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Cohort profile: protocol and baseline survey results for Stop the Spread Ottawa (SSO), a community-based prospective cohort study on antibody responses, antibody neutralization 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 (PASC) 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 November 2, 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 (i.e., 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 (e.g., healthcare, teaching, and transportation). 108 participants (10.5%) reported immunocompromising conditions or treatments at baseline (e.g., 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 maximize opportunities to track SARS-CoV-2 immunity and vaccine efficacy, detect and characterize emerging variants, and compare subgroup humoral and cellular response robustness and persistence.

Strengths and Limitations

- 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 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. 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]. Despite rigorous efforts by the international research community, uncertainty remains as to disparities in 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[10-13] and people with PASC (Post-Acute Sequelae of COVID-19)[14-17]. Subjects with an immunocompromised state may not elicit sufficient humoral and cellular response to vaccination[18-22]. 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[23-25]. There is still need for longitudinal analyses of SARS-CoV-2 immune response in cases of laboratory-confirmed or suspect COVID-19 disease[26-29]; infection and reinfection by emerging variants of concern (VOC)[30-34]; members of high-risk working groups[35-37], and by age, sex, and other characteristics relating to health inequities[38-43].

Most SARS-CoV-2 convalescents develop IgM, IgG, and IgA antibodies targeting the SARS-CoV-2 nucleocapsid (N) or spike (S) proteins between 7 to 14 days post-onset of symptoms[44-45]. Seroconversion is dependent on the virological and clinical profile over time[46]. The receptor binding domain (RBD) of the S protein is the primary target of neutralizing antibodies[47]. During the pandemic, several SARS-CoV-2 variants have become dominant in many countries in different periods[32-33,48]. These variants harbour mutations of the spike protein that can restrict antibody neutralization capacity and hinder vaccine efficacy[49-51]. Neutralizing antibodies comprise a core function of adaptive humoral immune response, predictive of COVID-19 severity and survival[52-53]. Substantial correlations have been found between neutralizing antibody profile and disease severity[54]; anti-S IgG and neutralizing titres[55-56]; anti-S/-N levels and PASC[57-58]; and immunosuppression and anti-S IgG non-response[22,59-62].

Research to date has focused on hospitalized 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[63-64], may confound interpretation of serological analyses. 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- 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]. Additionally, routine testing for SARS-CoV-2 viral RNA will aid surveillance of infections and reinfections, especially during shortages of supplies and staff required for large-scale testing. 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[30,32-33]. There remains an urgent need for large studies monitoring diverse groups over time[3,5,13-14,32]. Priority topics given emerging variants include the transmissibility, pathogenicity, and vaccine resistance of VOC[3,32,48], and the impacts of vaccination and VOC on post-infection symptoms[71-74].

To characterize 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 neutralization 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) Monitoring viral RNA kinetics in saliva in infected acute and convalescent patients;

5) Tracking participant protocol adherence and drop out;

6) Understanding the psychological and socioeconomic impacts of testing positive for COVID-19;

7) Assessing the seroprevalence of other common community-acquired viral respiratory illnesses by risk group; and

8) Comparing COVID-19 specific immunity derived from natural infection and from immunization.

All participants provide monthly collection of blood and saliva samples and complete extensive serial questionnaires, used to track health history (e.g., vaccinations), COVID-19 disease severity, persistent SARS-CoV-2 symptoms, risk factors of exposure, and socioeconomic and psychosocial impacts of the pandemic. This manuscript 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 September 14, 2020 to September 28, 2021. Since October 19, 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. 300 participants will continue to provide bimonthly blood samples for 24 months (i.e., 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 polymerase chain reaction (RT-PCR) or serology test were 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 utilized 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 organizations 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 hematologic 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.

Enrollment closed September 28, 2021. Data collection is ongoing. The expected duration of the study with extension is 60 months. Primary results should be known approximately six months after the last participant has been recruited and completed testing procedures. Conduct of this study was reviewed and approved by The Ottawa Health Science Network Research Ethics Board (2020-0481). All participants provided informed and written consent.

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 November 2, 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 November 2, 1026 participants had completed the baseline questionnaire 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 (5mL) tube with a separator gel with clot activator for serum and two (10mLx2) tubes with EDTA for lymphocyte isolation were drawn. 200 EDTA samples from the initial Surveillance participants (n=100) and Convalescent participants (n=100) were applied to dried blood spot cards and compared to finger-prick dried blood spots to ensure assay function. 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 two months over the next

24 months (Figure 1). During this time, ten (5mLx10) tubes with separator gel with clot activator will be collected every four months. Five (10mLx5) tubes with EDTA will be drawn every four 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 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 were provided with access to video demonstrations through the study website to aid self-collection. 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, extending participants will collect and submit one salivette (Sarstedt, 51.1534) for SARS-CoV-2 antibody testing every four months, starting month 16.

Questionnaires

Electronic study questionnaires are completed at baseline, and at 3- and 10-months post-baseline. 300 participants in extended follow-up complete questionnaires every 6 months (month 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 (e.g., age, ethnic group, gender)
- Health history (e.g., 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.

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. Neutralization 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 characterization 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 neutralizing 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 ELISA assay[78-79]. All viral antigens required for serological assessment and anti-human IgG-HRP fusion secondary antibody are provided by Yves Durocher at the National Research Council of Canada (NRC). Proteins are expressed in a CHO-DXB11-derived clone (CHOBRI/55E1) with yields estimated at 70-100 mg/L[80,81]. 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 humanized antibodies (clones CR3022, CR3018 & 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 inter-plate comparison, background corrected luminescence values are scaled in relation to the calibration curve. By using a large panel of pre-pandemic serum samples, we were able to generate thresholds to determine signal to cut-off ratios. Samples with S/CO values greater than 1.0 are considered positive. While positive and negative calls are interesting in the optics of seroprevalence surveys, quantification of antibodies titers enables more robust analyses. As such, we are establishing 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-neutralizing properties and abundance of anti-SARS-CoV-2 antibodies and correlate these with individual case severity in the Convalescent Cohort. Additionally, we will analyze 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 restriction on replicative SARS-CoV-2, we have implemented a high-throughput protein-based surrogate neutralization assay. In this assay, trimeric spike or RBD is coated in a 384 well plate and blocked. Afterwards, diluted serum samples are applied and incubated. Unbound antibodies are then removed and recombinant biotin-conjugated ACE2 is applied. If neutralizing antibodies are present, they will inhibit Spike (or RBD) ACE2 interaction. A streptavidin-HPR polymer is then applied to detect bound ACE2 and the plate developed using a chemiluminescent substrate. In this assay[82], the signal is inversely correlated to the neutralization efficiency. Results of this assay can be reported in titres using international units (IU/mL) as per World Health Organization (WHO) standards (NIBSC 20/136) or, alternatively, by reporting half maximal inhibitory dilution (ID50).

To maximize 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 (i) SARS-CoV-2 antibody measurements and neutralization efficiency in blood and (ii) viral diagnostics using reverse transcription droplet digital PCR technology (RT-ddPCR). Core architecture includes: i) a robotic liquid handler (Hamilton MicroLab Star) dedicated to isolating serum or plasma from clinical bar-coded tubes and performing an ELISA assay using an integrated plate washer (Biotek 405 TS/LS LHC2) and plate reader (Biotek NEO2); ii) 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); and 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, analyze, and report back on >3,200 blood samples and >2,000 NPS/sputum samples per 5-day work week.

Power calculations & analyses

We have recruited over 250 participants with current or past COVID-19 infection. We anticipate new infections and reinfections, given the spread of emerging variants. The proportion of IgG antibody in convalescent patients with and without comorbidities is estimated to be 70% and 90%, respectively, at month 6 post COVID-19 infection. To achieve our initially foreseen primary outcome, the comparison of proportions of IgG antibody between individuals with history of COVID-19 infection vs. individuals with no history of COVID-19 infection, a sample size of 250 convalescents provides 99% power to detect 20% difference in proportions using a two-sided hypothesis test with a significance level of 0.05. Given our large sample size, we will use a two-sample t-test to detect the difference in IgG antibody response between patients with and without comorbidities. If the data are not normally distributed and the median better represents the center of the distribution, we will use a two-sample Wilcoxon-Mann-Whitney test. The same statistical analyses will be performed for each within-subgroup analysis.

As a secondary outcome, we will also 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. A sample of 400 (200 women and 200 men) will provide 80% power to detect a 10% difference in IgG (80% vs. 90%) with a significance level of 0.05.

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. A total size of 240 convalescents, assuming 30% prevalence of PASC[83], would allow for the inclusion of five predictors (≥ 14.4 EPV) to achieve mean absolute prediction error (MAPE) <0.05 (Lasso)[84]. As prevalence estimates of PASC continue to vacillate, Bayesian methods may be used to derive updated posterior estimates[85]. Multiple imputation will be used to handle missing data, assumed to be MCAR or MAR. Potential over-fitting 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 version 9.4, GraphPad Prism 9.3.1 and R, 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 October 18, 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 November 2, 2021 (n=1026), 67.1% (n=688) are female, the median age is 44 years (IQR: 23, range 18-79, Table 1).

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

	Stop the Spread Ottawa cohort (n=1026)[a]
Age, median (IQR)	44 (23)
Sex, female (%)[b]	688 (67.1)
Ethnicity (%)	
Aboriginal (Inuit, Métis, North American Indian)	10 (1.0)
Arab/West Asian (e.g., Armenian, Egyptian, Iranian)	20 (1.9)
Black (e.g., 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)
	9 (0.9)

South East Asian	909 (88.6)
White (Caucasian)	26 (2.5)
Other	
Born in Canada (%)[b]	875 (85.3)
Smoking (%)	
Never	744 (72.5)
Former	231 (22.5)
Current	46 (4.5)
Currently employed (%)[b]	837 (81.6)
Annual household income (%)	
<$60,000	139 (13.5)
$60,000 - $89,999	179 (17.4)
$90,000 - $119,999	197 (19.2)
$120,000 to $149,999	110 (10.7)
$150,000 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)

[a]Number to complete baseline questionnaire as of November 2, 2021. Number missing for each variable: Ethnicity 2, Born in Canada 21, Smoking 5, Employed 23, Income 27 Education 23

[b]Binary response

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, 38.2% (n=392) report an annual household income ≥$120,000. 61.6% (n=632) have an undergraduate or graduate degree.

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 lab-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%).

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

	Convalescent Cohort (n=255)[a]	**Surveillance Cohort (n=771)[b]**
Age, median (IQR)	47 (26)*	43 (22)
Sex, female (%)[d]	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 (%)[d]	201 (78.8)	636 (82.5)

*P< 0.05 compared to Surveillance Cohort by chi-square/Fisher's test (categorical variables), or t-test (continuous variables)

[a]Number missing for each variable, Convalescent Cohort: Employed 5 Smoking 1

[b]Number missing for each variable, Surveillance Cohort: Ethnicity 2 , Smoking 5 , Employed 18

[c]Convalescent: history of SARS-CoV-2 infection by positive PCR test and/or serology

[d]Binary response

We enrolled priority populations with conditions of clinical significance, including members with self-report of immunocompromising conditions/treatments (e.g., 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.

Table 3: Baseline health conditions of Stop the Spread Ottawa participants

Health conditions, frequency (%) [b]	Participants (n=1026)[a]
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 hematological 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)

[a]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 hematological disorder 16, Chronic neurological impairment/disease 26, Organ or bone recipient 20, Other health condition(s) 24, Treatment that weakens immune system 9

[b]Binary response, unless stated otherwise

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

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 7.2% (n=15) were hospitalized for SARS-CoV-2 symptoms. 77.2% of all study participants report no impact of the pandemic on ability to meet essential/financial needs and a majority (69.9%) 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, 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 maximize opportunities to track SARS-CoV-2 immune and vaccine efficacy, detect and characterize emerging variants, and compare subgroup humoral response robustness and persistence.

Limitations include poor diversity in age, race, and income status. The sampling strategy of Stop the Spread Ottawa involved the enrollment of multiple at-risk groups for SARS-CoV-2 exposure (e.g., healthcare workers, transportation workers, teachers, immunocompromised patients, residents in retirement homes, elderly). Recruiting a high number of healthcare workers, for example, likely 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.

Other limitations include lags in availability of laboratory results given the immensity of this project, staffing shortages, a high number of ongoing COVID-19 studies, and the current use of signal-to-cut-off ratios (S/COs), which allow only for binary assessment of seroconversion. Going forward, binding antigen units (BAUs) will be used to quantify SARS-CoV-2 post-vaccine titres.

Future plans

Extended follow up of a subset of participants for Stop the Spread Ottawa launched September 30, 2021. The primary aims of study extension are to: 1) Evaluate and compare sub-group durability of SARS-CoV-2 immune responses over a lengthened time period; 2) Advance ongoing investigations of variants of concern (VOC) immunity and vaccine effectiveness; 3) Maximize 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)[86], 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- 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 with their expression of interest. 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 and The Coronavirus Variants Rapid Response Network (CoVaRR-Net) Biobank are the custodians of Stop the Spread Ottawa biological materials and data.

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Contributions

CLC and MAL conceived and designed the study. RB, CAB, AMC, JL, MM, and RS participated in the conceptual 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 LW 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 MAL coordinate all laboratory processing of cohort biological specimens. All authors critically reviewed and approved the final manuscript.

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Competing interests: None declared

Ethics approval: Ethics approval was obtained from the The Ottawa Health Science Network Research Ethics Board (Protocol ID Number: 20200481-01H), and access to the data sets was granted by relevant data custodians.

Data sharing statement: Direct access to the data and analytical files is not permitted without the expressed permission of the approving human research ethics committees and data custodians. Researchers interested in collaboration should contact the corresponding author.

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Figure Captions

Figure 1: Procedures for Stop the Spread Ottawa study participants, baseline to Month 10 and extension to Month 34

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

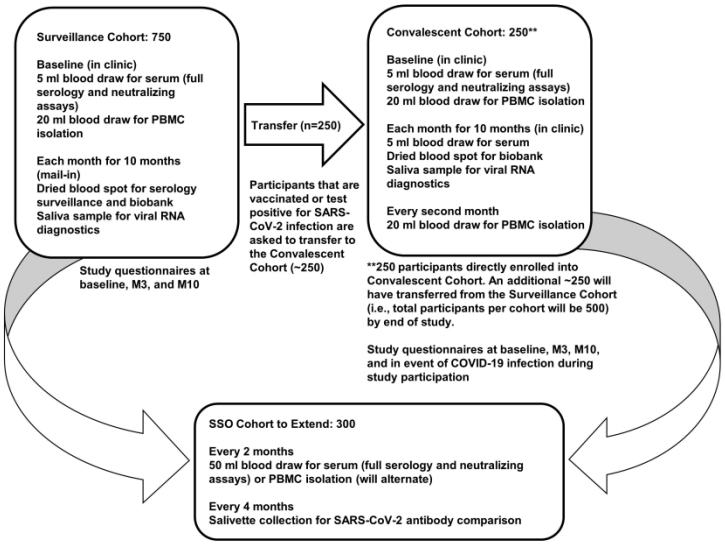

Figure 1: Procedures for Stop the Spread Ottawa study participants, baseline to Month 10 and extension to Month 34

338x190mm (300 x 300 DPI)

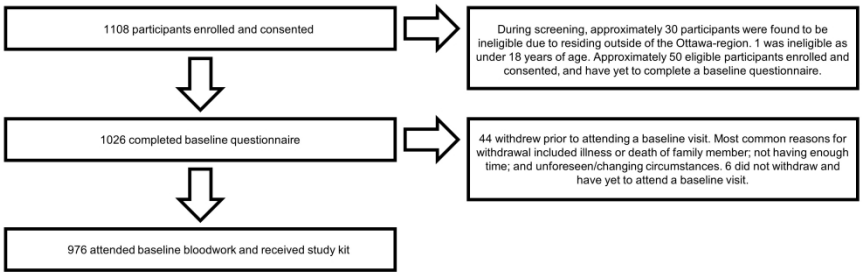

Figure 2. Flow diagram of enrolled participants, as of November 2, 2021

338x190mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7-8, Figure 2
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6, 10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-12
Bias	9	Describe any efforts to address potential sources of bias	16-17
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11, 14-16
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11
		(*b*) Describe any methods used to examine subgroups and interactions	11
		(*c*) Explain how missing data were addressed	11, Tables 1-3
		(*d*) If applicable, explain how loss to follow-up was addressed	5, 11
		(*e*) Describe any sensitivity analyses	n/a[a]
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7, Figure 2

		(b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6-7, Figure 2 Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	12-16, Tables 1-3 Tables 1-3 n/a[b]
Outcome data	15*	Report numbers of outcome events or summary measures over time	n/a[b]
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a[b] n/a[c] n/a[b]
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, 15-16
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

[a]Currently, sensitivity analyses are planned to compare serology results of participants with confirmed history of SARS-CoV-2 infection (by RT-PCR), with results of participants with suspect infection history only. However, this was not decided until after baseline assessment, and thus not included in this submission.

[b]This cohort profile only reports the study protocol and baseline results. No follow-up data is reported.

[c]No continuous variables were categorized.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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

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Cohort profile: Stop the Spread Ottawa (SSO)—a community-based prospective cohort study on antibody responses, antibody neutralization 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 (PASC) 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 November 2, 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 (i.e., 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 (e.g., healthcare, teaching, and transportation). 108 participants (10.5%) reported immunocompromising conditions or treatments at baseline (e.g., 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 maximize opportunities to track SARS-CoV-2 immunity and vaccine efficacy, detect and characterize 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 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]. Subjects 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 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 to 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 neutralizing 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 neutralization capacity and hinder vaccine efficacy [45-47]. Neutralizing 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 neutralizing antibody profile and disease severity [50]; anti-S IgG and neutralizing 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 hospitalized 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- 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 characterize 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 neutralization 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 immunization.

All participants provide monthly collection of blood and saliva samples and complete extensive serial questionnaires, used to track health history (e.g., vaccinations), COVID-19 disease severity,

persistent SARS-CoV-2 symptoms, risk factors of exposure, and socioeconomic and psychosocial impacts of the pandemic. This manuscript 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 September 14, 2020 to September 28, 2021. Since October 19, 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. 300 participants will continue to provide bimonthly blood samples for 24 months (i.e., 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 polymerase chain reaction (RT-PCR) or serology test were 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 utilized 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 organizations 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 hematologic 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.

Enrollment closed September 28, 2021. Data collection is ongoing. The expected duration of the study with extension is 60 months. Primary results should be known approximately six months after the last participant has been recruited and completed testing procedures. Conduct of this study was reviewed and approved by The Ottawa Health Science Network Research Ethics Board (2020-0481). All participants provided informed and written consent.

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 November 2, 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 November 2, 1026 participants had completed the baseline questionnaire 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 (5mL) tube with a separator gel with clot activator for serum and two (10mLx2) 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 two months over the next 24 months (Figure 1). During this time, ten (5mLx10) tubes with separator gel with clot activator will be collected every four months. Five (10mLx5) tubes with EDTA will be drawn every four 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, extending participants will collect and submit one salivette (Sarstedt, Numbrecht, Germany: 51.1534) for SARS-CoV-2 antibody testing every four 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. 300 participants in extended follow-up complete questionnaires every 6 months (month 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 (e.g., age, ethnic group, gender)
- Health history (e.g., 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. Neutralization 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 characterization 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 neutralizing 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 assay [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 fusion secondary antibody are provided by Yves

Durocher at the National Research Council of Canada (NRC). 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 humanized antibodies (clones CR3022, CR3018 & 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 inter-plate comparison, background corrected luminescence values are scaled in relation to the calibration curve. We used 123 serum samples and 320 dried blood spot (DBS) samples representative of pre-pandemic adults to generate thresholds to determine signal to cut-off ratios [80]. Samples with S/CO values greater than 1.0 are considered positive. While positive and negative calls are interesting in the optics of seroprevalence surveys, quantification of antibody titers 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-neutralizing properties and abundance of anti-SARS-CoV-2 antibodies and correlate these with individual case severity in the Convalescent Cohort. Additionally, we will analyze 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 neutralization assay, adapted from Abet et al., 2020 [94]. The protein-based surrogate neutralization was shown to correlate with lentiviral pseudo type-based neutralization 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 neutralizing antibodies will inhibit Spike - 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 [95], the signal is inversely correlated to the

neutralization efficiency. Results of this assay can be reported in titres using international units (IU/mL) as per World Health Organization (WHO) standards (NIBSC 20/136) or, alternatively, by reporting half maximal inhibitory dilution (ID50) or percent inhibition as compared to maximum ACE2 binding.

To maximize 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 (i) SARS-CoV-2 antibody measurements and neutralization efficiency in blood and (ii) viral diagnostics using reverse transcription droplet digital PCR technology (RT-ddPCR). Core architecture includes: i) a robotic liquid handler (Hamilton MicroLab Star) dedicated to isolating serum or plasma from clinical bar-coded collection tubes and performing ELISA assays using an integrated plate washer (Biotek 405 TS/LS LHC2) and plate reader (Biotek Synergy NEO2); ii) 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); and 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 >3,200 blood samples and >2,000 NPS/sputum samples per 5-day work week.

Power calculations & 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 number of required participants with history of COVID infection (n=250), and 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 variants of concern (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 neutralizing antibodies as (IU/mL) for collected serum. Plans to analyze 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 (MAPE) <0.05 (Lasso) [96]. As prevalence estimates of PASC continue to vacillate [97,98] we will use Bayesian updating to estimate the prevalence of PASC using the most current data available [99]. Multiple imputation will be used to handle missing data, assumed to be MCAR or MAR. Potential over-fitting 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 version 9.4, GraphPad Prism 9.3.1 and R, 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 October 18, 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 November 2, 2021 (n=1026), 67.1% (n=688) are female, the median age is 44 years (IQR: 23, range 18-79, Table 1).

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

	Stop the Spread Ottawa cohort (n=1026)[a]
Age, median (IQR)	44 (23)
Sex, female (%)[b]	688 (67.1)
Ethnicity (%)	
Aboriginal (Inuit, Métis, North American Indian)	10 (1.0)
Arab/West Asian (e.g., Armenian, Egyptian, Iranian)	20 (1.9)
Black (e.g., 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 (%)[b]	875 (85.3)
Smoking (%)	

Never	744 (72.5)
Former	231 (22.5)
Current	46 (4.5)
Currently employed (%) [b]	837 (81.6)
Annual household income (%)	
<$60,000	139 (13.5)
$60,000 - $89,999	179 (17.4)
$90,000 - $119,999	197 (19.2)
$120,000 to $149,999	110 (10.7)
$150,000 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 (%)	
Number of participants who received ≥ 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 (%)[c]	
≥ 1 dose BNT162b2 (Pfizer–BioNTech)	204 (19.9)
≥ 1 dose mRNA-1273 (Moderna)	57 (5.6)
≥ 1 dose AZD1222 (Oxford–AstraZeneca)	34 (3.3)

[a]Number to complete baseline questionnaire as of November 2, 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%.

[b]Binary response

[c]Participants to receive 2 doses of SARS-CoV-2 vaccine prior to baseline may have received different vaccine types.

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, 38.2% (n=392) report an annual household income ≥$120,000. 61.6% (n=632) have an undergraduate or graduate degree.

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 lab-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%).

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

	Convalescent Cohort (n=255)[a]	**Surveillance Cohort (n=771)[b]**
Age, median (IQR)	47 (26)*	43 (22)
Sex, female (%)[d]	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 (%)[d]	201 (78.8)	636 (82.5)

*P< 0.05 compared to Surveillance Cohort by chi-square/Fisher's test (categorical variables), or t-test (continuous variables)

[a]Number missing for each variable, Convalescent Cohort: Employed 5 Smoking 1

[b]Number missing for each variable, Surveillance Cohort: Ethnicity 2 , Smoking 5 , Employed 18

[c]Convalescent: history of SARS-CoV-2 infection by positive PCR test and/or serology

[d]Binary response

We enrolled priority populations with conditions of clinical significance, including members with self-report of immunocompromising conditions/treatments (e.g., 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.

Table 3: Baseline health conditions of Stop the Spread Ottawa participants

Health conditions, frequency (%)[b]	Participants (n=1026)[a]
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 hematological 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)

[a]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 hematological 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%

[b]Binary response, unless stated otherwise

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

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 7.2% (n=15) were hospitalized for SARS-CoV-2 symptoms. 77.2% of all study participants report no impact of the pandemic on ability to meet essential/financial needs and a majority (69.9%) 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, 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 maximize opportunities to track SARS-CoV-2 immune and vaccine efficacy, detect and characterize 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 Stop the Spread Ottawa involved the enrollment of multiple at-risk groups for SARS-CoV-2 exposure (e.g., healthcare workers, transportation workers, teachers, immunocompromised patients, residents in retirement homes, elderly). Recruiting a high number of healthcare workers likely 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 same. We anticipate that all data curation for the 10-month study will be completed six 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 Stop the Spread Ottawa launched September 30, 2021. The primary aims of study extension are to: 1) Evaluate and compare sub-group durability of SARS-CoV-2 immune responses over a lengthened time period; 2) Advance ongoing investigations of variants of concern (VOC) immunity and vaccine effectiveness; 3) Maximize 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) [100], 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- 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 and The Coronavirus Variants Rapid Response Network (CoVaRR-Net) Biobank are the custodians of Stop the Spread Ottawa biological materials and data.

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Contributors

CLC and MAL conceived and designed the study. RB, CAB, AMC, JL, MM, and RS participated in the conceptual 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 LW 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 MAL coordinate all laboratory processing of cohort biological specimens. All authors critically reviewed and approved the final manuscript.

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Data availability statement: Direct access to the data and analytical files is not permitted without the expressed permission of the approving human research ethics committees and data custodians. Researchers interested in collaboration should contact the corresponding author.

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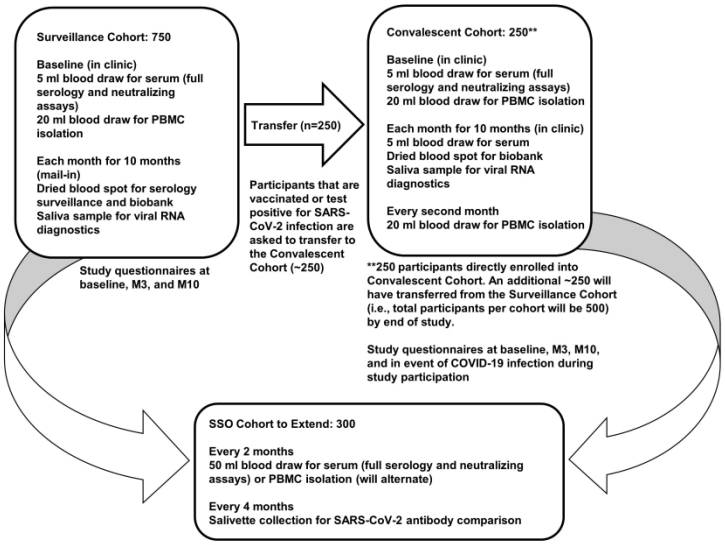
Figure titles

Figure 1: Procedures for Stop the Spread Ottawa study participants, baseline to Month 10 and extension to Month 34

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



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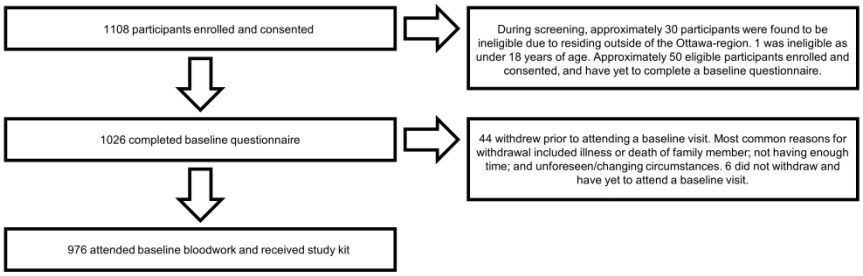

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STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7-8, Figure 2
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6, 10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-12
Bias	9	Describe any efforts to address potential sources of bias	16-17
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11, 14-16
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11
		(*b*) Describe any methods used to examine subgroups and interactions	11
		(*c*) Explain how missing data were addressed	11, Tables 1-3
		(*d*) If applicable, explain how loss to follow-up was addressed	5, 11
		(*e*) Describe any sensitivity analyses	n/a[a]
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7, Figure 2

		(b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6-7, Figure 2 Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	12-16, Tables 1-3 Tables 1-3 n/a[b]
Outcome data	15*	Report numbers of outcome events or summary measures over time	n/a[b]
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a[b] n/a[c] n/a[b]
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, 15-16
Discussion			
Key results	18	Summarise key results with reference to study objectives	12-16
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for exposed and unexposed groups.

[a]Currently, sensitivity analyses are planned to compare serology results of participants with confirmed history of SARS-CoV-2 infection (by RT-PCR), with results of participants with suspect infection history only. However, this was not decided until after baseline assessment, and thus not included in this submission.

[b]This cohort profile only reports the study protocol and baseline results. No follow-up data is reported.

[c]No continuous variables were categorized.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.