgal K, Gupta A, *et al*. Post-Acute COVID-19 syndrome. *Nat Med* 2021;27:601–15.
30 Buonfrate D, Piubelli C, Gobbi F, *et al*. Antibody response induced by the BNT162b2 mRNA COVID-19 vaccine in a cohort of health-care workers, with or without prior SARS-CoV-2 infection: a prospective study. *Clin Microbiol Infect* 2021;27:1845–50.
31 Government of Canada. Emerging COVID-19 research gaps and priorities. Available: https://www.canada.ca/en/institutes-health-research/news/2022/03/emerging-covid-19-research-gaps-and-priorities.html [Accessed 4 March 2022].
32 Dupont L, Snell LB, Graham C, *et al*. Neutralizing antibody activity in convalescent sera from infection in humans with SARS-CoV-2 and variants of concern. *Nat Microbiol* 2021;6:1433–42.
33 Jeffery-Smith A, Rowland TAJ, Patel M, *et al*. Reinfection with new variants of SARS-CoV-2 after natural infection: a prospective observational cohort in 13 care homes in England. *Lancet Healthy Longev* 2021;2:e811–9.
34 Tao K, Tzou PL, Nouhin J, *et al*. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet* 2021;22:757–73.
35 Karim SSA, Karim QA. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *Lancet* 2021;398:2126–8.
36 Sotoodeh Ghorbani S, Taherpour N, Bayat S, *et al*. Epidemiologic characteristics of cases with reinfection, recurrence, and hospital readmission due to COVID-19: a systematic review and meta-analysis. *J Med Virol* 2022;94:44–53.
37 Lio D, Scola L, Giarratana RM, *et al*. Sars CoV2 infection _The longevity study perspectives. *Ageing Res Rev* 2021;67:101299.
38 Grzelak L, Velay A, Madec Y, *et al*. Sex differences in the evolution of neutralizing antibodies to severe acute respiratory syndrome coronavirus 2. *J Infect Dis* 2021;224:983–8.
39 Antequera A, Lawson DO, Noorduyn SG, *et al*. Improving social justice in COVID-19 health research: interim guidelines for reporting health equity in observational studies. *Int J Environ Res Public Health* 2021;18:9357.
40 Wu L-X, Wang H, Gou D, *et al*. Clinical significance of the serum IgM and IgG to SARS-CoV-2 in coronavirus disease-2019. *J Clin Lab Anal* 2021;35:e23649.
41 Ma H, Zeng W, He H, *et al*. Serum IgA, IgM, and IgG responses in COVID-19. *Cell Mol Immunol* 2020;17:773–5.
42 Masiá M, Telenti G, Fernández M, *et al*. SARS-CoV-2 seroconversion and viral clearance in patients hospitalized with COVID-19: viral load predicts antibody response. *Open Forum Infect Dis* 2021;8:ofab005.
43 Jackson CB, Farzan M, Chen B, *et al*. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biol* 2022;23:3–20.
44 Dubey A, Choudhary S, Kumar P, *et al*. Emerging SARS-CoV-2 variants: genetic variability and clinical implications. *Curr Microbiol* 2022;79:1–18.
45 Greaney AJ, Starr TN, Gilchuk P, *et al*. Complete mapping of mutations to the SARS-CoV-2 spike receptor-binding domain that escape antibody recognition. *Cell Host Microbe* 2021;29:44–57.
46 Wang P, Nair MS, Liu L, *et al*. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* 2021;593:130–5.
47 Lipsitch M, Krammer F, Regev-Yochay G, *et al*. SARS-CoV-2 breakthrough infections in vaccinated individuals: measurement, causes and impact. *Nat Rev Immunol* 2022;22:57–65.
48 Vanshylla K, Di Cristanziano V, Kleipass F, *et al*. Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. *Cell Host Microbe* 2021;29:917–29.
49 Garcia-Beltran WF, Lam EC, Astudillo MG, *et al*. COVID-19-neutralizing antibodies predict disease severity and survival. *Cell* 2021;184:e11:476–88.
50 Chen W, Zhang J, Qin X, *et al*. SARS-CoV-2 neutralizing antibody levels are correlated with severity of COVID-19 pneumonia. *Biomed Pharmacother* 2020;130:110629.
51 Salazar E, Kuchipudi SV, Christensen PA, *et al*. Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor-binding domain IgG correlate with virus neutralization. *J Clin Invest* 2020;130:6728–38.
52 Dolscheid-Pommerich R, Bartok E, Renn M, *et al*. Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity. *J Med Virol* 2022;94:388–92.
53 Peghin M, Palese A, Venturini M, *et al*. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. *Clin Microbiol Infect* 2021;27:1507–13.
54 Seeßle J, Waterboer T, Hippchen T, et al. Persistent symptoms in adult patients 1 year after coronavirus disease 2019 (COVID-19): a prospective cohort study. *Clin Infect Dis* 2021.
55 Lindemann M, Klisanin V, Thümmler L, *et al*. Humoral and cellular vaccination responses against SARS-CoV-2 in hematopoietic stem cell transplant recipients. *Vaccines* 2021;9:1075.
56 Munro C. Covid-19: 40% of patients with weakened immune system mount lower response to vaccines. *BMJ* 2021;374:n2098.
57 Grinshpun A, Rottenberg Y, Ben-Dov IZ, *et al*. Serologic response to COVID-19 infection and/or vaccine in cancer patients on active treatment. *ESMO Open* 2021;6:100283.
58 Liu W, Fontanet A, Zhang P-H, *et al*. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. *J Infect Dis* 2006;193:792–5.

59 Anderson EM, Goodwin EC, Verma A, *et al*. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell* 2021;184:e10:1858–64.
60 Imai K, Matsuoka M, Tabata S, *et al*. Cross-Reactive humoral immune responses against seasonal human coronaviruses in COVID-19 patients with different disease severities. *Int J Infect Dis* 2021;111:68–75.
61 Lv H, Wu NC, Tsang OT-Y, *et al*. Cross-Reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Rep* 2020;31:107725.
62 Ladner JT, Henson SN, Boyle AS, *et al*. Epitope-resolved profiling of the SARS-CoV-2 antibody response identifies cross-reactivity with endemic human coronaviruses. *Cell Rep Med* 2021;2:100189.
63 Okba NMA, Müller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2–specific antibody responses in coronavirus disease patients. *Emerging Infectious Diseases journal - CDC* 2020;26.
64 Galipeau Y, Siragam V, Laroche G, *et al*. Relative ratios of human seasonal coronavirus antibodies predict the efficiency of cross-neutralization of SARS-CoV-2 spike binding to ACE2. *EBioMedicine* 2021;74:103700.
65 Lustig Y, Sapir E, Regev-Yochay G, *et al*. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir Med* 2021;9:999–1009.
66 Cox RJ, Brokstad KA. Not just antibodies: B cells and T cells mediate immunity to COVID-19. *Nat Rev Immunol* 2020;20:581–2.
67 Bertoletti A, Le Bert N, Qui M, *et al*. SARS-CoV-2-specific T cells in infection and vaccination. *Cell Mol Immunol* 2021;18:2307–12.
68 Geers D, Shamier MC, Bogers S, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. *Sci Immunol* 2021;6 doi:10.1126/sciimmunol.abj1750, PMID: 34035118
69 Grunau B, Asamoah-Boaheng M, Lavoie PM, et al. A higher antibody response is generated with a 6- to 7-week (vs standard) severe acute respiratory syndrome coronavirusHigher Antibody Response Is Generated With a 6- to 7-Week (vs Standard) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) vaccine dosing interval. *Clin Infect Dis* 2022;75:e888–91 doi: 10.1093/cid/ciab938, PMID: 34849655
70 Richardson CD. Heterologous ChAdOx1-nCoV19-BNT162b2 vaccination provides superior immunogenicity against COVID-19. *Lancet Respir Med* 2021;9:1207–9.
71 Powell AA, Power L, Westrop S, *et al*. Real-world data shows increased reactogenicity in adults after heterologous compared to homologous prime-boost COVID-19 vaccination, March–June 2021, England. *Eurosurveillance* 2021;26:2100634.
72 Tregoning JS, Flight KE, Higham SL, *et al*. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nat Rev Immunol* 2021;21:626–36.
73 Scherlinger M, Pijnenburg L, Chatelus E, *et al*. Effect of SARS-CoV-2 vaccination on symptoms from post-acute sequelae of COVID-19: results from the nationwide VAXILONG study. *Vaccines* 2022;10:46.
74 Desimmie BA, Raru YY, Awadh HM, *et al*. Insights into SARS-CoV-2 persistence and its relevance. *Viruses* 2021;13:1025.
75 Ibrahimi N, Delaunay-Moisan A, Hill C, *et al*. Screening for SARS-CoV-2 by RT-PCR: saliva or nasopharyngeal swab? Rapid review and meta-analysis. *PLoS One* 2021;16:e0253007.
76 Moreira VM, Mascarenhas P, Machado V, *et al*. Diagnosis of SARS-Cov-2 infection by RT-PCR using specimens other than naso- and oropharyngeal swabs: a systematic review and meta-analysis. *Diagnostics* 2021;11:363.
77 Warsi I, Khurshid Z, Shazam H, et al. Saliva exhibits high sensitivity and specificity for the detection of SARS-COV-2. *Diseases* 2021;9:38 doi: 10.3390/diseases9020038, PMID: 34065171
78 Morley GL, Taylor S, Jossi S, *et al*. Sensitive detection of SARS-CoV-2-specific antibodies in dried blood spot samples. *Emerg Infect Dis* 2020;26:2970–3.
79 Cholette F, Mesa C, Harris A, *et al*. Dried blood spot specimens for SARS-CoV-2 antibody testing: a multi-site, multi-assay comparison. *PLoS One* 2021;16:e0261003.
80 Colwill K, Galipeau Y, Stuible M, *et al*. A scalable serology solution for profiling humoral immune responses to SARS-CoV-2 infection and vaccination. *Clin Transl Immunology* 2022;11:e1380.
81 Kristiansen PA, Page M, Bernasconi V, *et al*. WHO international standard for anti-SARS-CoV-2 immunoglobulin. *Lancet* 2021;397:1347–8.
82 DNA Genotek - Saliva Microbiome DNA and RNA Collection Kit. Available: https://www.dnagenotek.com/ROW/products/collection-infectious-disease/omnigene-oral/OM-505.html [Accessed 26 Jun 2022].
83 Saliva collection handbook. Salimetrics., Published June 27, 2017. Available: https://salimetrics.com/saliva-collection-handbook/ [Accessed 26 Jun 2022].
84 Salivary detection of COVID-19 | Annals of internal medicine. Available: https://www.acpjournals.org/doi/full/10.7326/M20-4738 [Accessed 26 Jun 2022].
85 Sheikh-Mohamed S, Isho B, Chao GYC, *et al*. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. *Mucosal Immunol* 2022;15:799–808.
86 Bhatt M, Zemek RL, Tang K, *et al*. Antibody seronegativity in COVID-19 RT-PCR-positive children. *Pediatr Infect Dis J* 2022;41:e318–20.
87 Bhatt M, Plint AC, Tang K, *et al*. Household transmission of SARS-CoV-2 from unvaccinated asymptomatic and symptomatic household members with confirmed SARS-CoV-2 infection: an antibody-surveillance study. *CMAJ Open* 2022;10:E357–66.
88 Alibhai K, Fakhraei R, Erwin E, *et al*. Universal SARS-CoV-2 testing among obstetrical patients (UNIVERSE-OB) in Ottawa, Canada. *Journal of Obstetrics and Gynaecology Canada* 2022;44:600.
89 Vinh DC, Gouin J-P, Cruz-Santiago D, *et al*. Real-world serological responses to extended-interval and heterologous COVID-19 mRNA vaccination in frail, older people (uncover): an interim report from a prospective observational cohort study. *Lancet Healthy Longev* 2022;3:e166–75.
90 Anand SS, Arnold C, Bangdiwala S, et al. What factors converged to create a COVID-19 hot-spot? lessons from the South Asian community in Ontario. *medRxiv* 2022 https://doi.org/10.1101/2022.04.01.22273252
91 Government of Canada SC. COVID-19 infection in the Canadian household population, 2022. Available: https://www150.statcan.gc.ca/n1/pub/82-003-x/2022004/article/00003-eng.htm [Accessed 6 Jul 2022].
92 Poulain A, Perret S, Malenfant F, *et al*. Rapid protein production from stable CHO cell pools using plasmid vector and the cumate gene-switch. *J Biotechnol* 2017;255:16–27.
93 Poulain A, Mullick A, Massie B, *et al*. Reducing recombinant protein expression during CHO pool selection enhances frequency of high-producing cells. *J Biotechnol* 2019;296:32–41.
94 Abe KT, Li Z, Samson R, et al. A simple protein-based surrogate neutralization assay for SARS-CoV-2. *JCI Insight* 2020;5.
95 Riley RD, Ensor J, Snell KIE, *et al*. Calculating the sample size required for developing a clinical prediction model. *BMJ* 2020;368:m441.
96 Domingo FR, Waddell LA, Cheung AM, et al. Prevalence of long-term effects in individuals diagnosed with COVID-19: an updated living systematic review. *medRxiv* 2021 https://doi.org/10.1101/2021.06.03.21258317
97 Chen C, Haupert SR, Zimmermann L, et al. Global prevalence of post COVID-19 condition or long COVID: a meta-analysis and systematic review. *J Infect Dis* 2022 doi: 10.1093/infdis/jiac136
98 Gao X, Dong Q. A primer on Bayesian estimation of prevalence of COVID-19 patient outcomes. *JAMIA Open* 2021;3:628–31.
99 Costiniuk CT, Singer J, Langlois M-A, *et al*. CTN 328: immunogenicity outcomes in people living with HIV in Canada following vaccination for COVID-19 (HIV-COV): protocol for an observational cohort study. *BMJ Open* 2021;11:e054208.