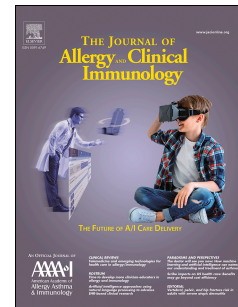


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SARS-CoV-2 specific B- and T-cell immunity in a population-based study of young Swedish adults

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SARS-CoV-2 specific B- and T-cell immunity in a population-based study of young Swedish adults

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Conflict of interest statement

The authors declare no conflict of interests.

ABSTRACT

Background: Young adults are now considered major spreaders of COVID-19 disease. Although most young individuals suffer from mild to moderate disease, there are concerns of long-term adverse health effects. The impact of COVID-19 disease and to which extent population-level immunity against SARS-CoV-2 exist in young adults remain unclear.

Objective: To conduct a population-based study on humoral and cellular immunity to SARS-CoV-2 and explore COVID-19 disease characteristics in young adults.

Methods: We invited participants from the Swedish BAMSE birth cohort (age 24-27 years) to take part in a COVID-19 follow-up. From 980 participants (October 2020-June 2021), we here present data on SARS-CoV-2 RBD-specific IgM, IgA and IgG titres measured by ELISA and on symptoms and epidemiological factors associated with seropositivity. Further, SARS-CoV-2-specific memory B- and T-cell responses were detected for a subpopulation (n=108) by ELISpot and Fluorospot.

Results: 28.4% of subjects were seropositive of which 18.4% were IgM single positive. One in seven seropositive subjects were asymptomatic. Seropositivity associated with use of public transport, but not with sex, asthma, rhinitis, IgE-sensitization, smoking or BMI. In a subset of representative samples, 20.7% and 35.0% had detectable SARS-CoV-2 specific B- and T-cell responses, respectively. B- and T-cell memory responses were clearly associated with seropositivity, but T-cell responses were also detected in 17.2% of seronegative subjects.

Conclusion: Assessment of IgM and T-cell responses may improve population-based estimations of SARS-CoV-2 infection. The pronounced surge of both symptomatic and asymptomatic infections among young adults should imply a continuation of the large-scale vaccination campaign.

CLINICAL IMPLICATIONS

Characterizing COVID-19 disease and measuring IgM and memory T-cell responses in young adults, in addition to IgG, improve estimations of SARS-CoV-2 specific immunity and create awareness of disease spread.

CAPSULE SUMMARY

Multifaceted investigation of SARS-CoV-2 adaptive immunity improves population-based infection estimations. The high incidence of both symptomatic and asymptomatic infections should imply a continuation of the large-scale vaccination campaign

KEY WORDS

SARS-CoV-2, COVID-19 disease, IgM, IgA, IgG, memory T-cells, memory B-cells, young adults, population-based cohort, asthma, risk factors

ABBREVIATIONS

BAMSE: Barn (Children), Allergy Milieu, Stockholm, Epidemiology

COVID-19: Coronavirus disease 2019

IFN: Interferon

IL: Interleukin

PBMC: Peripheral blood mononuclear cells

PCR: Polymerase chain reaction

RBD: Receptor-binding domain

SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2

S1: Spike 1 scanning peptide pool

S N M O: Spike protein (S), nucleoprotein (N), membrane protein (M) and open reading frame (ORF)-3a and ORF-7a proteins (O) peptide pool

INTRODUCTION

The COVID-19 (coronavirus disease 2019) pandemic, caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) poses a severe threat to public health worldwide. In Sweden, more than one million cases and 14,729 deaths have been confirmed up until September 15th 2021. Recently the death rate has decreased, mainly due to the vaccination campaign. Still, the number of patients at the intensive care units remains at relatively high levels, which may reflect an increased general spread of the infection potentially due to more transmissible new variants. Young adults have been considered as major spreaders of disease since last autumn¹. Even though the risk for severe illness and mortality increases with age it is feared that the number of younger adults requiring intensive care may increase. Furthermore, there are also concerns for long-term adverse health effects in infected individuals, including young adults. The impact of COVID-19 disease and to which extent population-level immunity against SARS-CoV-2 exist in young adults are currently unclear, and of particular interest since restrictions and recommendations suggested by WHO to reduce the disease spread was implemented rather late in Sweden and throughout the pandemic, schools have remained open and no formal lockdown was enforced.

SARS-CoV-2 infection elicits robust humoral and cellular adaptive immune responses to 4 major structural proteins encoded in the viral genome, i.e. the spike (S) protein, nucleoprotein (N), membrane (M) protein and envelope (E) protein, and several other minor proteins^{2,3,4}. T-cell responses against SARS-CoV-2 have been described in mild or asymptomatic cases, also without seroconversion^{5,6}. However, the prevalence of SARS-CoV-2 cellular memory responses at the population level is not well-studied, especially among young adults.

We therefore conducted a population-based study on the presence of humoral and cellular immunity to SARS-CoV-2 and explored disease characteristics in young adults (age 24-27 years). We invited participants from the ongoing BAMSE (Swedish abbreviation for Children, Allergy Milieu, Stockholm, Epidemiology) birth cohort study^{7,8} to participate in a COVID-19 follow-up. We here present data on SARS-CoV-2 receptor-binding domain (RBD)-specific IgM, IgA and IgG titres and on symptoms and epidemiological factors associated with seropositivity in all unvaccinated participants (October 2020 to June 2021 (n=980)). In addition, we present data on memory B- and T-cell responses in 108 unvaccinated participants (October 2020 to January 2021).

METHODS

Study population, study design and variables

The study population includes participants from the prospective birth cohort BAMSE, which originally included 4089 newborns between 1994-1996⁸. A 24-year follow-up was conducted 2016-2019 with a total of 2270 participants attending a clinical examination⁷. These participants were invited to an ongoing COVID-19 follow-up for which a phase 1 web-questionnaire was answered August - November 2020 (n=1645). 1453/1645 subjects were invited to phase 2 (start October 6th 2020) which included a clinical examination and new web-questionnaire.

From the phase 1 and phase 2 questionnaires, we extracted self-reported data on COVID-19-related symptoms, SARS-CoV-2 polymerase chain reaction (PCR)- and/or antibody-test, household members with COVID-19, use of public transport, face mask usage, interactions at work, asthma diagnosis and rhinitis⁹. The presence of symptoms was evaluated by the question "Have you had symptoms of suspected COVID-19" and follow-up questions including type and duration of symptoms and being bedbound or hospitalized. Weight and body fat percentage were measured using a Tanita MC 780 body composition monitor and body mass index (BMI) was defined from measured weight and height.

PCR and antibody-tests were reported through the questions "When was the last time you performed a nose- or throat test" with the follow-up question "What was the result?" (PCR) and "When was the last time you performed a blood test" with the follow-up question "What was the result?" (antibody-test). Positive PCR-tests (primarily) and positive antibody-tests (secondary) were used to estimate the time in months that elapsed between presumed COVID-19 disease and the clinical examination.

Data on IgE-sensitization was obtained from the 24-year cohort follow-up. Further details are available in the Methods section in this article's Online Repository.

The present study included all 1028 participants who completed the clinical examination (6th October 2020 – 23rd of June 2021), rendering a study population of 1011 subjects after exclusion of subjects with insufficient sample material (n=17). 980 unvaccinated subjects were included in the main study population and 31 vaccinated subjects in a sub analysis (Fig E1). The study was approved by the Swedish Ethical Review Authority (Ref: 2020-02922). Participants provided written informed consent.

Sample preparation

Venous blood was collected in serum- and sodium heparin tubes (BD Vacutainer) at the clinical examination (Södersjukhuset, Stockholm, Sweden). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples by density centrifugation with Lymphoprep (STEMCELL Technologies). PBMCs were cryopreserved in 90% FBS + 10% DMSO and stored in liquid nitrogen. Serum was stored at -20°C.

Detection of anti-SARS-CoV-2 antibodies

Serum titres of anti-SARS-CoV-2 antibodies were measured for the whole study population (980 unvaccinated subjects and 31 vaccinated subjects). Serum samples were diluted 1:400 and levels of anti-RBD IgM, IgA and IgG antibodies were determined by an in-house ELISA as previously described⁴. In-house standards made by pooled highly positive serum were calibrated by using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC, 20/136). 1 arbitrary unit (AU)/ml of in-house serum standard equal to 7.55 AU/ml IgM, 46.4 AU/ml IgA and 1.03 AU/ml IgG of the WHO international standards, respectively. Cut-off values for antibody positivity were determined based on receiver operating characteristic curves with data from convalescent COVID-19 patients and 108 negative historical control samples (outside BAMSE)⁴. The value with the highest sensitivity and a specificity of at least 99% was selected as the cut-off for each isotype: 14.42 AU/ml for IgM, 2.61 AU/ml for IgA and 25.09 AU/ml for IgG.

Detection of SARS-CoV-2 specific memory B- and T-cell responses

SARS-CoV-2 specific memory B- and T-cell responses were analysed for the first five samples collected per/study week (n=58-60, October 2020 to January 2021) and for an additional 49 subjects from the same time-period to increase group sizes. The number of B-cells secreting SARS-CoV-2 RBD-specific IgG was measured using the Human IgG SARS-CoV-2 RBD ELISpot^{PLUS} kit (Mabtech AB) and the number of S1 and S N M O specific IFN- γ - and IL-2-secreting T-cells were detected using the Human IFN- γ /IL-2 SARS-CoV-2 FluoroSpot^{PLUS} kit (Mabtech AB) (details in this article's Online Repository⁴). The results are expressed as the number of spots per 300 000 cells, after subtracting the background. The cut-off values were set at the highest number of spots detected in 11 pre-pandemic PBMC control samples⁴.

Statistical analyses and graphical presentation

The Chi-Square test, Fisher's exact test or multiple logistic regression were used for categorical data and the Mann-Whitney U-test or the Kruskal-Wallis test was used for continuous data. For multiple comparisons, Dunn's test with Benjamini-Hochberg correction was used. Categorical data are presented as number (n) and percentage (%) while continuous data are presented as median and interquartile range. Spearman rank correlation was used for associations between two continuous variables. Statistical analysis was made with STATA (version 16.0, Stata Corp). P-values <0.05 were considered statistically significant. Graphical presentations were made with GraphPad Prism 9.1.0 and RStudio version 1.3.1093.

RESULTS

The study flow chart is presented in Fig E1. 277 serum samples (28.4%) were positive for at least one SARS-CoV-2 anti-RBD antibody isotype (seropositive), while 700 samples (71.6%) were triple negative (Fig 1, A). IgM-, IgA- and IgG antibodies were detected in 11.8%, 6.8% and 22.4% of samples, respectively (Table I). Individual data from BAMSE participants and pre-pandemic control samples⁴ are displayed in Fig 1, B. Among seropositive samples, 8.7% were triple positive and 27.8% were positive for two isotypes (14.1% IgM⁺IgG⁺, 13.0% IgA⁺IgG⁺, 0.7% IgM⁺IgA⁺). The remaining samples were single positive (18.4% IgM⁺, 1.8% IgA⁺, 43.3% IgG⁺) (Table I, Fig 1, C). Analysis of seroprevalence by month revealed a notable increase in the proportion of IgG and IgA seropositive subjects in January 2021 (33.7% and 15.7%) which is also reflected in a corresponding peak in the overall seropositivity (Fig 1, D). The proportion of IgM seropositive subjects notably increased during April-June 2021 and the overall seropositivity per month increased during the study period (Fig 1, D). Taken together, more than one in four young adult participants were seropositive and assessment of IgM in addition to IgG can better estimate the prevalence of infection.

The seropositive and seronegative groups did not differ significantly by age, sex, asthma diagnosis, rhinitis, IgE-sensitization, smoking, snuff use, BMI or body fat percentage (Table II). A higher proportion of seropositive subjects reported suspected/confirmed COVID-19 disease in the household ($p<0.001$) and small but significant differences were noted for regular use of the public transport system and face mask usage ($p<0.05$), while the frequencies of regular interactions with people at work was rather similar (Table II). The seropositive group more often reported to have taken an PCR- or antibody-test ($p<0.0001$, $p=0.010$), and more often reported positive results from these tests ($p<0.0001$) (Table II). Among seropositive and seronegative subjects, 85.2% and 59.6% reported at least one occasion with possible COVID-19 related-symptoms between February 2020 and the clinical visit ($p<0.0001$, Table III, Fig 2, A). 14.8% of seropositive subjects were asymptomatic during the whole study period. When seropositive subjects were divided into IgG⁺ and IgM⁺IgA⁻IgG⁻, we noticed a higher prevalence of asymptomatic subjects ($p<0.001$) and a shorter duration of symptoms ($p=0.047$) in the latter group (Table E1).

One third of seronegative and seropositive symptomatic subjects reported symptoms more than once, resulting in a total of 314 and 554 occasions with symptoms, respectively. The

seropositive group more often reported to have been bedbound due to symptoms (51.8% vs. 39.3%) ($p<0.001$), while hospitalization was rare in both groups (Table III). Symptom duration did not differ (Table III), while seropositive subjects significantly more often reported reduced taste and smell ($p<0.0001$), fever ($p<0.001$) and pain in bone and muscles ($p=0.001$), but less often sore throat ($p<0.0001$) (Fig 2, B, Table E2). After rating the symptoms, the most notable observation was the high impact on taste and smell in the seropositive group (Fig 2, C, Table E3). The proportion of subjects who reported at least one occasion with symptoms significantly differed among seropositive subjects with different antibody profiles ($p<0.001$) (Fig 2, D). Among seropositive subjects, positive titres of IgA and IgG, but not IgM, were significantly higher in the symptomatic group ($p=0.048$, $p=0.044$) (Fig 2, E). Overall, seropositive subjects reported significantly more often to have been bedbound, substantial effects on taste and smell, and one in seven were asymptomatic.

We then investigated SARS-CoV-2 specific memory B- and T-cell responses by ELISpot and FluoroSpot assays, respectively. We first analysed cellular immunity on a population-based level by restricting the analyses to the first five samples collected during each week of the first four months of the study period. Among these 60 samples, 30.0%, 20.7% and 35.0% were positive for specific antibodies, B- and T-cell responses, respectively. The proportions of subjects with positive memory T-cell responses were 66.7% and 21.4% among seropositive and seronegative subjects respectively, and for memory B-cell responses, the corresponding numbers were 58.8% and 4.9%.

To increase sample size, we included additional samples from seropositive ($n=31$) and seronegative ($n=17$) subjects rendering a total of 107 and 108 samples analysed for B- and T-cell responses respectively. The IgG⁺ group had a significantly higher proportion of subjects with positive memory B-cell response (68.1%), a higher median number of IgG-producing B-cells (50 spot-forming cells/300000 PBMC) and a higher proportion of individuals with at least one S1- or SNMO-specific IFN- γ and/or IL-2 T-cell responses (55.1%) as compared to the IgM⁺IgA⁻IgG⁻ group (positive B-cells: 0.0%, positive T-cells: 42.9%) and the seronegative group (positive B-cells: 3.7%, positive T-cells: 17.3%) ($p<0.05$ for all parameters) (Table IV, Fig 3, A). Further investigation of S1- or SNMO-specific IFN- γ T-cell responses revealed similar patterns regarding both the proportion of positive subjects and the median number of spot-forming cells ($p<0.05$ for all parameters) (Table IV, Fig 3, B, C). Of note, IgM⁺IgA⁻IgG⁻ and seronegative subjects that were positive for memory T-cell responses

mainly produced IL-2 (Table IV, Fig 3, B, C). The number of IgG-producing B-cells and the proportion of subjects with a detectable memory B-cell response were higher in the symptomatic group, although not statistically significant (Fig 3, D). T-cell responses did not associate with the presence of symptoms (Fig 3, E, similar results obtained for SNMO are not shown).

Ten subjects had positive memory B-cell and/or T-cell responses within the seronegative group. Among these, two had a positive B-cell response of which one was borderline positive for all three antibody isotypes, T-cell positive, reported a positive PCR-test and suspected COVID-19 disease in the household. Among the remaining eight subjects with T-cell responses, seven had IgG and/or IgM titres exceeding the median value in the seronegative group, none reported a positive PCR-test, three reported suspected/confirmed COVID-19 in the household and five reported a history of COVID-19-related symptoms (Table E4). Taken together, SARS-CoV-2 B- and T-cell memory responses were detected in 68.1% and 55.1% of seropositive individuals, respectively. A memory B-cell response was almost exclusively associated with seropositivity while memory T-cell responses were found in one in five seronegative subjects.

Data on all three “arms” of adaptive immunity (virus-specific antibodies, memory B- and T-cells) were available for 104 subjects. Among these, 60.6% of subjects were positive for at least one investigated immune parameter with highly variable patterns, suggesting a high heterogeneity of adaptive immune responses in young adults (Fig E2).

We correlated measured antibody titres and cellular responses at the time of the clinical examination with the time in months that elapsed between presumed COVID-19 disease based on primarily self-reported positive PCR-test ($n=99$ for antibody-titres and $n=12-13$ for cellular responses) and secondarily self-reported positive antibody-test (additional $n=56$ for antibody-titres and $n=6-8$ for cellular responses). Of note, the estimation based on self-reported positive antibody-tests gives a minimum number of elapsed months. IgM, IgA and IgG titres negatively correlated with time elapsed since presumed disease regardless of if the estimation was solely based on positive PCR-tests (Fig 4, A, IgM: $r=-0.31$, $p=0.002$, IgA: $r=-0.42$, $p<0.0001$, IgG: $r=-0.36$, $p<0.001$) and when combining positive PCR- and antibody-tests (Fig E3, A). The number of IgG-producing B-cells positively correlated with time elapsed since presumed disease (Fig E3, B, $p=0.007$) while T-cell responses did not show

306 clear associations (Fig E3, C, D), however all investigated cellular responses, except S1-
307 specific IL-2, were measurable 6 months after presumed disease.
308 Finally, we investigated antibody-levels among 31 subjects who had received the first dose of
309 a SARS-CoV-2 vaccine (Astra Zeneca n=6, Moderna n=1, Pfizer n=24) of whom 18/31 had
310 also received the second vaccine dose. Few vaccinated subjects were IgM⁺ while IgA and
311 IgG-responses were robust after the second dose (Fig 4, B).

DISCUSSION

In this population-based study, we show that more than one in four young adults were SARS-CoV-2 seropositive and with a significant proportion of subjects being IgM single positive. Seropositivity was associated with COVID-19 disease in the household and weakly with use of the public transport system. Memory B-cell and T-cell responses were observed in the majority of IgG⁺ subjects, but T-cell responses were also observed in one in five seronegative subjects.

The proportion of seropositive subjects remained relatively stable October-December 2020, increased to over 30% in January 2021, declined during early spring and increased again to above 40% close to the summer of 2021. The latter increase was mostly due to an increased number of IgM-positive subjects, suggesting that we detected more cases with recent or ongoing infections during the late spring. These subjects may have been asymptomatic at the time of the clinical examination considering that the participants were asked not to attend if symptomatic. If this reflects a higher degree of asymptomatic infections due to virus variants during this time-period or less cautiousness is not known. Data from the Swedish Public Health Agency collected late May-early June 2021 report the national IgG seroprevalence to be 52% for subjects aged 20-64 years, however this heterogeneous age group also includes a large proportion of vaccinated subjects¹⁰. Our data suggest that the spread of infection was, and still is, substantial in young adults.

Our results indicate that measuring IgM likely captures a significant proportion of recent or ongoing SARS-CoV-2 infections. However, anti-RBD IgM was less frequently observed in acute and convalescent COVID-19 patients, as compared to IgG¹¹, and studies of anti-SARS-CoV-2 IgM indicate the longevity to be four months or less among symptomatic individuals^{4,12}. Nevertheless, our result suggests that using only IgG as a readout underestimates the prevalence of SARS-CoV-2 infection in the population, especially when the epidemic is resurgent.

In this cohort, seropositive subjects had more frequently used the public transport system and more often had household members with suspected or confirmed COVID-19 disease as compared to seronegative subjects. These associations have been described by others^{13,14}. Notably, even though the public transport area is likely to contribute to viral spreading¹⁵, we cannot conclude that participants were infected while using the public transport system since

we do not have detailed data on viral spread. Also, we adjusted for potential confounders in the regression model (Table II) but cannot exclude residual confounding effects (e.g., contact with confirmed cases at work or at social events). In Sweden, public face mask usage was not recommended until the beginning of 2021. We found a weak but significant inverse association between face mask usage and seropositivity, while other observational studies have found no association^{16,17}. Of note, our study was not designed to evaluate the effect of face mask usage or use of public transport. Seroprevalence did not associate with asthma diagnosis, similar to other reports¹⁸. We observed mostly mild COVID-19 disease in young adults defined as not requiring hospital care and less than one in ten seropositive subjects experienced symptoms for >4 weeks. A high degree of lost taste and/or smell was the most evident symptom among seropositive subjects, in accordance with other data¹⁹.

SARS-CoV-2-specific cellular responses develop in most subjects with confirmed infection^{3,4}. We observed memory B-cell and T-cell responses in 68.1% and 55.1% of IgG⁺ subjects, respectively. This is somewhat different from other studies showing that memory B-cell and T-cell responses occur in more or less all subjects with confirmed COVID-19 infection^{3,4,11,20,21}. Of note, our participants are younger than included subjects in the above-mentioned studies, had mild disease and were sampled in a population-based manner. We have not investigated CD4 and CD8 T-cell responses separately, analysed additional modes of activation besides cytokine expression or performed intracellular cytokine staining.

One in ten IgG⁺ subjects and one in three IgM single positive subjects reported to have been asymptomatic throughout the study and these results suggest that undetected infection may be a major public health issue in young adults. Data regarding associations between the magnitude and duration of immune responses and symptom severity during COVID-19 disease are conflicting^{11,22–24}. A connection between mild symptoms and lower T-cell responses has been suggested²⁰, while other studies have failed to find this association^{4,5,22}, and we and others show that specific T-cell responses are similar in symptomatic and asymptomatic subjects^{25,26}. Furthermore, IgG-titres were significantly higher and the median number of memory B-cells tended to be higher in symptomatic subjects, also reported elsewhere²². Others show that individuals with mild or severe COVID-19 disease mount comparable and equally durable memory B-cell responses²⁷.

We found that almost one in five seronegative subjects tested positive in at least one T-cell assay. Studies report that 40-60% of non-exposed pre-pandemic subjects had SARS-CoV-2 reactive T-cells, most likely due to cross-reactivity²⁰, and that cross-reactive T-cells exist to a

higher extent in young adults²⁸. However, the protective effect of such cross-reactive memory T-cells to newly encountered infections like SARS-CoV-2 is unknown. Our data show that most seronegative subjects with positive T-cell responses have higher titres (though still below the cut-offs) of anti-SARS-CoV-2 antibodies than seronegative subjects without T-cell responses, suggesting that some subjects mount low antibody responses which are not detected by available assays. Since the full dynamics of the SARS-CoV-2 immune response is not understood, a missing overlap between seropositivity and cellular memory responses may be due to sampling timing. One recent review highlights an urgent need for the development of SARS-CoV-2 T-cell assays²⁹ and Sweden is now one of the first countries to offer T-cell tests to the community. Still, it is unclear how broad scale T-cell testing can increase the understanding of population immunity, guide restrictions and behavioural recommendations or evaluate the vaccine response. Finally, the question on which T-cell assay would be the most appropriate to choose for broad scale testing of a variety of age groups remains to be addressed.

Several studies have assessed the maintenance of protective levels of antibodies and the durability of cellular responses to SARS-CoV-2^{3,4,20}, partly due to concerns regarding re-infections. Some studies suggest that antibody-titres wane within a few months^{22,30}, while other show that titres are maintained at a relatively high level for at least 6 months after onset of symptoms^{3,4,31}. We used self-reported PCR- and antibody-tests to make a presumption on when participants had COVID-19 disease. Our results suggest that even though the levels of antibodies declined, they were measurable up until 8 months after infection, and in some cases longer for IgG. For the 14 subjects who were seronegative at the clinical examination but reported a previous positive PCR-test, seven subjects had ≥ 6 months, while one subject had < 1 month, that elapsed between presumed disease and the clinical examination, and their IgG- or IgM-levels were measurable but below the assay cut-offs. Four subjects had 3-5 months in between presumed disease and clinical examination. Our data indicate that antibody-titres decline more rapidly in some individuals, which may be worth to notice in the light of vaccination.

The strengths of this study are the population-based approach targeting young adults and the consecutive follow-up over an extended time-period. We measured IgM and IgA in addition to IgG, as well as specific B- and T-cell responses. We included only unvaccinated subjects in our main analyses to gain knowledge about virus spread, risk factors and the level of adaptive

immunity among young adults. Vaccination campaigns for our target age group are ongoing in Sweden, and our pilot analyses of 31 vaccinated participants show adequate serological responses. Limitations of this study include that we were only able to study mild COVID-19 disease, lack information of exact disease dates for some subjects and that we investigated cellular memory responses in a relatively small group.

In summary, this is to our knowledge the first population-based cohort study to investigate different arms of adaptive immunity against SARS-CoV-2 among young adults. Our study provides new information regarding COVID-19 disease characteristics and protective immunity among young, non-hospitalized adults.

AUTHOR CONTRIBUTIONS

Conceptualization: SB, AG, AB, IK, LH, EM, QPH, the BAMSE COVID-19 Study Group

Methodology: LD, FZ, NS

Data curation: SB, LD, FZ, SE, LS, YW, HW

Investigation: SB, LD, FZ, HW, HM, LH, EM, QPH

Formal Analysis: SB, NA

Visualization: SB, LD, FZ, HW

Interpretation: SB, LD, FZ, HW, HM, LH, EM, QPH

Resources: JA

Funding acquisition: EM, QPH

Project administration: AG, AB, IK, EM, QPH

Supervision: LH, EM, QPH

Writing - original draft: SB, EM, QPH

Writing - review and editing: All authors

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FIGURE LEGENDS

FIG 1. SARS-CoV-2 anti-RBD IgM, IgA and IgG prevalence and titres

(A) The proportions of SARS-CoV-2 seropositive and seronegative subjects. (B) The titres of SARS-CoV-2 anti-RBD IgM, IgA and IgG in samples from historical controls collected before the pandemic (n=108) and BAMSE participants (n=980) expressed in arbitrary units and prevalence of IgM, IgA and IgG displayed as pie-charts for the BAMSE participants. (C) Venn-diagram showing the overlap of IgM-, IgA- and IgG seropositivity. (D) The percentages of IgM-, IgA- and IgG-positive subjects at each month of the study period. χ^2 -test was used for statistical analysis. Red lines represent median values, green lines represent assay cut off values.

FIG 2. Symptom prevalence and characteristics

(A) The proportions of SARS-CoV-2 seropositive and seronegative subjects who reported at least one occasion with symptoms throughout the study. (B, C) The proportions of SARS-CoV-2 seropositive and seronegative subjects reporting to have experienced the indicated symptoms (B) and grading of symptom severity (C). (D) The proportion of seropositive subjects who reported at least one occasion with symptoms throughout the study stratified by antibody profiles. (E) The titres of IgM, IgA and IgG expressed in arbitrary units in symptomatic and asymptomatic SARS-CoV-2 seropositive subjects. Fisher's exact test, χ^2 -test, or Mann-Whitney U-test were used for statistical analysis. Red lines represent median values, green lines represent assay cut off values.

FIG 3. SARS-CoV-2 anti-RBD memory B-cell responses and anti-S1 and anti-SNMO memory T-cell responses

(A-C) The numbers of RBD-specific IgG⁺ B-cells (A), S1-specific IFN- γ ⁺, IL-2⁺ and IFN- γ ⁺IL-2⁺ T-cells (B) and SNMO-specific IFN- γ ⁺, IL-2⁺ and IFN- γ ⁺IL-2⁺ T-cells (C) per 300000 PBMC within the SARS-CoV-2 IgG⁺, IgM⁺IgA⁻IgG⁻ and seronegative groups. (D-E) The numbers of RBD-specific IgG⁺ B-cells (D) and S1-specific IFN- γ ⁺, IL-2⁺ and IFN- γ ⁺IL-2⁺ T-cells (E). (F) The percentages of B-cell- and T-cell positive subjects within the SARS-CoV-2 seropositive symptomatic and asymptomatic groups. Dunn's test, Mann-Whitney U-test, or Fisher's exact test were used for statistical analysis. Red lines represent median values, green lines represent assay cut off values.

571

572 **FIG 4. Correlation of antibody titres and elapsed time since presumed COVID-19**
573 **disease or SARS-CoV-2 vaccination**

574 **(A)** Correlation between the number of months that elapsed between self-reported positive
575 PCR-test to clinical examination and the titres of anti-SARS-CoV-2 IgM, IgA or IgG. **(B)**
576 Correlation between the number of days that elapsed since the date of receiving dose 1 of a
577 SARS-CoV-2 vaccine and the titres of anti-SARS-CoV-2 IgM, IgA or IgG (open symbols: 1
578 dose received, half-filled symbols: 2 doses received). Spearman rank correlation was used for
579 statistical analysis. Green lines represent assay cut off values.

Table I. Proportions of SARS-CoV-2 anti-RBD IgM, IgA and IgG-positive and negative subjects

		n (%)	Median antibody titre, AU/mL (IQR)
Anti-RBD antibody	Positive	277 (28.4)	NA
	Negative	700 (71.6)	
Anti-RBD IgM	Positive	116 (11.8)	21.7 (14.5-568.4)
	Negative	864 (88.2)	3.1 (0.0-14.4)
Anti-RBD IgA	Positive	67 (6.8)	5.3 (2.7-429.4)
	Negative	913 (93.2)	0.0 (0.0-2.6)
Anti-RBD IgG	Positive	219 (22.4)	60.3 (25.9-1407.4)
	Negative	758 (77.6)	3.7 (0.0-24.8)
IgG⁺IgM⁺IgA⁺	Positive/total	24/277 (8.7)	NA
IgG⁺IgM⁺IgA⁻	Positive/total	39/277 (14.1)	NA
IgG⁺IgM⁻IgA⁺	Positive/total	36/277 (13.0)	NA
IgG⁻IgM⁺IgA⁺	Positive/total	2/277 (0.7)	NA
IgG⁻IgM⁺IgA⁻	Positive/total	51/277 (18.4)	NA
IgG⁻IgM⁻IgA⁺	Positive/total	5/277 (1.8)	NA
IgG⁺IgM⁻IgA⁻	Positive/total	120/277 (43.3)	NA

NA: not applicable

IQR: Interquartile range

AU: Arbitrary unit

Table II. Background characteristics of SARS-CoV-2 seropositive and seronegative subjects

		Seropositive		Seronegative		p-value ^a
		Median	IQR	Median	IQR	
Age in years		25.8	23.9-27.3	25.8	24.0-27.2	ns
BMI		22.9	15.2-48.3	22.5	14.8-59.9	ns
Body fat percentage		23.9	18.9-28.4	22.8	17.7-27.2	ns
		n	%	n	%	p-value ^b
Sex	Female	177	63.9	428	61.2	ns
	Male	100	36.1	271	38.8	
Asthma	Yes	37	13.4	100	14.3	ns
	No	240	86.6	600	85.7	
Rhinitis	Yes	45	16.2	103	14.8	ns
	No	232	83.8	595	85.2	
IgE-sensitization	Yes	111	40.2	313	45.0	ns
	No	165	59.8	382	55.0	
Smoking	Yes	39	14.2	78	11.2	ns
	No	236	85.8	619	88.8	
Snuff	Yes	67	24.4	157	22.5	ns
	No	208	75.6	541	77.5	
BMI categories ^c	Overweight/obese	75	28.2	157	23.1	ns
	No overweight	191	71.8	526	76.9	
COVID-19 in household ^d						
Feb 2020-Jul 2020	Yes/No/Not sure	94/167/14	34.2/60.7/5.1	125/519/56	17.9/74.1/8.0	<0.0001
Aug 2020-June 2021	Yes/No/Not sure	84/181/8	30.8/66.3/2.9	103/578/17	14.8/82.8/2.4	<0.0001
Do you use face mask	Yes, often	35	12.7	102	14.6	0.031
	Yes, sometimes	162	57.9	346	49.6	
	No	78	28.4	249	35.7	
Regular interactions with people at work						
Feb 2020-Jul 2020	Yes/No	204/73	73.6/26.4	497/203	71.0/29.0	ns
Aug 2020-June 2021	Yes/No	211/64	76.7/23.3	491/207	70.3/29.7	0.045
Regular use of public transport to work						
Feb 2020-Jul 2020	Yes/No	90/187	32.5/67.5	176/524	25.1/74.9	0.020 ^e
Aug 2020-June 2021	Yes/No	91/184	33.1/66.9	174/523	25.0/75.0	0.010 ^f
PCR-test performed prior to clinical examination (self-reported)	No	112	40.4	378	54.0	<0.0001
	Yes	165	59.6	322	46.0	<0.0001
	Positive	86	52.1	14	4.4	
	Negative	79	47.9	308	95.6	

Antibody-test performed prior to clinical examination (self-reported)	No	171	61.7	492	70.3	0.010
	Yes	106	38.3	208	29.7	
	<i>Positive</i>	59	55.7	16	7.7	<i><0.0001</i>
	<i>Negative</i>	47	44.3	192	92.3	

^aMann-Whitney U-test

^bChi-square test or Fisher's exact test

^cOverweight/obese defined as BMI ≥ 25 kg/m², no overweight defined as BMI < 25 kg/m²

^dSuspected or confirmed

^eCorresponding odds ratio 1.5 [95% confidence interval 1.1-2.0] for regular use of public transport to work during phase 1 after adjustment for age, sex, smoking, having regular interactions at work and occupation.

^fCorresponding odds ratio 1.4 [95% confidence interval 1.0-1.9] for regular use of public transport to work during phase 2 after adjustment for age, sex, smoking, face mask usage, having regular interactions at work and occupation.

IQR: Interquartile range

BMI: Body mass index

Table III. Self-reported symptom and disease characteristics reported by SARS-CoV-2 seropositive and seronegative subjects

		Seropositive		Seronegative		p-value ^a
		n	%	n	%	
Any COVID-19 related symptoms between February 2020 and June 2021	Yes	236	85.2	415	59.6	<0.0001
	No	41	14.8	281	40.4	
Disease symptoms experienced more than once between February 2020 and June 2021	>Once	78	33.1	139	33.5	ns
	Once	158	66.9	276	66.5	
Total number of occasions with symptoms		314		554		NA
Bedbound during disease	Yes, ≥ 7 days	25	8.0	26	4.7	0.001
	Yes, <7 days	137	43.8	191	34.6	
	No	151	48.2	335	60.7	
Hospitalization	Yes	3	1.0	3	0.5	ns
	No	310	99.0	549	99.5	
When were symptoms experienced?	Feb-20	13	4.2	52	9.4	NA
	Mar-20	56	18.0	124	22.5	
	Apr-20	38	12.2	51	9.2	
	May-20	24	7.7	9	1.6	
	Jun-20	16	5.1	14	2.5	
	Jul-20	7	2.3	22	4.0	
	Aug-20	7	2.3	41	7.4	
	Sep-20	19	6.1	56	10.1	
	Oct-20	30	9.6	52	9.4	
	Nov-20	31	10.0	45	8.2	
	Dec-20	23	7.4	31	5.6	
	Jan-21	11	3.5	19	3.4	
	Feb-21	7	2.3	14	2.5	
	Mar-21	17	5.5	12	2.2	
	Apr-21	9	2.9	8	1.5	
	May-21	3	1.0	2	0.4	
Duration of symptoms	<1 week	82	26.2	160	28.9	ns
	1 week	89	28.4	164	29.7	
	2 weeks	84	26.8	138	25.0	
	3 weeks	27	8.6	44	8.0	
	4 weeks	11	3.5	19	3.4	
	5-7 weeks	8	2.6	9	1.6	
	≥ 8 weeks	12	3.8	19	3.4	

^aChi-Square-test or Fisher's exact test

NA: not applicable

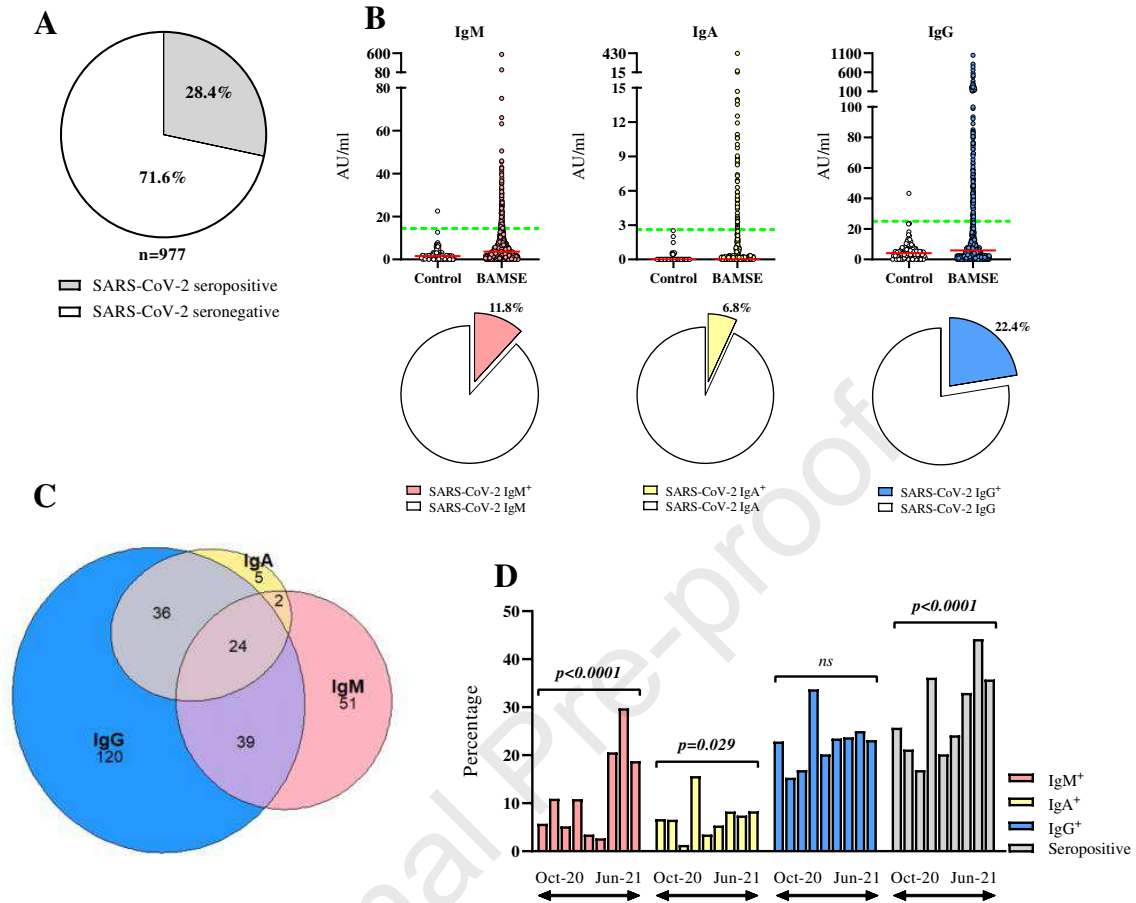
Table IV. SARS-Cov-2 memory B-cell and T-cell responses among SARS-CoV-2 IgG⁺, IgM⁺IgA⁻IgG⁻ and seronegative subjects

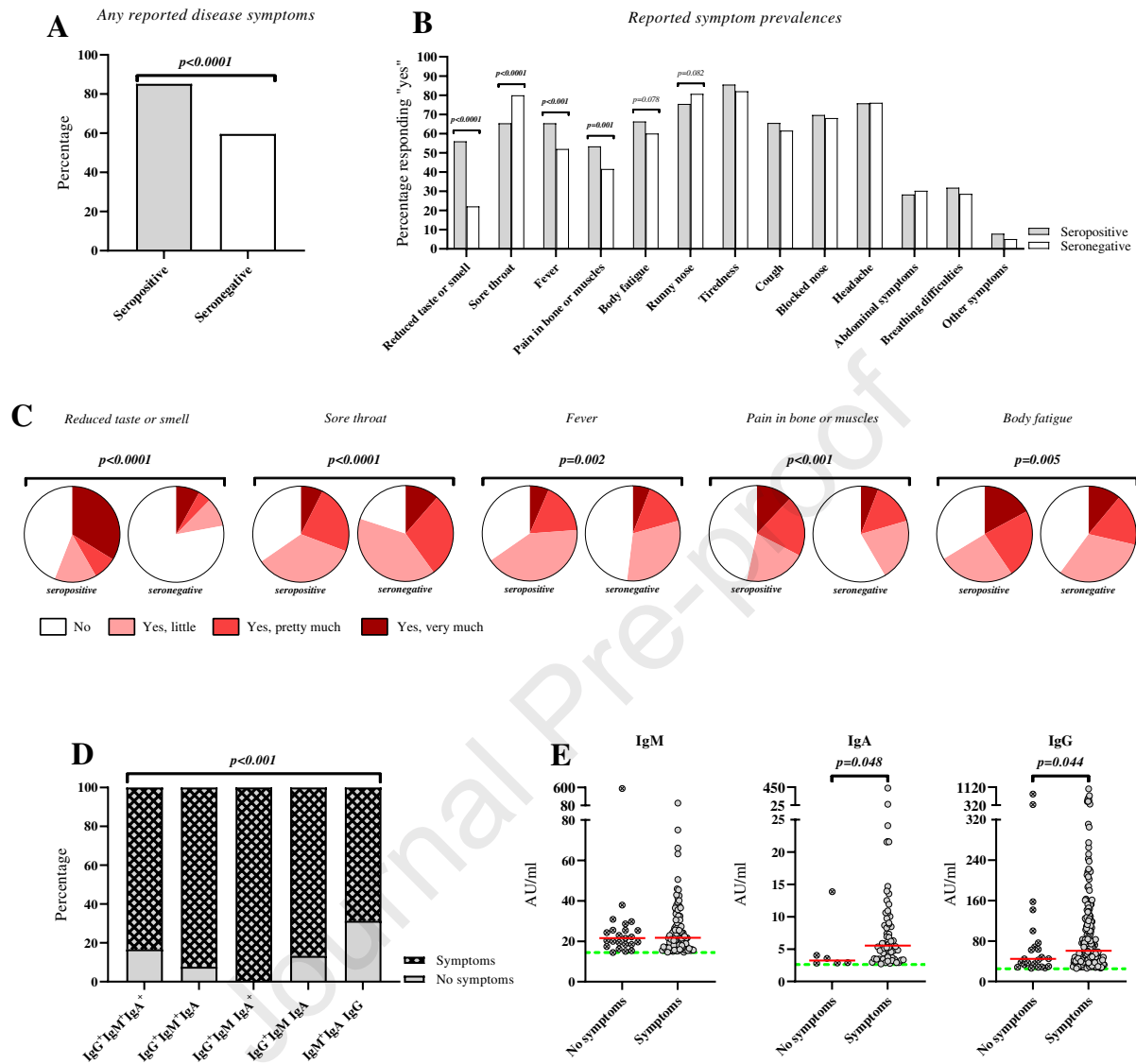
	IgG ⁺		IgM ⁺ IgA ⁻ IgG ⁻		Seronegative		p-value ^b
	n (%)	Median (IQR) ^a	n (%)	Median (IQR) ^a	n (%)	Median (IQR) ^a	
B-cell response	32/47 (68.1)	50 (4-109)	0/6 (0.0)	0 (0-3)	2/54 (3.7)	0 (0-2)	<0.0001/<0.001
T-cell response (any)	27/49 (55.1)	NA	3/7 (42.9)	NA	9/52 (17.3)	NA	<0.0001/NA
T-cell response S1	21/49 (42.9)	NA	3/7 (42.9)	NA	5/52 (9.6)	NA	0.001/NA
IFN- γ	16/49 (32.7)	3 (0-11)	1/7 (14.3)	2 (2-5)	1/52 (1.9)	0 (0-1)	<0.0001/<0.001
IL-2	14/49 (28.6)	1 (0-11)	3/7 (42.9)	7 (0-30)	4/52 (7.7)	0 (0-0.5)	0.008/0.010
IFN- γ /IL-2	7/49 (14.3)	1 (0-3)	1/7 (14.3)	2 (0-3)	0/52 (0.0)	0 (0-0)	0.018/0.0001
T-cell response SNMO	26/49 (53.1)	NA	3/7 (42.9)	NA	7/52 (13.5)	NA	<0.0001/NA
IFN- γ	23/49 (44.9)	9 (2-26)	2/7 (28.6)	2 (2-15)	1/52 (1.9)	0 (0-2)	<0.0001/<0.001
IL-2	17/49 (34.7)	1 (0-14)	3/7 (42.9)	6 (1-20)	6/52 (11.5)	0 (0-4.5)	0.012/<0.028
IFN- γ /IL-2	17/49 (34.7)	3 (0-8)	1/7 (14.2)	1 (0-5)	1/52 (1.9)	0 (0-1)	<0.0001/<0.001

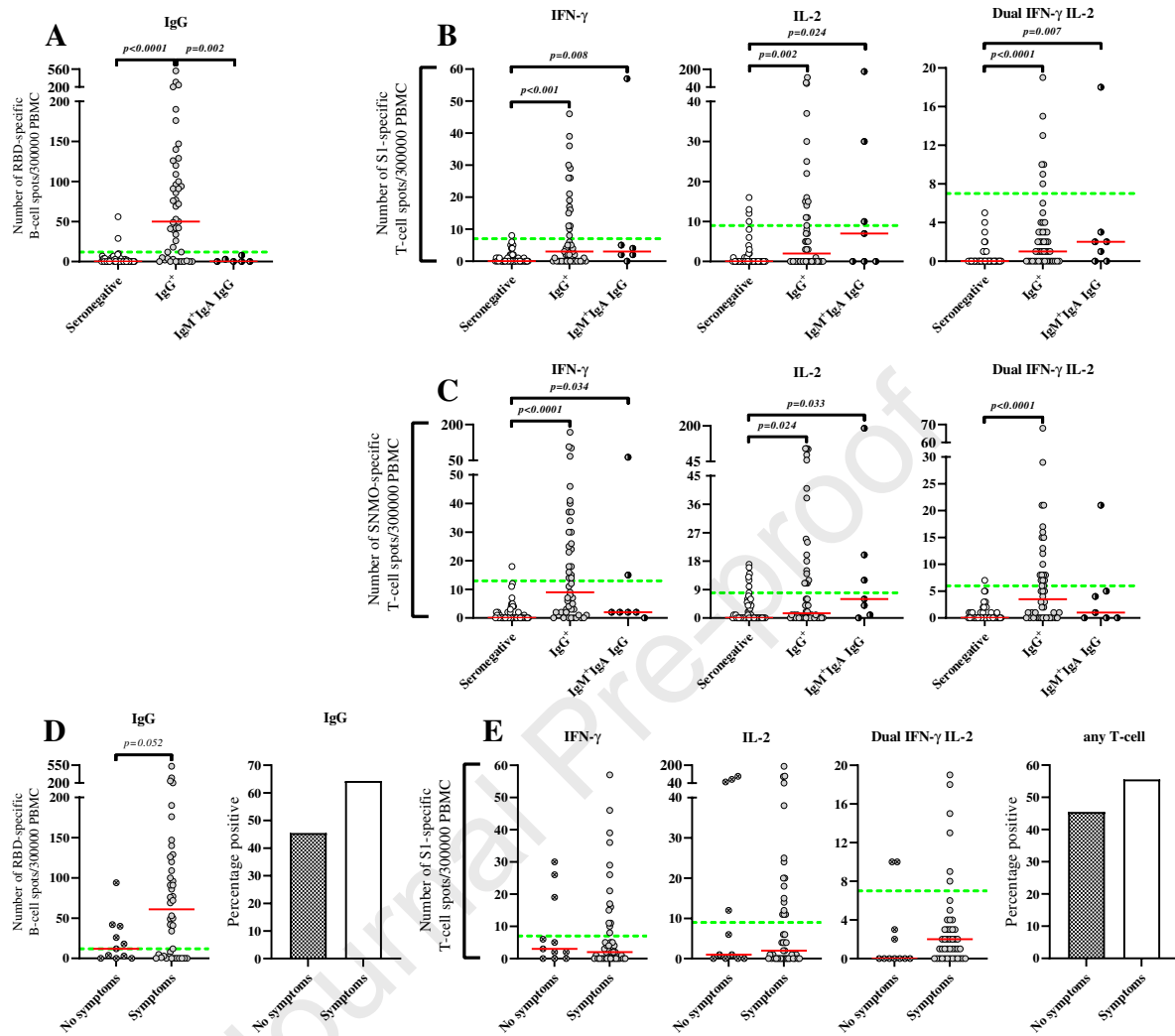
^aMedian number of positive cells^bChi-square test/Kruskal-Wallis test

NA: not applicable

IQR: Interquartile range







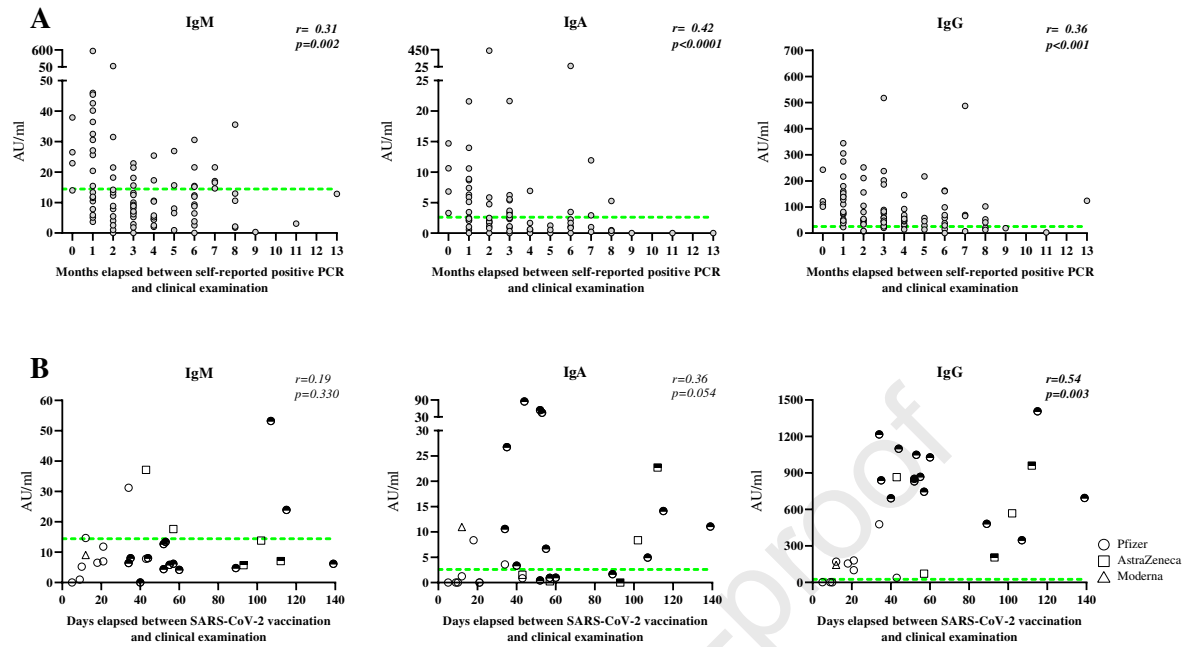


Table E1. Self-reported symptom and disease characteristics reported by SARS-CoV-2 IgG⁺ and IgM⁺IgA⁻IgG⁻ subjects

		IgG ⁺		IgM ⁺ IgA ⁻ IgG ⁻		p-value ^a
		n	%	n	%	
Any COVID-19 related symptoms between Feb-20 and June-21	Yes	196	89.5	35	68.6	<0.0001
	No	23	10.5	16	31.4	
Disease symptoms experienced more than once between Feb-20 and June-21	>Once	64	32.7	14	40.0	ns
	Once	132	67.3	21	60.0	
<i>Total number of occasions with symptoms</i>		260		49		
Bedbound during disease	Yes, ≥7 days	23	8.8	1	2.1	ns
	Yes, 1-6 days	114	43.8	21	43.8	
	No	123	47.3	26	54.2	
Hospitalization	Yes	3	1.2	0	0.0	
	No	257	98.8	48	100.0	
When were symptoms experienced?	Feb-20	12	4.7	1	2.1	<0.0001
	Mar-20	47	18.2	7	14.6	
	Apr-20	31	12.0	6	12.5	
	May-20	20	7.8	3	6.3	
	Jun-20	13	5.0	3	6.3	
	Jul-20	4	1.6	3	6.3	
	Aug-20	4	1.6	3	6.3	
	Sep-20	15	5.8	3	6.3	
	Oct-20	20	7.8	10	20.8	
	Nov-20	31	12.0	0	0.0	
	Dec-20	22	8.5	1	2.1	
	Jan-21	10	3.9	1	2.1	
	Feb-21	7	2.7	0	0.0	
	Mar-21	13	5.0	4	8.3	
	Apr-21	6	2.3	3	6.3	
	May-21	3	1.2	0	0.0	
Duration of symptoms	<1 week	60	23.1	21	43.8	ns
	1 week	75	28.8	12	25.0	
	2 weeks	73	28.1	10	20.8	
	3 weeks	24	9.2	2	4.2	
	≥4 weeks	28	10.8	3	6.3	

^aChi-Square-test or Fisher's exact test

NA: not applicable

Table E2. Prevalence of symptoms for all disease occasions reported by SARS-CoV-2 seropositive and seronegative subjects

		Seropositive		Seronegative		p-value ^a
		n	%	n	%	
Reduced taste or smell	Yes	169	56.0	118	22.1	<0.0001
	No	133	44.0	416	77.9	
Sore throat	Yes	202	65.4	440	80.0	<0.0001
	No	107	34.6	110	20.0	
Fever	Yes	200	65.4	279	52.0	<0.001
	No	106	34.6	258	48.0	
Pain in bone or muscles	Yes	164	53.4	225	41.6	0.001
	No	143	46.6	316	58.4	
Body fatigue	Yes	205	66.3	330	60.1	0.078
	No	104	33.7	219	39.9	
Runny nose	Yes	234	75.5	442	80.7	0.082
	No	76	24.5	106	19.3	
Tiredness	Yes	262	85.6	453	82.1	ns
	No	44	14.4	99	17.9	
Cough	Yes	204	65.6	338	61.6	ns
	No	107	34.4	211	38.4	
Blocked nose	Yes	216	69.7	370	68.1	ns
	No	94	30.3	173	31.9	
Headache	Yes	235	75.8	417	76.1	ns
	No	75	24.2	131	23.9	
Abdominal symptoms: stomach pain, nausea, vomiting, diarrhoea	Yes	87	28.3	164	30.3	ns
	No	220	71.7	378	69.7	
Breathing difficulties	Yes	98	31.9	158	28.7	ns
	No	209	68.1	392	71.3	
Other symptoms	Yes	22	7.9	26	5.1	ns
	No	257	92.1	484	94.9	

Table E3. Symptom rating for all disease occasions reported by SARS-CoV-2 seropositive and seronegative subjects

		Seropositive		Seronegative		p-value ^a
		n	%	n	%	
Reduced taste or smell	Yes, very much	102	33.8	43	8.1	<0.0001
	Yes, pretty much	24	8.0	23	4.3	
	Yes, a little	43	14.2	52	9.7	
	No	133	44.0	416	77.9	
Sore throat	Yes, very much	23	7.4	63	11.5	<0.0001
	Yes, pretty much	72	23.3	157	28.5	
	Yes, a little	107	34.6	220	40.0	
	No	107	34.6	110	20.0	
Fever	Yes, very much	20	6.5	32	6.0	0.002
	Yes, pretty much	53	17.3	79	14.7	
	Yes, a little	127	41.5	168	31.3	
	No	106	34.6	258	48.0	
Pain in bone or muscles	Yes, very much	37	12.1	32	5.9	<0.001
	Yes, pretty much	63	20.5	79	14.6	
	Yes, a little	64	20.8	114	21.1	
	No	143	46.6	316	58.4	
Body fatigue	Yes, very much	53	17.2	61	11.1	0.005
	Yes, pretty much	72	23.3	96	17.5	
	Yes, a little	80	25.9	173	31.5	
	No	104	33.7	219	39.9	
Runny nose	Yes, very much	31	10.0	69	12.6	ns
	Yes, pretty much	78	25.2	162	29.6	
	Yes, a little	125	40.3	211	38.5	
	No	76	24.5	106	19.3	

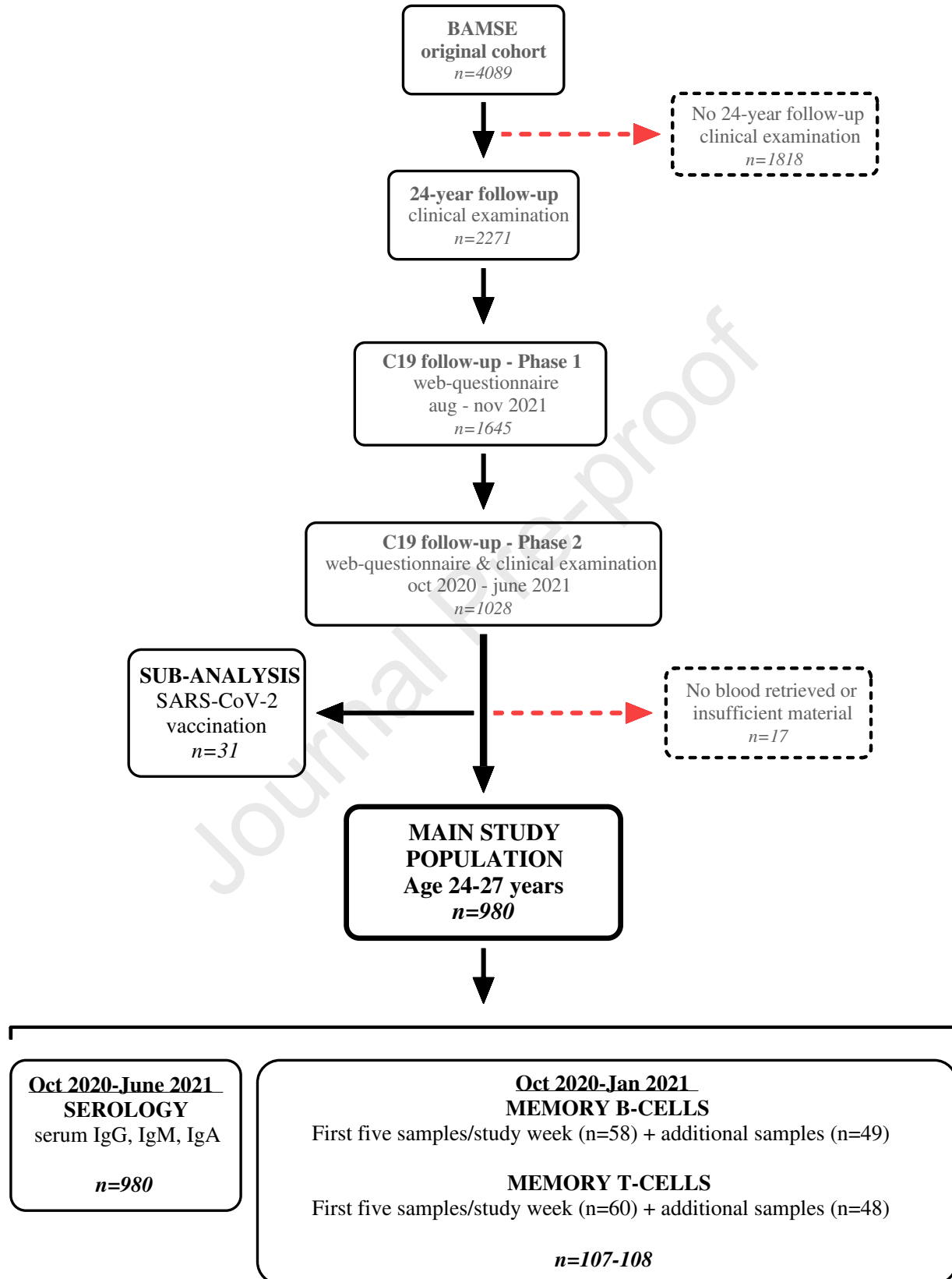
Table E4. Characterization of seronegative subjects (n=10) with SARS-CoV-2 memory B-cell and T-cell responses

	1A	2B	3C	4D	5E	6F	7G	8H	9I	10J
Cellular memory immunity										
Memory B-cell response	No	Yes	No	No	No	No	No	No	Yes	No
Memory T-cell response ^a	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antibody titers^{b,c}										
(cut of/borderline/median)										
IgG (25.1/22.5/6.5)	9.7 ^c	6.6 ^c	8.6 ^c	7.5 ^c	3.1	11.5 ^c	9.2 ^c	16.2 ^c	23.2 ^b	9.6 ^c
IgM (14.4/7.8/3.0)	3.7 ^c	7.0 ^c	4.3 ^c	4.6 ^c	2.5	6.5 ^c	2.2	11.2 ^b	10.5 ^b	3.7 ^c
IgA (2.6/1.4/0.1)	0.0	0.0	2.1 ^b	0.0	0.0	0.0	1.1 ^c	0.5 ^c	2.1 ^b	0.0
Symptoms										
Any symptoms Feb-20 to Jan-21	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	No
Time: symptoms to clinical visit ^e	0	NA	0	NA	NA	2	8	10	1	NA
Other factors										
Self-reported positive PCR test	No	No	No	No	No	No	No	No	Yes	No
COVID-19 in household ^f	No	No	Yes	No	No	No	Yes	No	Yes	Yes

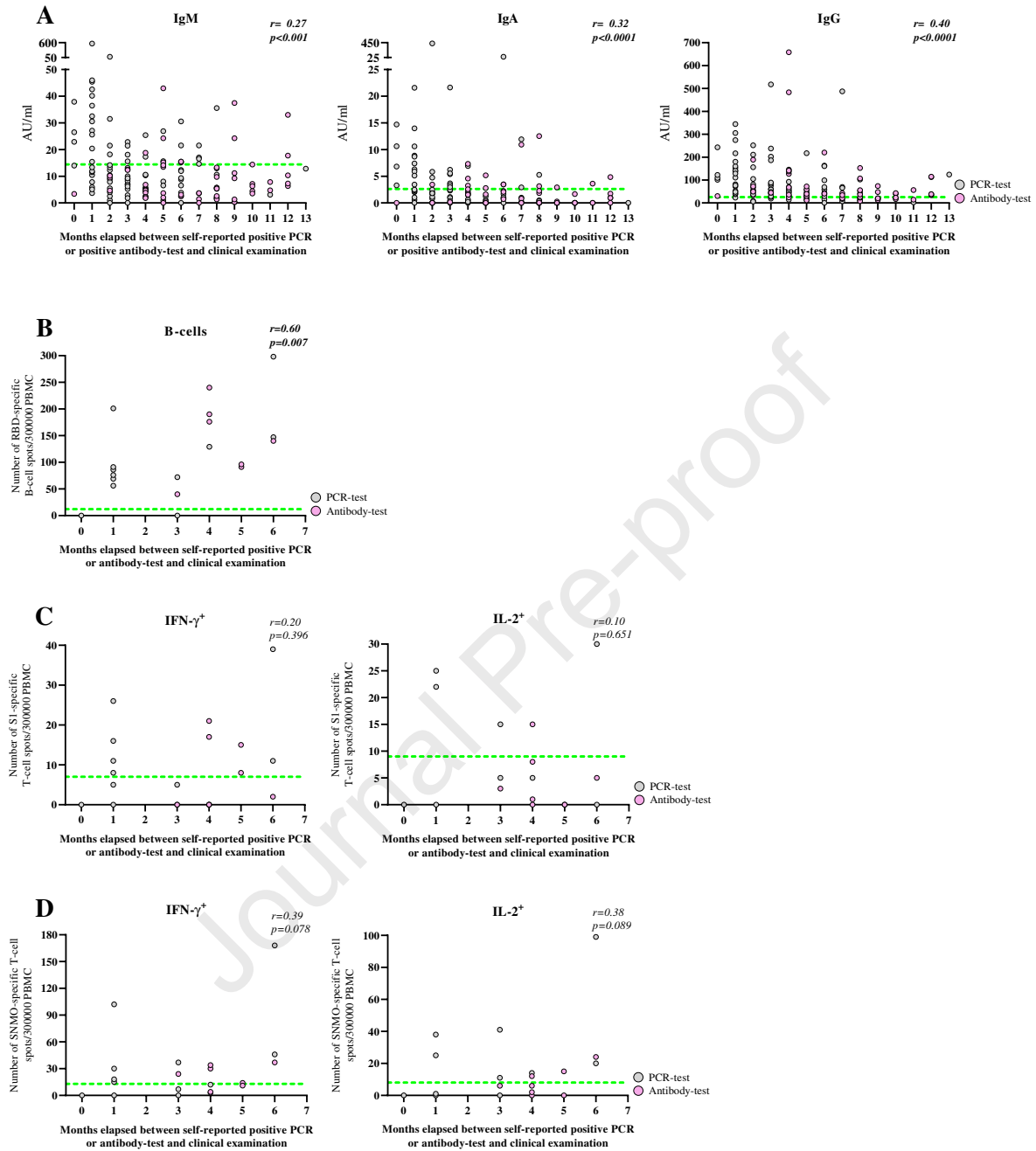
^aPositive in at least one T-cell assay^bBordeline positive antibody-titre^cTitre exceeding the median antibody titre value in the seronegative group^dTime in months between latest episode of reported symptoms and clinical visit^eSuspected or confirmed COVID-19 disease in the household

NA: not applicable

Participant cohort identification numbers are re-named by one number and one letter which cannot be connected to their original identification number.







ONLINE REPOSITORY

METHODS

Extended definition of variables obtained from questionnaires

From the phase 1 and phase 2 web-questionnaires, we obtained information regarding type of symptoms: fever, cough, reduced taste or smell, sore throat, running nose, blocked nose, headache, joint or muscle pain, abdominal symptoms, breathing difficulties, tiredness, body fatigue and from the questions: “Has anyone in your household had suspected or confirmed COVID-19 during the pandemic/since August 1st 2020?”, “Do you use face mask?” (asked only during phase 2, when national face mask recommendations were issued), “Have you regularly met other people at work?” and “What is your main use of transport to your daily work?”. PCR and antibody-tests were reported through the questions “When was the last time you performed a nose- or throat test” with the follow-up question “What was the result?” (PCR) and “When was the last time you performed a blood test” with the follow-up question “What was the result?” (antibody-test). Asthma was defined as doctor’s diagnosis (ever) together with symptoms of breathing difficulties or asthma medication occasionally or regularly in the last 12 months^{E1}. Rhinitis was defined as doctor’s diagnosis (ever) together with nasal or eye problems without having a cold in the last 12 months. The phase 1 questionnaire covers February 2020 until November 2020 and the phase 2 questionnaire covers August 1st 2020 until the clinical examination^{E2}.

IgE-sensitization was analysed during the 24-year follow-up and defined as ≥ 0.35 kU/L of soluble serum IgE to at least one of the tested allergens using ImmunoCAP System (Thermo Fisher Scientific)^{E3}.

Detection of SARS-CoV-2 specific memory B- and T-cell responses

To detect SARS-CoV-2 RBD-specific IgG-secreting memory B-cells, PBMCs were cultured for four days in RPMI-1640 medium with 10% FBS and 1% penicillin-streptomycin, supplemented with 1 μ g/ml R848 (resiquimod; a TLR7/8 agonist) and 10 ng/ml recombinant human IL-2. 300 000 cells/well were then loaded to ELISpot plates pre-coated with monoclonal anti-human IgG antibodies (Human IgG SARS-CoV-2 RBD ELISpot^{PLUS} kit, Mabtech AB). IFN- γ - and IL-2-secreting T-cells were detected using the Human IFN- γ /IL-2 SARS-CoV-2 FluoroSpot^{PLUS} kit (Mabtech AB). 300 000 PBMCs/well were added to FluoroSpot plates pre-coated with monoclonal anti-IFN- γ and anti-IL-2 antibodies together

with the S1 scanning pool containing 16 peptides (#3629-1, Mabtech AB) or the S N M O defined peptide pool containing 47 synthetic peptides binding to human HLA (#3620-1, Mabtech AB), and 100 ng/ml anti-CD28. The plates were incubated overnight and developed the following day, according to the manufacturer's protocol. ELISpot and FluoroSpot images and spot counts were obtained using an IRIS plate reader. The results are expressed as the number of spots per 300 000 cells, after subtracting the background. The cut-off value was set at the highest number of spots detected in 11 pre-pandemic PBMC control samples^{E4}.

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SUPPLEMENTAL FIGURE LEGENDS

FIG E1. Flow chart of the study protocol

From the BAMSE original cohort (n=4089), we invited all subjects that participated in the clinical phase of the 24-year follow-up to the COVID-19 follow-up (m=2270). Out of these, 1644 participants answered the Phase 1 web-questionnaire and were then invited to the clinical examination. Out of the 1026 participants that attended the COVID-19 clinical examination, 980 subjects constituted the main study population, whereas 32 vaccinated subjects were grouped for sub-analysis. 14 subjects were excluded after the clinical examination due to lack of or insufficient sample material.

FIG E2. Polar barplot representation of the SARS-CoV-2 specific adaptive immune responses

For subjects with available data on all three “arms” of adaptive immunity (virus-specific antibodies, memory B- and T-cells). The bars, from top in a clockwise direction, indicate the serum anti-RBD IgA, IgG and IgM titers (red bar), the number of RBD-specific memory B-cells (green bar), and the number of T-cells (blue bar) specific for the virus protein derived peptides pools S1 and S N M O that produce IFN- γ or IL-2 or both (Dual) (see example). The axis was scaled from 0 to 1 using minimum and maximum log normalized values. The headers shown in the center of each plot is unrelated to the study participants identification number used within the BAMSE cohort and cannot be connected to any individual.

FIG E3. Correlation of antibody titres or cellular responses with the time in months that elapsed since presumed COVID-19 disease

(A) Correlation between the number of months that elapsed between self-reported positive PCR-test or antibody-test to clinical examination and the titres of anti-SARS-CoV-2 IgM, IgA or IgG (grey circles: PCR-test, purple circles: antibody-test). (B-D) Correlation between the number of months that elapsed since presumed COVID-19 disease among seropositive subjects based on self-reported positive PCR-test (grey circles) or antibody-test (pink circles) and the numbers of RBD-specific IgG⁺ B-cells (A), the numbers of S1-specific IFN- γ ⁺ or IL-2⁺ T-cells (B), or the numbers of SNMO-specific IFN- γ ⁺ or IL-2⁺ T-cells (C). Spearman rank correlation was used for statistical analysis. Green lines represent assay cut off values.