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SARS-CoV-2 infection induces long-lived B and T-cell responses up to 15 months post-infection, irrespective of disease severity.

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Running title:
Durable B- and T-cell responses one-year after SARS-CoV-2 infection

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Dear Editor,

Policies for deployment of SARS-CoV2 vaccines depends on detailed knowledge of immunity after natural infection. With hybrid immunity in the global population, after the combination of infection and vaccination, it is no longer possible to characterize infection induced immunity. Recently Shaw and colleagues conducted a head-to-head comparison of different heterologous mRNA and ChAdOx1 COVID vaccination schedules finding similar humoral and cellular responses six months after vaccination irrespective of regime[1]. They recommended easing logistical challenges by using heterologous vaccine regimes in future pandemics.

We compared long-term humoral and cellular immune responses in age and gender matched SARS-CoV-2 infected hospitalized(n=22) and non-hospitalized(n=22) patients during the Wuhan-like wave, prior to available treatment or vaccines with a 12-15 month (m) follow-up (Supplementary Table 1). Patients (41% female, aged 24–87years) were classified according to disease severity, based on the need for oxygen treatment and respiratory failure [2].

Detailed immunological analyses were conducted of humoral (Spike-specific SARS-CoV-2 IgG, memory B-cells (MBC)) and T-cell responses. Despite less severe disease, non-hospitalized patients achieved comparable long-term MBC and T-cellular responses for >1 year, although often higher in patients requiring oxygen. In both groups, spike-specific IgG peaked at 3-9 weeks(w), and declined over time, remaining detectable at 12-15m. Significantly more hospitalized patients had comorbidities (68%vs9%) and reported persisting post COVID condition symptoms 12-15m post-infection (59%vs32%, $p<0.001$) (Supplementary Table 1).

Strain-specific antibodies decrease within six months of vaccination, limiting their protection and necessitating boosters in risk groups[3]. However, we found patients still had detectable levels after 12-15m, although the highest antibody levels declined most rapidly, similarly to the antibody decay seen after heterologous vaccination in the Com-COV2 study[1].

In the hospitalized cohort, there was a significant increase in IgG titers from 0-3w peaking at 3-5m (GMT 9632, $p<0.01$) (Figure 1A), declining by 49% at 12-15 months (ns)(GMT 4899). In the non-hospitalized cohort, IgG was highest at 3-9w (GMT 5999), maintained at 3-5m, followed by a significant decrease (40%) at 12-15m(GMT 3571)(Figure 1A). Overall, we found no significant difference in the magnitude of spike-specific IgGs between patient groups, or by severity score, although higher titers were observed in hospitalized patients receiving oxygen (Figure1A,1B,1E).

In the acute phase, spike-specific IgG MBCs were not detected in 67% hospitalized patients, indicating that antibodies mainly originated from naïve B-cells, not cross-reactive coronavirus MBCs(Figure 1C+D). MBCs then increased significantly, peaking at 3-5m($p<0.0001$) for both groups, and were maintained at 12-15m significantly higher than the acute phase for the hospitalized group($p<0.001$)(Figure 1C)[4]. The frequencies of spike-specific MBC decreased most in hospitalized cases indicating immune contraction moving to a resting state over time. The magnitude and kinetics of the MBC response at 3-5m and 12-15m was similar in the two groups, irrespective of disease severity(Figure 1C+1E). Nevertheless, hospitalized patients requiring oxygen had the highest IgG MBC responses, with the most significant increase between 0-3w and 3-5m($p<0.0001$) (Figure 1D+1E). There was heterogeneity of responses within both hospitalized and non-hospitalized groups (Figure 1D). Interestingly RBD-specific MBC frequencies increased relative to spike-specific MBCs up to 12-15 months in both hospitalized and non-hospitalized individuals (Figure 1F), perhaps suggesting antigen persistence, prolonged activity in germinal centers, and affinity maturation. Supporting this, antibody maturation favors epitopes overlapping the ACE2 binding site on the RBD[5]. Interestingly, four hospitalized patients showed immune activation with detectable levels of spike-specific IgG MBC at admission, >10 days from symptom onset suggesting rapid, early memory responses. A less likely explanation is pre-existing cross-reactive MBC from earlier coronavirus infections.

Patients with severe and fatal COVID-19 may have impaired germinal centers, associated with extrafollicular B-cell responses, high magnitudes of antibody secreting cells, and neutralizing antibodies [6]. Consistent with our results, hospitalized COVID-19 patients showed higher antibody and MBC responses compared to milder infections [4, 7]. However, our findings of robust and long-lived MBC responses after infection in non-hospitalized patients supports findings indicating that lymph node function is either maintained or restored, at least in survivors with less severe disease [8]. This long-lived immunity may protect against severe disease upon reinfection from SARS-CoV-2 variants.

The grade of COVID-19 severity may influence the function of central and effector memory T-cells[4]. In non-hospitalized patients the frequencies of single cytokine (IFN-γ/IL-2) and double positive IFN-γ+IL-2+ producing T-cell peaked at 3-5m and were maintained at 12-15m(Figure 2A+D). There was a significant increase in IFN-γ+IL-2+ producing T-cells from 3-9w to 3-5m, not observed for single IFN-γ/ IL-2 cytokine producing T-cells (Figure 2A-C).

In contrast, in hospitalized patients, the frequencies of all three T-cell subsets increased significantly from 0-3w to 3-5m($p<0.001$), remaining significantly higher at 12-15m($p<0.05$)(Figure 2A-C). Although in the acute phase, only low numbers of double positive IFN-γ+IL-2+ T-cells were found(Figure 2A-C). Overall, no statistically significant differences of T-cell frequencies were observed by disease severity (Figure 2A-C), or by oxygen treatment(Figure 2E). As expected, T-cells were boosted in patients(n=4) vaccinated prior to sampling at 12m(Figure 2D, excluded from other analyses).

The cytokines IL-2 and IFN-γ are specific biomarkers of SARS-CoV-2 T-cell responses supporting our earlier findings of T-cell responses at 6 months which were significantly stronger in hospitalized than non-hospitalized patients [9]. However, our 15 m data show similar T-cell frequencies independently of disease severity. The quality of the T cell response, with increased IL-2 and decreased IFN-γ production in hospitalized patients, may indicate higher levels of central memory T cells relative to effector memory T cells in hospitalized compared to non-hospitalized patients[10]. Of importance, frequencies of IFN-γ T-cells for both hospitalized and non-hospitalized patients at 12-15m were equivalent to the most superior heterologous booster regime (80 and 79 SFCs/10^6 PBMC) found by Shaw and colleagues[1].

The strength of our study is the longitudinal follow-up of two matched, unvaccinated cohorts, with differing disease severity following natural infection. Our findings of durable SARS-CoV-2 antibodies, MBC and T-cellular protective immune responses more than one-year post-infection, contribute to detailed knowledge of long-term memory responses, and are important for developing future vaccination strategies. Similar kinetics of durable B and T cell respiratory responses were found irrespective of disease severity between non-hospitalised and hospitalised which were comparable to heterologous vaccination schedules.

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Figure legends

Supplementary table 1

Demographics and patient clinical characteristics. None of the patients were treated with experimental drugs, immunosuppressants or corticosteroids**.** Four patients (3 hospitalized and 1 non-hospitalized) were vaccinated prior to the last blood sampling (12-15 month), and not included in statistical analysis. Reported persistent symptoms of long-Covid; such as: dyspnea, arthralgia, tiredness, reduced concentration, and chemosensory dysfunction.

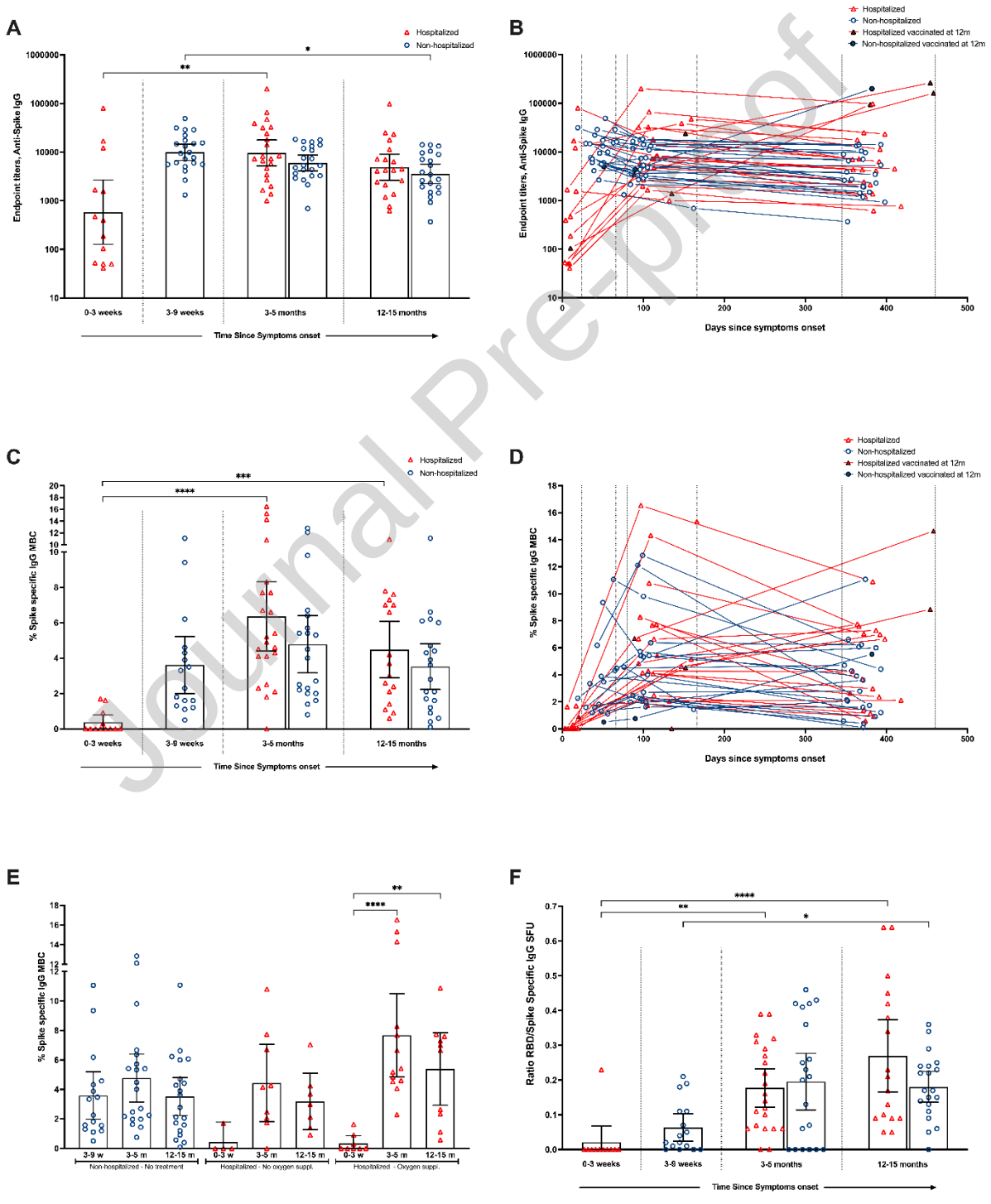

Figure 1. **Frequencies of SARS CoV-2 Spike specific IgG antibody and IgG Memory B cell (MBC) responses 12-15 months post-infection:**

Longitudinal comparison of the SARS-CoV-2 spike IgG titers between hospitalized and non-hospitalized patients is shown at 0-3 weeks (hospitalized only), 3-9 weeks (non-hospitalized only), 3-5 months and 12 months post symptoms onset (A). Each symbol represents individual antibody titers. The horizontal bars show the geometric mean of the antibody titers ± 95% CI. The kinetics of the individual titers of SARS-CoV-2 spike IgG antibody titers since symptom onset (PSO) (days) (B). Triangles represent hospitalized cases and circles represent non-hospitalized cases. Closed triangles and circles indicate cases that were vaccinated against SARS-CoV-2 prior to sampling at 12 months and are not included in the analysis at this timepoint (B,D).

Comparison of the SARS-CoV-2 spike IgG MBC response between non-hospitalized and hospitalized patients is shown (C). Results are presented as percentage of spike specific IgG MBC out of total IgG MBC. The kinetics of the individual MBC responses from symptom onset PSO (days)(D). Comparison of the frequency of MBC according to disease severity (non-hospitalized and hospitalized with/without oxygen supplementation during acute phase)(E). Ratio of RBD specific IgG MBC versus spike specific IgG MBC (F). The horizontal bars show the mean of the MBC frequencies ± 95% CI (C,E,F). A nonparametric Kruskal–Wallis multiple comparisons test was used to compare the different timepoints within each cohort (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

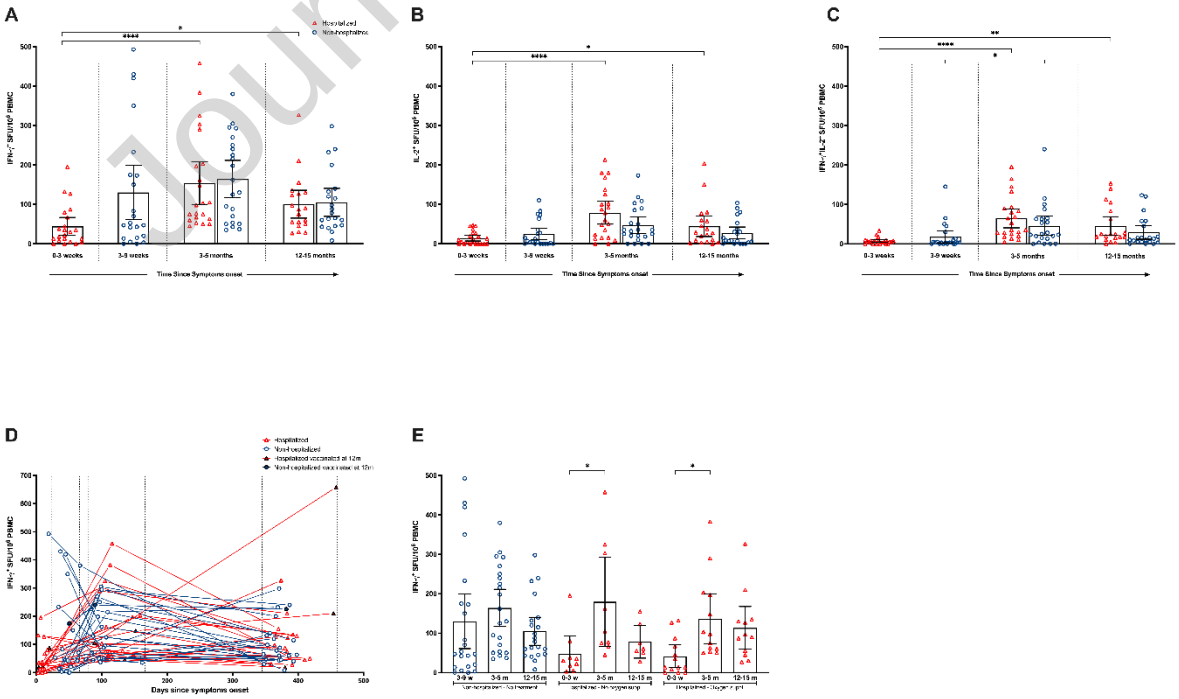

Figure 2: SARS CoV-2 specific T-cell cytokine responses 12-15 months post-infection

T-cell immune responses were evaluated by measuring the number of SARS-CoV-2 specific IFN-γ (A), IL-2 (B) and IFN-γ/ IL-2 (C) secreting T-cells after infection. The triangles represent hospitalized cases and circles represent non-hospitalized cases. Each symbol represents one individual and the combined number of spot forming units (SFU) per 1×10^6cells) after stimulation with virus spike antigen (spike, non-spike (nucleocapsid and matrix peptides). For the individual responses the time from symptom onset is indicated (D). Closed triangles and circles represent cases that were vaccinated against SARS-CoV-2 prior to sampling at 12 months, not included in the analysis at this timepoint. Comparison of the frequency of T-cell responses according to disease severity (non-hospitalized and hospitalized with/without oxygen supplementation during acute phase)(E). Disease severity is divided into three scores: 1) non- hospitalized (scores 1-2), 2) hospitalized cases with no oxygen supplementation (scores 3-4) and 3) hospitalized cases with oxygen supplementation (score 5). The horizontal bars show the mean of the SFU ± 95% confidence interval (CI). Statistical differences between hospitalized and non-hospitalized subjects were determined by the nonparametric Kruskal–Wallis multiple comparisons test (* = P<0.05, ** = P<0.01, ***P<0.001, ****P<0.0001).

Declaration of Competing Interest

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Kristin G-I Mohn has received honoraria from Takeda and IQVIA. All other authors declare no competing interests

Graphical abstract