Symptom burden and immune dynamics 6 to 18 months following mild SARS-CoV-2 infection -a case-control study

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Abstract

Background: The burden and duration of persistent symptoms after non-severe COVID-19 remains uncertain. This study aimed to assess post-infection symptom trajectories in home-isolated COVID-19 cases compared to age- and time-period matched seronegative controls, and investigate immunological correlates of long COVID.

Methods: A prospective case-control study conducted between February 28th and April 4th 2020 included home-isolated COVID-19 cases followed for 12 (n=233) to 18 (n=149) months, and 189 age-matched SARS-CoV-2 naive controls. We collected clinical data at baseline, 6, 12 and 18 months post-infection, and blood samples at 2, 4, 6 and 12 months for analysis of SARS-CoV-2 specific humoral and cellular responses.

Results: Overall, 46% (108/233) had persisting symptoms 12 months after COVID-19. Compared to controls, adult cases had a high risk of fatigue (27% excess risk, gender and comorbidity adjusted odds ratio [aOR] 5.86, 95% confidence interval [CI]3.27-10.5), memory problems (21% excess risk, aOR 7.42, CI 3.51-15.67), concentration problems (20% excess risk, aOR 8.88, CI 3.88-20.35), and dyspnea (10% excess risk, aOR 2.66, CI 1.22-5.79). The prevalence of memory problems increased overall from 6 to 18 months (excess risk 11.5%, CI 1.5, 21.5, p=0.024) and among women (excess risk 18.7%, CI 4.4, 32.9, p=0.010). Longitudinal spike IgG was significantly associated with dyspnea at 12 months. The spike-specific clonal CD4^{+}TCRβ depth was significantly associated with both dyspnea and number of symptoms at 12 months.

Conclusions: This study documents a high burden of persisting symptoms after mild COVID-19, and suggest that infection induced SARS-CoV-2 specific immune responses may influence long-term symptoms.

Keywords: long COVID, PASC, SARS CoV-2, antibodies, T-cells.

Introduction

Prolonged complications after Coronavirus disease 2019 (COVID-19) are a major health concern in the ongoing pandemic. New and persisting symptoms beyond 3 months after acute COVID-19, without other medical explanations[1-4], are referred to as long COVID. Long COVID significantly overlaps with the post-intensive care syndrome (PICS) observed in survivors of severe COVID-19[5, 6]. Although the burden of long COVID is greater after severe disease, long COVID can also develop after mild illness, with 39-77% of hospitalized and-non-hospitalized patients reporting persisting symptoms 12 months after COVID-19[7-13]. In two year longitudinal follow-up studies, symptom burden decreased with time, but residual symptoms persisted in 55% of hospitalized patients[14] and 38% of non-hospitalized patients[15]. Frequent persisting symptoms are fatigue, dyspnea, neurocognitive problems and mental health problems[16], but due to methodological heterogeneity, uncertainty remains about the true burden. Symptoms of long COVID may be wrongly attributed to infection as only a few studies included controls[14, 17, 18], making it difficult to identify any confounders[10, 15]. Online surveys where participants are included on their own initiative likely overestimate the symptom burden of long COVID[19]. In contrast, registry data may fail to pick up on symptoms that do not result in contact with health service, and may consequently underestimate symptom prevalence[20, 21]. Previously, we reported higher SARS-CoV-2 spike-specific antibodies associated with long COVID in a prospective cohort of home-isolated patients at 6 months[22]. Others have found potent antibody responses, aberrant T-cellular responses and pre-existing illness are associated with symptom sequelae[22-26]. Knowledge of the pathophysiology of long COVID is still evolving. In this study, we aimed to investigate symptom trajectories up to 18 months post-infection, assess the excess risk of symptoms in COVID-19 cases compared to age- and time-matched SARS-CoV-2 naïve controls, and explore the immunological and clinical correlates of long COVID.

Methods

Study population

Cases included home-isolated patients with Reverse transcription-polymerase chain reaction (RT-PCR) confirmed SARS-CoV-2 infection, tested at the city´s centralized testing facility (Bergen Municipality Emergency Clinic, BMEC) between February 28th, 2020, and April 4th, 2020. Household contacts of confirmed cases were invited to participate in a study of household attack rates during the same period[27], and those testing positive for SARS-CoV-2 spike antibodies within 2 months after recruitment were included as cases in the current study. One patient who was hospitalized in the weeks after acute infection was excluded from this cohort. All cases were assessed by clinical follow-up for 12 months (n=233), and a subgroup of adult cases agreeing to further follow-up (n=149) were followed for 18 months.

A control group was assessed at the clinic and recruited in two ways. Firstly, household contacts without symptoms, who did not seroconvert, and had no history of RT-PCR positivity, were included, and considered socioeconomically matched to the cases. Secondly, age-matched controls were recruited between January and March 2021 from the population of individuals who were prioritized for vaccination due to either age, comorbidity or occupation. All controls were seronegative at the time of symptom assessment. Hence, the seasonal timing of assessment, and the degree of national and local restrictions, were similar for cases at the 12-month follow-up and controls. The matching was therefore primarily chosen for comparison to the 12-month patient data.

Ethical considerations

The study was approved by the Regional Ethics Committee of Western Norway (#118664 and # 218629). All eligible individuals received both oral and written information about the study protocol and provided written informed consent upon inclusion. For children <16years old, parents provided consent.

Clinical data collection

Participant data were entered in electronic case report forms (eCRFs) using the Research Electronic Data Capture database (REDCap®, Vanderbilt University, Nashville, Tennessee) software, and subsequently stored on a secure research server.

All cases recruited at Bergen Municipality Emergency Clinic were followed up for 12 months (Interquartile Range [IQR] 11.5-12.4 months) with systematic interviews at baseline, 2, 6, and 12 months (supplementary methods), and blood samples at 2, 4, 6 and 12 months. 149 cases had an additional follow-up at 18 months (Figure 1). All subjects provided information on demographics and comorbidities, prescription drug use, and COVID-19 related symptoms at baseline and follow-up visits. Comorbidities recorded were asthma, chronic obstructive pulmonary disease (COPD), hypertension, chronic heart disease, rheumatic disease, diabetes, cancer, neurological disease, immunosuppressive conditions, or other severe or chronic disorders.

The baseline symptom questionnaire was limited to fatigue, headache, fever, myalgia and dyspnea. At 6- 12- and 18-month follow-up of cases, a dichotomized yes/no questionnaire was conducted for the following persistent symptoms: dyspnea, sleep problems, headache, dizziness, tingling, palpitations, gastrointestinal problems or low-grade fever. A general

questionnaire with dichotomized answers was used to assess fatigue, concentration, and memory problems in children ≤15 years old. For adult cases, the validated 11-item Chalder Fatigue Scale (CFS) was used. This CFS questionnaire identifies symptoms associated with both physical and mental fatigue, with graded responses that can be reported according to a Likert scale (0,1,2,3) or as a bimodal score (0,0,1,1)[28]. The prevalence of fatigue, impaired concentration, and memory problems was derived from the corresponding bimodal score of the CFS item 1, 8, and 11, respectively (supplementary table 1). We used a definition of long COVID as persistent or new onset symptoms at 3 months after COVID-19 [4].

Controls provided blood samples and replied to a survey including demographic and clinical information on comorbidities, assessment of dyspnea, and the 11-item CFS concomitantly with the 12-month follow-up of cases.

Blood sampling

Sera were stored at -80°C and heat-inactivated for 1 hour at 56°C after thawing before use.

Enzyme-linked Immunosorbent Assay (ELISA)

A two-step ELISA for detection of IgG was used, first by antibody screening for the Wuhan receptor-binding domain (RBD), followed by endpoint Wuhan spike ELISA, as previously described[27, 29] (supplementary methods).

Microneutralization assay

The microneutralization (MN) assay was performed using a local SARS-CoV-2 isolate from March 2020, as previously described [27, 29] (supplementary methods).

Identification of SARS-CoV-2 associated T-cell receptor β (TCRβ) sequences

Genomic DNA was extracted from EDTA blood using the Qiagen DNeasy Blood Extraction Kit (QIAGEN, Germantown, MD) and amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. SARS-CoV-2 associated CDR3 regions of TCRβ chains were sequenced using the ImmunoSEQ Assay T-MAP™ COVID platform (Adaptive Biotechnologies, Seattle, WA) as previously described[30]. The relative number of SARS-CoV-2-associated TCRs was defined as the *clonal breadth*, and the relative proportion of SARS-CoV-2-associated TCRs as the *clonal depth*.

Statistical analysis

Data analysis and visualization were performed in R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) (Figure 2,3 & 4) and IBM SPSS Statistics version 26 (New York, US) (Table 1 & Supplementary Table 1-4). Age-stratified analysis was performed using 15-year intervals to provide sufficient group sizes. Pearson's chi-square test and Fisher's exact test were used to compare proportions. The Mann-Whitney U-test was used to compare continuous variables between two groups. Confidence intervals (CI) and p-values for risk differences were calculated using the fmsb-package in R. Correlations between antibody titers and T-cell breadth and depth were assessed by Spearman´s rho. Multivariate binomial logistic regression was used for analyses of binary outcome variables and negative

binomial regression was used for the count outcome "number of symptoms". Regression models are presented with adjusted odds ratios (aOR) and 95% CI or risk ratio (RR) with 95% CI or standard error (SE) and p-values. Scaling of TCR breadth was applied due to significant difference in the range between the depth and the breadth of the TCR variables. Microneutralization and IgG antibody titers were log(10) -transformed to adjust for non-normality. Generalized estimating equations (GEE) were used to compare longitudinal spike IgG antibody measurements between two groups (geepack package (v 1.3.3 in R).

Results.

Study population

A population of 233 home-isolated COVID-19 cases were followed for 12 months, and 189 controls were assessed at the time when cases had their 12 months follow-up. Cases and controls had similar median age (44 vs 41 years, p=0.576), 16/233 cases and 7/189 controls were ≤18 years. There were fewer females among cases (53% vs 66%, p=0.010). Overall, more cases reported comorbidities than controls (53% vs 42%, p=.026), most frequently chronic lung disease (12% vs 8%, p=0.168), hypertension (11% vs 7%, p=0.241), rheumatic disease (7% vs 3% p=0.047), and chronic heart disease (6% vs 6%, p=0.915) (Supplementary table 1).

Symptom burden in cases at 12-month follow-up compared to controls

Compared to controls, adult cases had excess risk, and higher gender and comorbidity adjusted odds of fatigue (37% vs 9%, aOR 5.86, CI 3.27-10.5, p<0.001), impaired

concentration (24% vs 4%, aOR 8.88, CI 3.88-20.35, p<0.001), memory problems (26% vs 5%, aOR 7.42, CI 3.51-15.67, p<0.001), and dyspnea (15% vs 5%, aOR 2.66, CI 1.22-5.79, p=0.014). Children 0-15 years old reported no symptoms at 12 months follow-up in either cases or controls. Cases aged 16-30, 31-45, and 46-60 had the highest risk of memory problems and impaired concentration (p<0.05) (Table 1). Fatigue, on the other hand, was more frequently reported by cases aged 46-60 (41% vs 2% in controls, p<0.001) and 61-81 (42% vs 13% in controls, p=0.033). Age-stratified prevalence of 11 symptoms is presented in Figure 2.

Longitudinal symptom development

We assessed the trajectories of 11 symptoms in a subgroup of 149 cases followed for 18 months (Figure 3a-c). The prevalence of reported memory difficulties increased overall from 6 to 18 months follow-up, with an excess risk of 11.5% (CI 1.5, 21.5, p=0.024), the excess risk was significant among women (excess risk 18.7%, CI 4.4, 32.9, p=0.010), but not among males (9.6%, CI -3.6, 22.8, p=0.154). The risk difference from 6 to 18 months for other specific symptoms and symptoms overall was not statistically significant (Figure 3a).

Compared to males, women had excess risk of having symptoms overall at 18 months (17.5%, CI: 1.6, 33.3, p=0.030, Fig 4b) and at 12 months follow-up (20.2%, CI: 4.5, 36.0, p=0.012), but not at 6 months (6.8%, CI: -9.3, 22.8, p=0.41). There was no statistically significant risk difference between the sexes for each specific symptom at 18 months follow-up (Figure 4b), although women had more memory problem at 12 month and scored higher on Chalder fatigue score at 6 and 12 months (Supplementary Table 2 and 3).

Assessing different intensities according to the Likert-scale ("more than usual" versus "much more than usual") we found that cases had excess risk of fatigue, memory problems, impaired concentration and dyspnea compared to controls at all three time points (Table 2). However, the proportion with severe symptoms was low, and there was no significantly increased risk of severe cognitive symptoms at 12 and 18 months.

Association between acute-phase symptoms and long COVID

The majority of cases were symptomatic in the acute phase (226/233 cases). When adjusted for age, gender and comorbidities, acute-phase dyspnea was associated with an increased risk of fatigue, (OR 2.14, CI 1.16-3.95, p=0.010) and dyspnea (OR 8.55, CI 2.77-26.32, p=0.002) at 12 months follow-up, and acute-phase headache was associated with impaired concentration (OR 2.34, CI 1.03-5.29, p=0.040) (Table 3).

Association of antibody titers and long COVID

We measured SARS-COV-2 spike-specific IgG antibody titers at 2, 4, 6, and 12 months after infection. Antibodies waned over time (supplementary table 4), and antibody titers measured at 2 months were considered to reflect the peak of humoral response[31]. Peak spike-binding IgG (geometric mean titer 6128, range 50-98924) and longitudinal antibody titers from 2-12 months, were associated with dyspnea at 12 months and persistent dyspnea from 6 to 12 months, in adjusted analysis (p=0.02 and p=0.05)(Table 3, Figure 4a). Longitudinal antibody responses were not significantly higher in cases with ≥3 symptoms at 12 months compared to those with no symptoms, or in cases with persistent fatigue at 6 and 12 months compared to cases without fatigue (Figure 4b-c).

Association of persisting symptoms and T-cell responses

We measured the correlations between SARS-CoV-2 associated class I restricted ($CD8^+$) or class II restricted ($CD4^+$) TCRs and spike IgG titers from the same time points. Spike IgG antibodies correlated more strongly with $CD4^+$ than $CD8^+$ spike-specific TCRs. Significant correlations between spike IgG and $CD4^+$ clonal breadth and depth were observed at 2 months ($r = 0.371$, $p<0.0001$ and $r=0.315$, $p<0.001$), respectively, and at 6 months ($r=0.276$, $p<0.001$ and $r=0.251$, $p<0.001$). Whereas only the spike IgG and $CD8^+$ clonal depth correlation at 2 months was significant ($r=0.139$, $p=0.039$). SARS-CoV-2 specific clonal depth, (Total, $CD4^+$, and spike-specific $CD4^+$) at 6 months was associated with increased symptom burden at 12 months, when adjusted for age, gender, and the reciprocal TCR breadth (Table 4). Total $CD4^+$ spike-specific clonal depth was also associated with dyspnea at 12 months.

Discussion

In this longitudinal observational case-control study, we found that half of the home-isolated cases still had at least one residual symptom 12 and 18 months post-infection. Compared to controls, cases had significant excess risk of the dominant long COVID symptoms; fatigue, memory- and concentration problems, and dyspnea.

A key strength of our study is the inclusion of age-matched, seronegative controls recruited from the same geographical location and during the same time-period as the cases. Both cases and controls, therefore, had similar exposures to pandemic-related public infection control measures, disrupted social services, and psychosocial stress. We show that the excess fatigue, cognitive symptoms, and dyspnea reported by cases are likely sequelae of mild SARS-CoV-2

infection. Other case-control studies find excess burden of main long COVID symptoms in cases compared to influenza controls[32], healthy adults[14], and children, but the quality-of-life scores were lower in pediatric controls[17], suggesting that pandemic circumstances have affected the health of young people considerably.

Investigating longitudinal symptoms trajectories is important to predict the long COVID burden. In our study, specific symptoms evolved differently over time in individual cases, supporting the fluctuating nature of long COVID previously described[33] . Symptom debut later than 6 months post-infection could also reflect a coincidental overlap with emerging symptoms attributable to other causes or personal circumstances.

In non-controlled studies, the proportion of patients with residual symptoms at 12 months varies considerably (39%-77%)[7, 9-13], and we found a prevalence of 46% in our cases. The prevalence of fatigue, a dominating long COVID sequelae, ranges from 27%[12] in non-hospitalized, 16%-53% in mixed populations[11, 13] to 10% -33%[7, 8, 10] in hospitalized patients, partly reflecting differences in patient selection and symptom assessment[9]. In our subgroup of cases followed for 18 months, the prevalence of most symptoms remained at similar levels throughout, while memory difficulties increased, particularly among women. Although a body of research essentially describe improvement of long COVID over time, studies have described durable symptoms concerning mental health and cognition[14, 15] . Our finding of a lack of improvement in memory difficulties over time is of concern. Although sometimes perceived as vague symptoms, not always being recognized by the health care systems, cognitive symptoms may have significant impact on daily activity and work performance. Our study provides some reassurance for patients with persistent cognitive symptoms in that most cases reported *moderate* symptoms, and that there was no significant excess risk of *severe* cognitive symptoms at 12 and 18 months.

SARS-CoV-2 infection leads to sustained alteration of immune responses and spike-specific IgG titers appears to be associated with long COVID in both hospitalized and home-isolated patients[22, 25, 34]. Our study found that higher peak and longitudinal spike-specific IgG was associated with persistent dyspnea at 12 months. Interestingly, neutralizing antibodies levels were not associated with long-term symptoms, suggesting that other antibody effector mechanisms such as complement activation, Fc receptor binding or cross-reactivity to autoantigens, could be involved in long COVID[35-37]. No association was observed between spike-specific IgG and cognitive symptoms. The role of antibodies in this pathology remains unclear, although SARS-CoV-2 specific antibodies have been discovered in the cerebrospinal fluid (CSF) of COVID-19 patients[38], with abnormal oligoclonal banding patterns found in mild COVID-19 with cognitive sequelae[39]. Furthermore, cerebral elevated cytokine levels and brain abnormalities found in long COVID patients are compatible with inflammatory damage[40].

Dysregulation of T-cell activation and their associated cytokine mediators suggest an aberrant systemic immune response in long COVID patients[26]. Here, we found that the spike specific $CD4^+$ TCR clonal depth at 6 months was associated with increased number of long COVID symptoms and dyspnea at 12 months, suggesting a role for $CD4^+$ T-cells in long COVID. This may indicate an extensive immune stimulation driving T-cell proliferation, resulting in an increased magnitude and duration of circulating spike-specific T-cells and their associated antibodies. T-cell mediated tissue damage, disruption of cytokines and cell signalling homeostasis, may thus be involved in the pathogenesis of long COVID. Further studies should investigate the role of antigen-driven dysregulation of T-cells in long COVID including functional and phenotypic characteristics of T-cell subsets.

Our study is limited by the small size hampering subgroup analysis, potential bias in self-reported symptoms, suboptimal gender- and comorbidity-matching for controls, and lack of information for controls on certain variables of interest for long COVID, such as smoking and BMI. Strengths of our study are the inclusion of a near-complete geographical cohort from the first pandemic wave and the personalized follow up to detect long COVID symptoms, which may be missed in healthcare-based registry studies. All cases were infected with the ancestral Wuhan-like strain, and the prevalence of long COVID may differ after infection with subsequent variants of concern, which have increased infectivity and cause a different range of organ-specific symptoms.

Overall, our findings should be considered as intermediate, as longer follow-up will be required to understand the nature and chronicity of long COVID. Nonetheless, it is worrisome that fatigue, dyspnea, and cognitive problems post-infection have affected an important portion of the working-age population over this extensive period.

Conclusion

The positive association between spike IgG antibodies and $CD4^+$ associated SARS-CoV-2 specific TCR sequences with long-term symptoms, supports previous published results linking immune responses to long COVID pathogenesis. Hallmark long COVID symptoms occurred far more frequently in cases than in time- and age-matched confirmed seronegative controls, suggesting a causal relationship between COVID-19 and sequelae. The high proportion of symptomatic patients at 18 months, particularly those with cognitive symptoms is concerning. It is somewhat reassuring that few patients perceived their cognitive symptoms as severe at 18 months.

NOTES

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Author contributions

N.L. and R.J.C. conceived and designed the study. K.K. and E.B.F. performed literature search. K.K., E.B.F., C.T. and K.G.I.-M., recruited the participants and followed them up. F.Z. developed and ran the neutralization assays. T.B.O conducted the ELISA assays. K.A.B. organized sample collection and the TCR analyses. R.E. and I.M.K. conducted the TCR analyses. T.Ö. and E.B.F. analyzed the data. B.B., R.J.C., N.L. and E.B.F. interpreted the data. N.L., R.J.C., B.B and E.B.F. wrote the manuscript. Members of the COVID-19 research group contributed to the study follow-up, data collection and laboratory assays. All authors reviewed, edited and approved the final version of the manuscript.

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Potential conflict of interest

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Tables

Table 1 Risk of frequently reported symptoms at 12-months in age-stratified COVID-19 cases aged ≥16 compared to non-infected controls.

	All (16-81 years)				16-30 years			31-45 years			46-60 years			61-81 years		
	Case % (n) n=220	Control % (n) n=182	aOR* (95% CI) p-value	Crude risk difference 95% CI p-value	Case % (n) n=55	Control % (n) n=38	aOR* (95% CI) p-value	Case % (n) n=56	Control % (n) n=78	aOR* (95% CI) p-value	Case % (n) n=68	Control % (n) n=43	aOR* (95% CI) p-value	Case % (n) n=41	Control % (n) n=23	aOR* (95% CI) p-value
Fatigue	37% (81)	9% (17)	5.86 (3.3-10.5) <0.001	27% (20- 35) <0.001	26% (14)	13% (5)	2.95 (0.9-9.5) 0.070	39% (22)	10% (8)	5.67 (2.3-14.2) <0.001	41% (28)	2% (1)	32.98 (4.2-260.8) 0.001	42% (17)	13% (3)	4.64 (1.1-19.1) 0.033
Memory problems	26% (57)	5% (9)	7.42 (3.5-15.7) <0.001	21% (14, 28) <0.001	18% (10)	3% (1)	12.97 (1.5-110.0) 0.019	25% (14)	8% (6)	4.62 (1.6-13.2) 0.005	32% (22)	0% (0)	NA**	27% (11)	9% (2)	3.42 (0.7-17.3) 0.137
Impaired concentration	24% (53)	4% (7)	8.88 (3.9-20.4) <0.001	20% (14, 27) <0.001	26% (14)	5% (2)	7.03 (1.4-34.6) 0.016	23% (13)	3% (2)	12.66 (2.7-59.8) 0.001	29% (20)	0% (0)	NA**	15% (6)	14% (3)	1.15 (0.3-5.3) 0.860
Dyspnea	15% (34)	5% (9)	2.66 (1.2-5.8) 0.014	11% (5,16) <0.001	13% (7)	0% (0)	NA**	14% (8)	5% (4)	2.61 (0.72-9.5) 0.144	18% (12)	4% (2)	3.62 (0.7-18.0) 0.115	17% (7)	14% (3)	1.19 (0.3-5.4) 0.827

*OR=Gender and comorbidity adjusted odds ratios with 95% confidence intervals. Comorbidity=includes the presence of any comorbidity. Corresponding 2-sided p-values <0.05 are shown in bold.

**NA=not applicable

Table 2 Longitudinal data on crude risk difference of long COVID symptoms† in 149 cases aged ≥16 years who came for 6-, 12- and 18-months follow-up compared to non-infected controls

					Excess risk compared to controls, % (CI) p		
	Controls	Cases 6m	Cases 12m	Cases 18m	6m	12m	18m
	N=182	N=148	N=149	N=149			
Fatigue	9% (17)	39% (58)	41% (61)	36% (53)	30% (21, 39) <0.001	32% (23, 41) <0.001	26% (17, 35) <0.001
more than usual	9% (17)	32% (47)	36% (53)	32% (48)	22% (14, 31) <0.001	26% (17, 35) <0.001	23% (14, 31) <0.001
much more than usual	0% (0)	7% (11)	5% (8))	3% (5)	7% (3, 12) <0.001	5% (2, 9) 0.004	3% (0, 6) 0.023
Concentration problems	4% (7)	22% (33)	29% (43)	26% (38)	18% (11, 26) <0.001	25% (17, 33) <0.001	22% (14, 29) <0.001
more than usual	4% (7)	18% (27)	27% (40)	23% (35)	14% (8, 21) <0.001	23% (15, 31) <0.001	20% (12, 27) <0.001
much more than usual	0% (0)	4% (6)	2% (3)	2% (3)	4% (0, 7) 0.012	2% (0, 4) 0.080	2% (0, 4) 0.080
Memory problems	5% (9)	21% (31)	29% (43)	33% (49)	16% (9, 23) <0.001	24% (16, 32) <0.001	28% (20, 36) <0.001
more than usual	4% (8)	20% (29)	27% (40)	30% (45)	15% (8, 22) <0.001	22% (15, 30) <0.001	26% (18, 34) <0.001
much more than usual	1% (1)	1% (2)	2% (3)	3% (4)	1% (-1, 3) 0.227	1% (-1, 4) 0.251	2% (-1, 5) 0.136
Dyspnea†	5% (9)	16% (23)	17% (25)	16% (24)	11% (4,17) 0.002	12% (5,19) <0.001	11% (4, 18) 0.001

† Severity of dyspnea was not recorded at 6 and 12 months.

CI = 95% confidence interval, p = p-values

Table 3 Associations between acute symptoms, early immune responses and long COVID symptoms at 12 months in adult cases†.

	Fatigue **aOR (CI)** **p-value**	**Memory problems** **aOR (CI)** **p-value**	**Impaired concentration** **aOR (CI)** **p-value**	**Dyspnea** **aOR (CI)** **p-value**
Acute phase headache (n=196)	1.37(0.69-2.74)	1.38 (0.65-2.93)	**2.34 (1.03-5.29)**	1.40 (0.55-3.53)
	0.3700	0.4100	**0.0400**	0.4800
Acute phase dyspnea (n=198)	**2.14 (1.16-3.95)**	1.85 (0.95-3.59)	1.11(0.58-2.12)	**8.55 (2.77-26.32)**
	0.0100	0.0700	0.7600	**0.0002**
Acute phase fever (n=198)	1.40 (0.72-2.70)	1.17 (0.58-2.39)	1.50 (0.73-3.08)	0.90 (0.39-2.09)
	0.3200	0.6600	0.2700	0.8100
Acute phase myalgia (n=198)	1.51 (0.80-2.86)	1.48 (0.73-2.98)	1.46 (0.73-2.93)	**3.73 (1.34-10.35)**
	0.2100	0.2800	0.2900	**0.0100**
Spike IgG titer at 2 months** (n=209)	1.16 (0.64-2.1)	1.14 (0.58-2.22)	1.39 (0.71-2.73)	**3.06 (1.23-7.61)**
	0.6300	0.7100	0.3300	**0.0200**
Microneutralizing antibody titer	0.95 (0.55-1.64)	0.80 (0.43-1.49)	0.98 (0.54-1.8)	1.21 (0.59-2.48)
at 2 months** (n=195)	0.8500	0.4800	0.9600	0.6000

† Presented as age, gender, and comorbidity adjusted odds ratios, with corresponding 2-sided p-values <0.05 shown in bold.
CI= 95% confidence interval
** IgG titer range: 50-98924. Samples with undetectable spike IgG titers were given a value of 50. Titers were log(10) transformed for calculation purposes.
** MN titers range: 10-16096. Samples with undetectable microneutralizing (MN) antibodies were given a value of 50. Titers were log(10) transformed for calculation purposes.

Table 4 Associations between SARS-CoV-2 associated T-cell clonal depth† and fatigue, memory/concentration, dyspnea and number of symptoms at 12 months.

SARS-CoV-2 associated T-cell receptor sequences	Fatigue aOR (SE)* p-value	Memory problems + impaired concentration aOR (SE)* p-value	Dyspnea aOR (SE)* p-value	Number of symptoms at 12 months¤ aRR (SE)** p-value
Total T-cell clonal depth	1.55 (0.332)	1.86 (0.378)	1.49 (0.497)	**1.71 (0.274)**
	0.1880	0.102	0.4250	**0.0499**
Total $CD4^+$ T-cell clonal depth	1.80 (0.362)	1.98 (0.395)	1.85 (0.568)	**1.94 (0.294)**
	0.1060	0.0827	0.2780	**0.0242**
Total spike specific $CD4^+$ T-cell clonal depth	2.70 (0.583)	2.77 (0.608)	**7.12 (0.969)**	**3.15 (0.483)**
	0.0889	0.0943	**0.0427**	**0.0176**

†One-tailed Fisher's exact test identified 8630 SARS-CoV-2-associated TCRβ sequences, and potential false positive TCRβ sequences associated with cytomegalovirus (CMV) or human leukocyte antigen (HLA) alleles were removed. SARS CoV-2 associated TCRβ sequences subsets were classified as Class I associated ($CD8^+$ T-cells) or Class II associated ($CD4^+$ T-cells), and spike or non-spike-associated. T-cell depth corresponded to the relative expansion of SARS-CoV-2 associated T-cell clonal subtypes

¤Number of symptoms at 12 months were encoded as integers from 0 to 11 including the total of 11 symptoms assessed.

*Odds ratio (OR), adjusted for age, gender and scaled reciprocal SARS-CoV-2 associated T-cell breadth, with standard error (SE), corresponding 2-sided p-value <0.05 are shown in bold.

** aRisk ratio (RR), adjusted for age, gender and scaled reciprocal SARS-CoV-2 associated T-cell breadth, with standard error (SE), corresponding 2-sided p-value <0.05 are shown in bold.

Figure legends

Figure 1 Study population.
Inclusion of SARS-CoV-2 cases (left) and control group (right). Eligible participants tested for SARS-CoV-2 infection by RT-PCR at Bergen Municipality Emergency Clinic (BMEC) were recruited between February 28th and April 4th, 2020. Only one case (the first most symptomatic) from each household was tested due to limited testing capacity, thus individuals living with COVID-19 positive study participants were included as household contacts. If household contact had positive SARS-CoV-2 serology (RBD and spike-IgG ELISA) within 2 months after recruitment, they were registered as cases. Seronegative household contact without a history of COVID-19 symptoms were included as controls. Additional controls were recruited amongst individuals who were prioritized for vaccination, either because of their age, comorbidity or occupation. At the time of symptom recording, all controls were confirmed seronegative. LTF = Lost to follow-up

Figure 2 Age-stratified symptom prevalence at 12 months post infection.
Bar plot representing the proportion of cases reporting 11 key symptoms at 12 months follow-up. The cases reported a mean of 1.4 symptoms overall. The age group 0-15 years old (n=13) is not shown due to absence of symptoms. The light gray area in the bar charts represent the overall proportion with any of the 11 symptom in the current age group. The colored areas represent the proportion with the specified symptoms.

Figure 3 Longitudinal symptom changes up to 18 months post-infection.
Dumbbell charts present longitudinal data on development of 11 specified symptoms in a subcohort of patients followed for 18 months (n=148, one patient was excluded due to missing data on all symptoms at 6 months). 3a) presents the overall symptom change from 6 to 12 months, 3b) presents the overall symptom change from 6 to 18 months and 3c) presents the symptom change in men (n=73) and women (n=75) from 6 to 18 months.

Figure 4 Kinetics of the spike IgG antibody response in relation to symptoms at 6 and 12 months.
The relationship between longitudinal antibody titers and a) persistent dyspnea versus no dyspnea, b) 3 or more symptoms at 12 months versus no symptoms and c) persistent fatigue versus no fatigue. The generalized estimating equation (GEE) coefficients with 95% Confidence intervals (CI) are adjusted for age, gender, comorbidity and time of measurement. All cases who had been vaccinated against SARS-CoV-2 during the follow-up period (n=20) were excluded from the analysis of immunological parameters at 12 months.

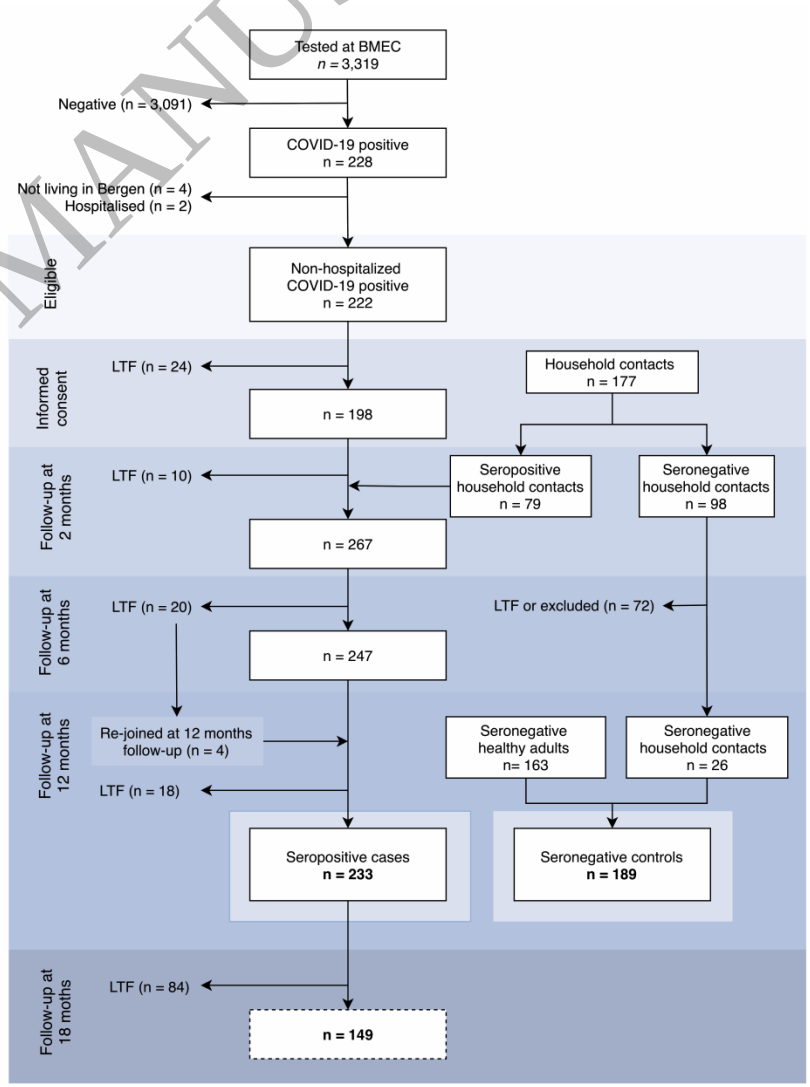

Figure 1
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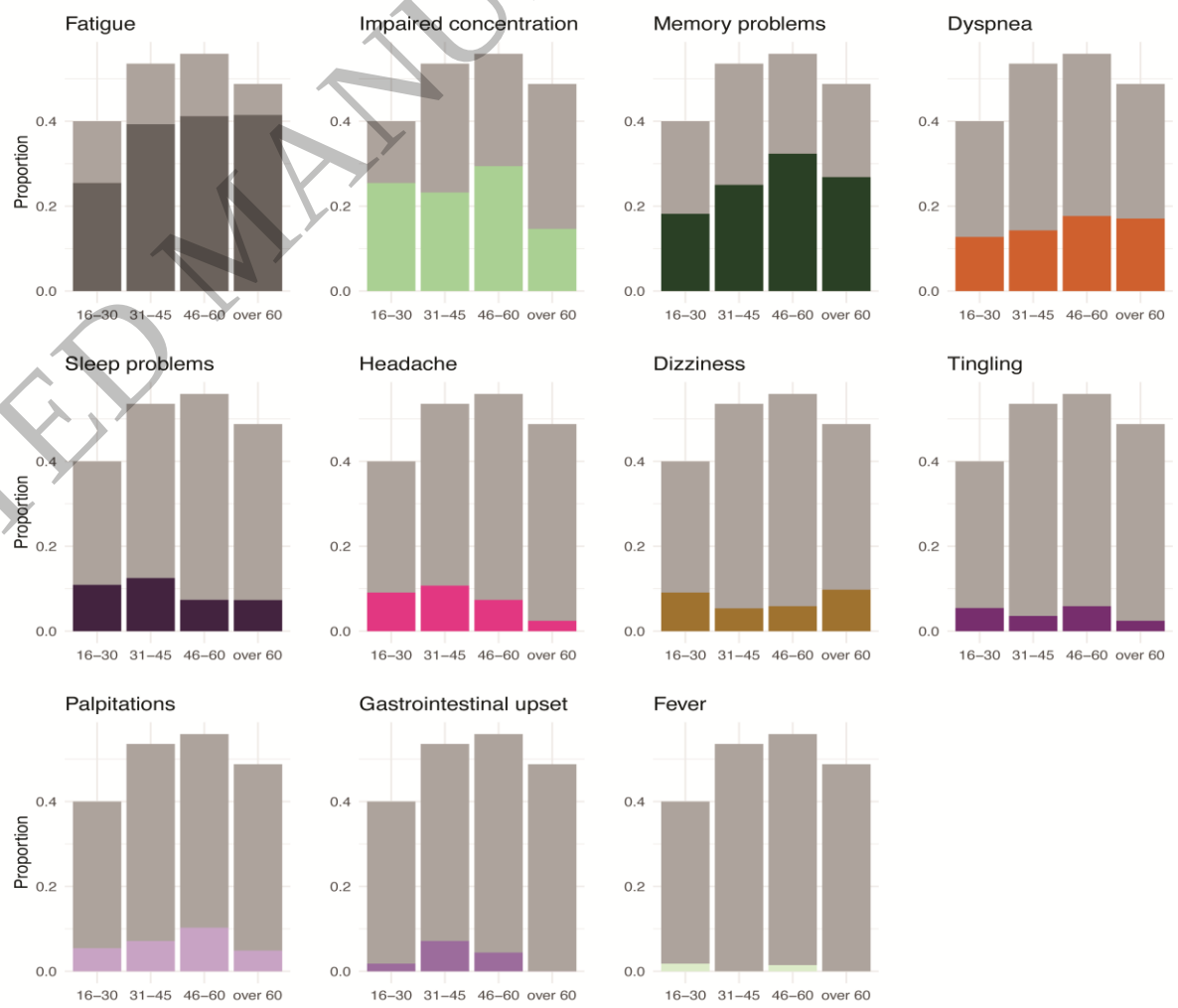

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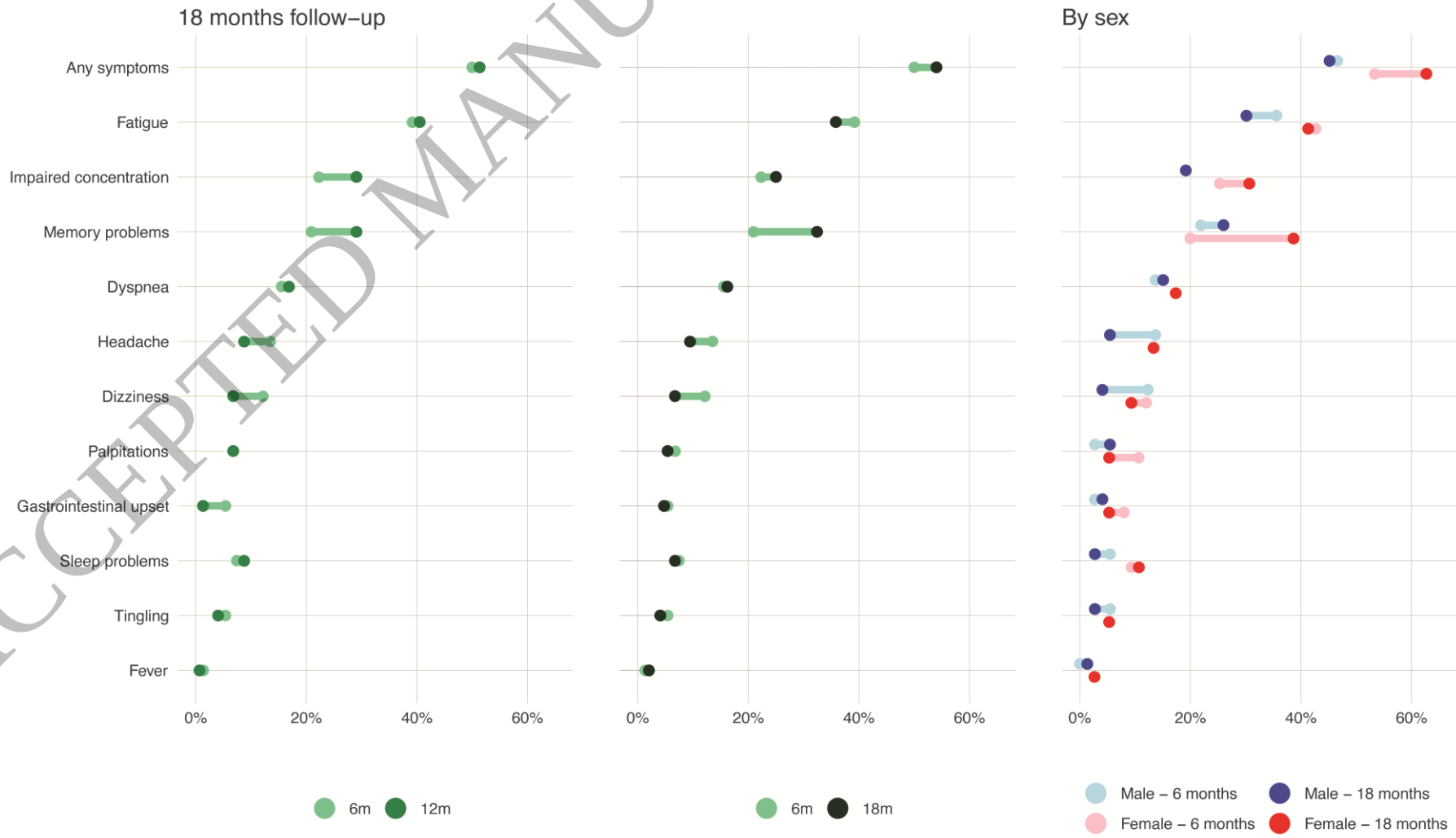

Figure 3
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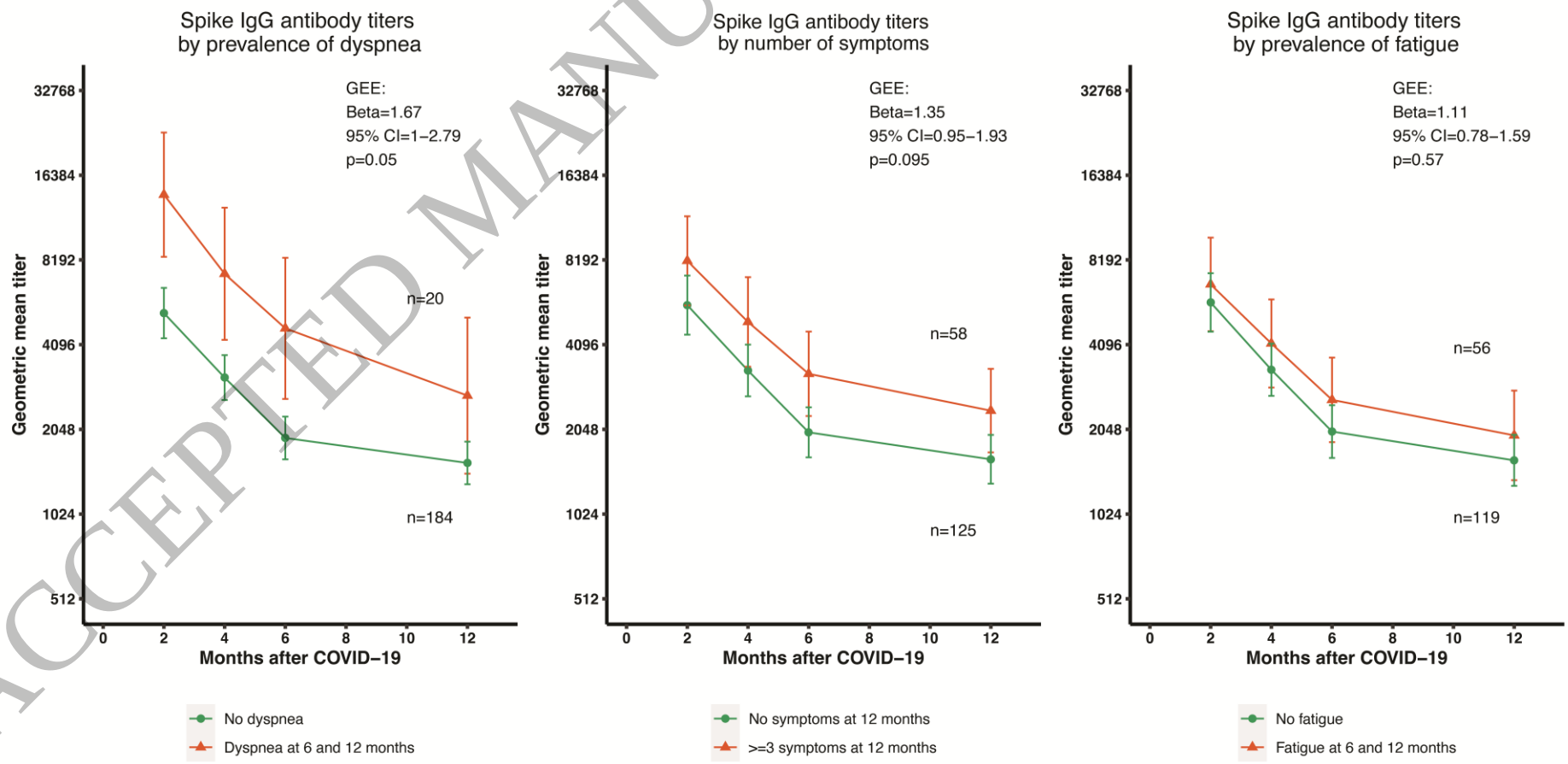

Figure 4
339x170 mm (.78 x DPI)