Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letters to the Editor

Quantitative SARS-CoV-2 antigen test as a tool able to predict the stage of the infection

Dear Editor,

Fowler et al.[1] proposed RNA RT-LAMP as a rapid and accurate tool to promptly identify highly contagious individuals during the pandemic era. In the current vaccination era, it would be useful to have a reliable tool to provide information on the infectivity/contagiousness of individuals.

FDA approved antigen (Ag) test as a fast and convenient alternative to PCR but, as known, this approach can be effective at symptoms onset,[2] when viral antigen is abundant,[3] otherwise false negative results can occur; moreover, positive antigenic results need to be confirmed by molecular test[4].

These assays are mostly qualitative and, even when a numerical value is provided, no straightforward correlation with the virological and clinical parameters has ever been demonstrated.

We evaluated an Ag test based on chemiluminescence (CLEIA), Lumipulse®G SARS-CoV-2 Ag (Fujirebio INC), in an extensive population with different characteristics.

This comparative study included 1000 nasopharyngeal samples (NPS), analyzed during the period fall/winter 2020-2021 at the Virology laboratory of Bambino Gesù Pediatric Hospital.

NPS were collected in Universal Transport Medium (UTM, Copan) and immediately analyzed for molecular SARS-CoV-2 detection by Allplex™ SARS-CoV-2 Assay (Seegene), according to which, 850 samples resulted positive (Cycle threshold, Ct<40) and 150 negative. Referring to an external standard curve ($y = -3.179x + 42.28$; R2 = 0.939), defined on the basis of serial dilutions of a commercial standard (EDX SARS-CoV-2 Standard, Exact Diagnostics LLC), for each Ct, the corresponding RNA viral load was calculated and expressed in log10 copies/ml.

Antigen detection was performed by Lumipulse®G SARS-CoV-2 Ag, using the automated Lumipulse G1200 System (Fujirebio).

Samples were considered negative when SARS-CoV-2 Ag concentration was <1 pg/ml, in gray zone when ≥1.00 and <10 pg/ml and positive when ≥ 10 pg/ml, according to manufacturer's instruction.

Considering molecular test as the reference standard, Ag showed a specificity of 95.33% (143/150 samples resulted negative, 7/150 resulted positive, 6 of which, included in the gray zone) and a sensitivity of 64% (541/850 samples had a SARS-CoV-2 Ag concentration <1 pg/ml, 131/850 between 1.00 and < 10 pg/ml, and 178/850 ≥ 10 pg/ml).

SARS-CoV-2 RNA viral load distribution versus antigen concentration, showed a clear difference in mean CTs and viral loads between the three groups (Fig. 1): negative (<1 pg/ml), gray-zone (≥ 1 and < 10 pg/ml) and positive (≥10 pg/ml) antigen, corresponded to 1.84, 3.15 and 6.31 log10 copies/ml, respectively (*P value* for trend < 0.001).

Of interest, only 5/541 samples with a negative antigen value showed >4 log10 copies/ml RNA viral load (0.9%). Lumipulse Ag assay showed a remarkable high sensitivity (97.4%) when considering samples with medium-high viral load (>4 log10 copies/ml).

A strong positive correlation ($R^2 = 0.841$) was evident between RNA viral load (log10 copies/ml) and antigen concentration (log10 pg/ml).

For 278 patients, it was possible to reconstruct the history of infection and to correlate antigen detection with days after first SARS-CoV-2 detection (Fig. 2): all 58 samples with antigen levels ≥ 50 pg/ml were collected from patients tested within ten days from first positivity. Of interest, only 3/58 samples referred to antigen detected from 8 to 10 days from first positivity.

Conversely, 205/207 samples with Ag <1 pg/ml, referred to samples collected later than 10 days from the first SARS-CoV-2 detection, the remaining 2/207 samples were collected at the 9th day from diagnosis.

Finally, the 20 samples with antigen levels ≥1 pg/ml and <50 pg/ml, were distributed over a period ranging from the acute to the convalescence phase.

Both in symptomatic and asymptomatic subjects, SARS-CoV-2 RNA can be detectable up to 3,4 weeks or longer in nasopharynx.[5,6] During convalescence, in presence of low amount of RNA (Ct > 35), only in 2–5% of cases virus isolation is possible and the risk to transmit infection is negligible.[7] However, it is fundamental to find a tool able to give indication on timing of infection.

Our data indicate that Lumipulse antigen quantification allows a definition of the period of the infection: antigen levels > 50 pg/ml characterize the early/acute phase, while antigen levels <1 pg/ml the late/convalescent phase.

The 99% of samples presenting an antigen concentration <1 pg/ml (538/543) had a viral load <4 log10 copies/ml, reported as associated to a post-acute phase[8] and were collected in a late/convalescent period of the infection. Analyzing the clinical course of the infection in the patients with a viral load >4 log10 copies/ml (5/543), negative antigen NPS were collected at least two weeks after the first SARS-CoV-2 detection, thus, in the post-acute phase.

Moreover, samples with Ag concentration ≥10 pg/ml showed a strong linear correlation with the corresponding RNA viral load (R2 = 0.841). Since high viral load is related to the early stages of infection,[9] we could assume the same for antigen detection. In support to this hypothesis, all samples with antigen levels >50 pg/ml were taken within 10 days from the first positivity (infection onset).

Overall, Lumipulse® Ag results well correlate to the timing of infection, showing a net demarcation (*P value* < 0.001) be-

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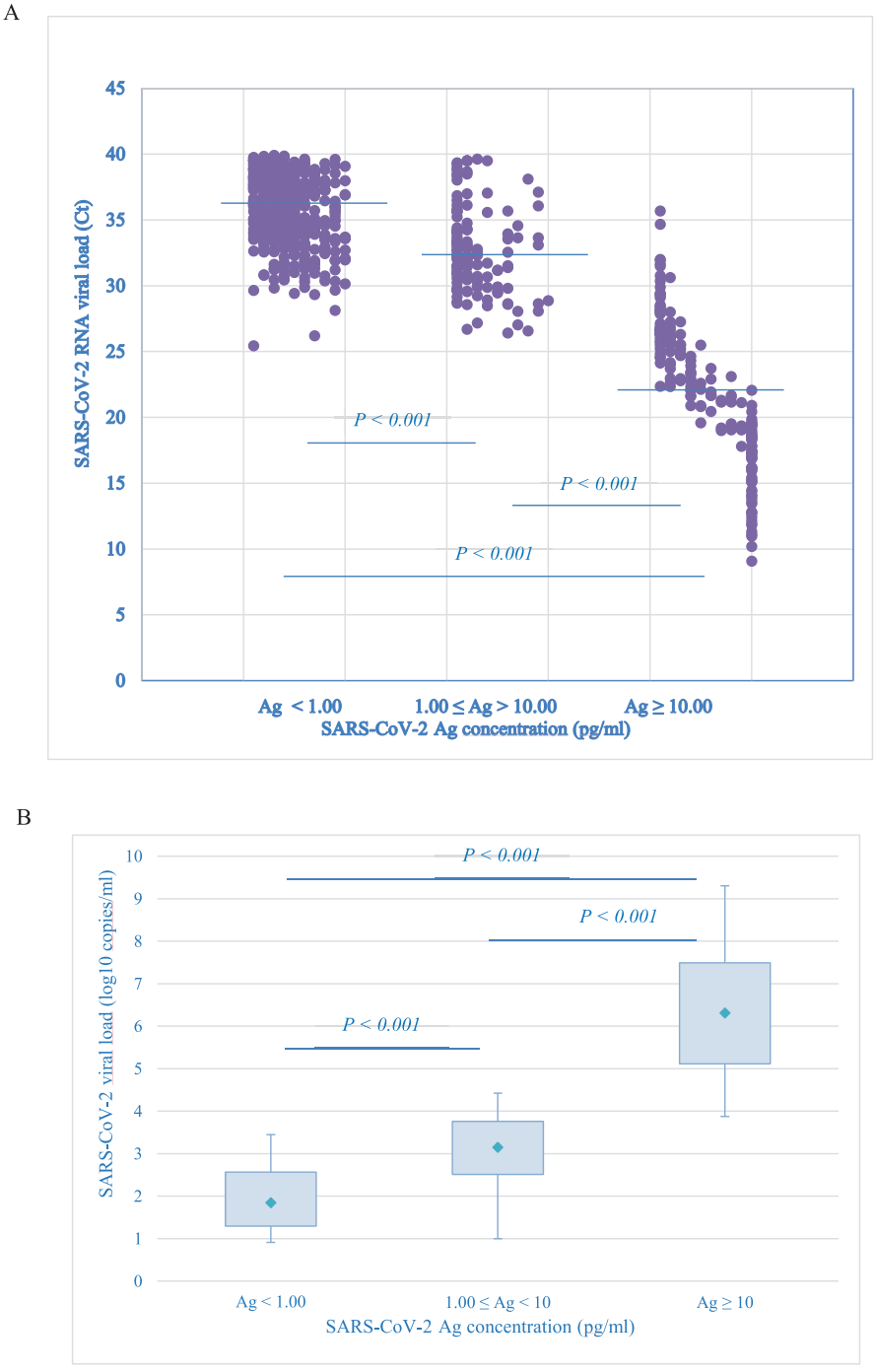

Fig. 1. SARS-CoV-2 RNA viral load (Ct or copies/ml) versus Ag concentration (pg/ml)
Samples were grouped according to antigen concentration in: Ag <1.00 pg/ml, Ag ≥ 1.00 and <10 pg/ml and Ag ≥ 10 pg/ml.
(A) Lines represent median SARS-CoV-2 RNA viral load expressed in Ct in the three groups. It was 35.86 (IQR: Ct 34.12–38.19), 32.28 (IQR: Ct 30.37–34.30) and 22.23 (IQR: Ct 18.48–26.05), respectively. *P value* for trend <0.001
(B) Box plots represent medians and quartiles of SARS-CoV-2 RNA viral load expressed in copies/ml in the three groups. It was 1.84 log10 copies/ml (IQR: 1.29–2.57 log10 copies/ml), 3.15 log10 copies/ml (IQR: 2.51–3.76 log10 copies/ml) and 6.31 log10 copies/ml (IQR: 5.11–7.49 log10 copies/ml), respectively. The ends of the box are the upper and lower quartiles, the median is marked by a rhombus inside the box. The whiskers extend to 5–95%. *P value* for trend < 0.001.
IQR abbreviation for interquartile range.

tween samples with Ag concentration >50 pg/ml, associated with early stages, and those with Ag concentration <1 pg/ml, related to late/convalescent phases.

Our results go beyond the classical utilization of the qualitative antigen test, as reported by Young et al. in their letter,[10] and offer a new and clinically relevant role for the quantitative antigen, as a parameter able to define the timing of the infection. This might be particularly useful in those patients with unknown status of infection, and/or for those without a molecular test at symptoms onset, and/or for those asymptomatic with a positive molecular test and/or for vaccinated subjects with low viral shedding.

In conclusion, while real time RT-PCR remains the cornerstone for diagnosis of SARS-CoV-2 infection, Lumipulse quantitative Ag

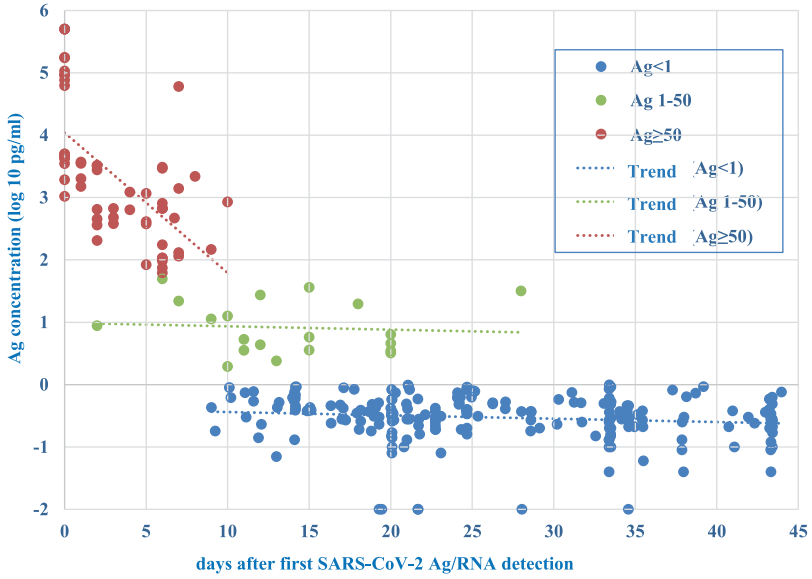

Fig. 2. Antigen concentration and timing of infection
Representation of the day of sample collection in relation to timing of infection and Ag concentration (log10 pg/ml) for 58 samples with Ag ≥ 50 pg/ml (orange dots), 20 samples with Ag ≥1 pg/ml and <50 pg/ml (gray dots) and 207 samples with Ag <1 pg/ml (blue dots). For each group, trend between Ag concentration and days after first SARS-CoV-2 Ag/RNA detection is indicated by dot line. *P value* for the two major groups < 0.001.

can be useful to define the stage of the disease. In particular, a positive molecular test with a negative Ag test can reasonably indicate a convalescent phase, identifying those subjects with low chances of being contagious.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.12.013.

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Luana Coltella, Stefania Ranno, Livia Piccioni, Giulia Linardos, Luna Colagrossi, Marilena Agosta, Cristina Russo, Carlo Concato
Department of Diagnostic and Laboratory Medicine, Unit of Microbiology and Diagnostic Immunology, Bambino Gesù Children Hospital IRCCS, Piazza Sant'Onofrio, Rome 400165, Italy

Andrea Campana
Department of Emergency, Acceptance and General Pediatrics, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio, Rome 400165, Italy

Andrea Onetti Muda
Department of Diagnostic and Laboratory Medicine, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio, Rome 400165, Italy

Alberto Villani
Department of Emergency, Acceptance and General Pediatrics, Bambino Gesù Children's Hospital, IRCCS, Piazza Sant'Onofrio, Rome 400165, Italy

Carlo Federico Perno
Department of Diagnostic and Laboratory Medicine, Unit of Microbiology and Diagnostic Immunology, Bambino Gesù Children Hospital IRCCS, Piazza Sant'Onofrio, Rome 400165, Italy

*Corresponding author.
E-mail address: stefania.ranno@opbg.net (S. Ranno)

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The COVID-19 susceptibility of cancer patients might due to the high expression of SARS-CoV-2 required host factors

Dear Editor,

Cancer patients have been disproportionately affected by the severe coronavirus disease 2019 (COVID-19) pandemic. Accumulating evidence suggests that patients with cancer are more likely to be infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and more likely develop a severe COVID-19[1]. Some recent published researches in *Journal of Infection* have shown that the susceptibility of cancer patients to SARS-CoV-2 infection is closely associated with the expression of SARS-CoV-2 cell receptors[2,3]. However, the host receptor mediated initial attachment and entry of the virion to the host cell is only the first step of SARS-CoV-2 viral life cycle, the required host factors for the other key step of viral life cycle may also impact the susceptibility to SARS-CoV-2 infection of cancer patients.

Zharko Daniloski etc.[4] have recently identified a series of required host factors for SARS-CoV-2 infection, which involve in regulating multiple steps of viral life cycle, including initial attachment (ACE2 and TMPRSS2) and endosomal entry (ACTR2, ACTR3, RAB7A, CCZ1B, ARPC3, ARPC4 and UVRAG), Spike cleavage and membrane fusion (ATP6AP1, ATP6AP2, ATP6V0B, ATP6V0C, ATP6V0D1, ATP6V1A, ATP6V1E1, ATP6V1G1, ATP6V1H, TMEM199 and CTSL), endosome recycling (VPS26A, VPS29, VPS35, SNX27, PIK3C3, WDR81, ACP5, COMMD2, COMMD3, COMMD3-BMI1 and COMMD4), viral RNA transcription (SLTM and SPEN), endoplasmic reticulum (DPM3 and ERMP1), and endoplasmic reticulum-Golgi trafficking (DPM3, ERMP1, PPID, and CHST14).

In this study, we investigated the expression patterns of all 37 SARS-CoV-2 required host factors in 31 available cancer types in the TCGA pan-cancer database from (Gene Expression Profiling Interactive Analysis) GEPIA[5] to explain the potential reasons why cancer patients have higher risks of SARS-CoV-2 infection and severe outcomes. We found that the SARS-CoV-2 required host factors are significant up-regulated in the majority of cancer types (Fig. 1), especially in Lymphoid Neoplasm Diffuse Large B-cell

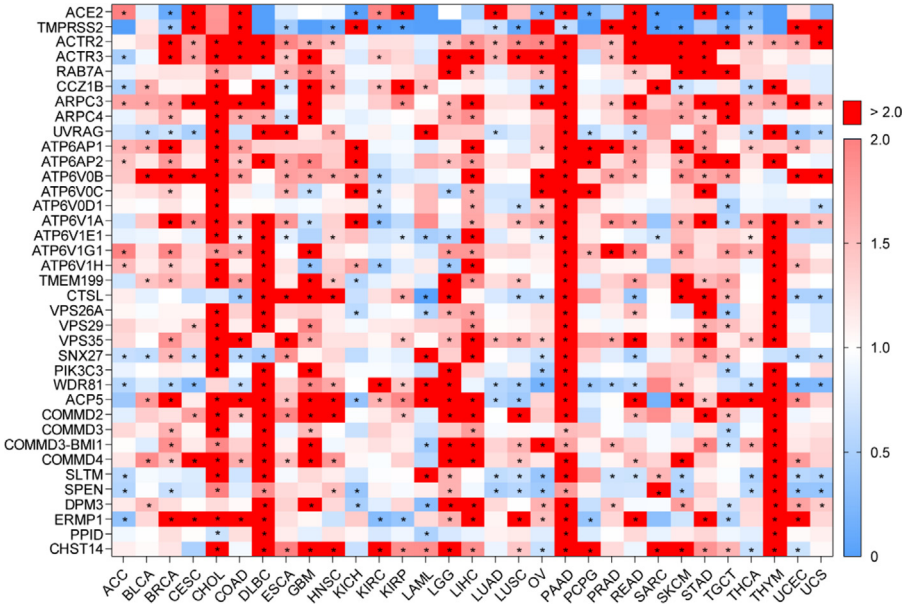

Fig. 1. Heatmap showing the difference in the 37 SARS-CoV-2 required host factors expression between the primary tumor and the normal tissues of 31 cancer types from GEPIA. Red (> 1.0) indicated up-regulation of factor expression in tumor tissues; Blue (< 1.0) indicated down-regulation of factor expression in tumor tissues; $^{*}P < 0.05$.

Abbreviations: COVID-19, the severe coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; GEPIA, Gene Expression Profiling Interactive Analysis); DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; PAAD, Pancreatic adenocarcinoma; THYM, Thymoma; CHOL, Cholangiocarcinoma.

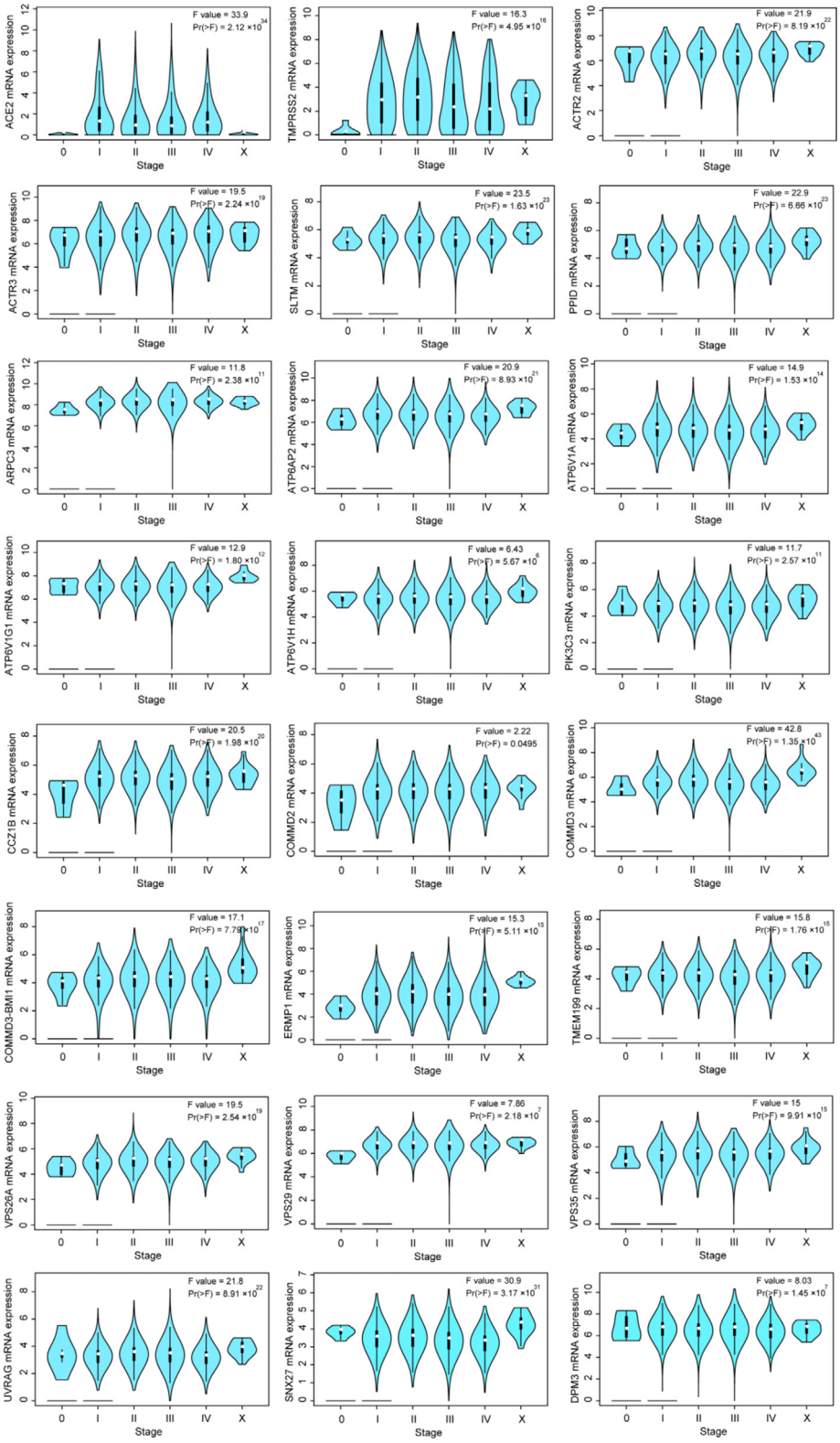

Fig. 2. Correlation between the expression levels of 24 SARS-CoV-2 required host factors and tumor stage in human pan-cancer (GEPIA).

Lymphoma (DLBC), Pancreatic adenocarcinoma (PAAD), Thymoma (THYM), Cholangiocarcinoma (CHOL), suggesting the high expression of these factors may increase the risk of SARS-CoV-2 infection in cancer patients. In contrast, many of these factors expression were decreased in female genital cancers (Fig. 1), which is consistent with the prior report that patients with female genital cancers show relatively low risks of SARS-CoV-2 infection[6].

It has been shown that patients with late-stage cancer have higher risks of SARS-CoV-2 infection and a more severe COVID-19 trajectory. Thus, we analyzed the correlation between these factors

expression and the tumor stage in human pan-cancer. Among all the 37 factors, the high expression levels of 24 factors were found to be correlated significantly with late pathological stage (Fig. 2), suggesting that the COVID-19 susceptibility of patients with late-stage cancer may due to the high expression of SARS-CoV-2 required host factors.

In summary, the up-regulation of the required host factors for SARS-COV-2 infection in tumor tissues made cancer patients more likely to be infected by SARS-COV-2. We also suggested that most of these factors expression in tumor tissues increases with tumor stage, which may be one of the underlying mechanisms mediating the high risks of SARS-CoV-2 infection and severe outcomes observed in patients with late-stage cancer. We provided new insights into the biological linkage between SARS-CoV-2 and cancer. We hoped that our findings will help develop novel therapies for all patients with COVID-19.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Geng Qin, Jie Yang, Chuanqi Zhao, Jinsong Ren, Xiaogang Qu*
Laboratory of Chemical Biology and State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry Chinese Academy of Science, Changchun, Jilin 130022, P. R. China
University of Science and Technology of China, Hefei, Anhui 230026, P. R. China

*Corresponding author.
E-mail address: xqu@ciac.ac.cn (X. Qu)

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Significant association of pre-existing asthma with an increased risk for ICU admission among COVID-19 patients: Evidence based on a meta-analysis

Dear Editor,

In this Journal, Fernadez-de-las-Penas et al. reported a similar prevalence of long-term post-coronavirus disease (COVID) symptoms in patients with asthma compared to non-asthmatics,[1] which suggests that asthma seems not to be a risk factor for more severe long-term post-COVID symptoms but also either was a "protective" factor for that. [1] We have had a valuable opportunity to carefully read this interesting paper and additional published articles regarding the relationship between pre-existing asthma and clinical outcomes of patients with coronavirus disease 2019 (COVID-19). We noticed that a number of published studies have explored the impact of pre-existing asthma on the risk for intensive care unit (ICU) admission among patients with COVID-19, however, the conclusions drawn for the previous individual studies were inconsistent. Although, several meta-analyses have been performed to address this issue, they uniformly failed to find the significant association between pre-existing asthma and the risk for ICU admission among patients with COVID-19.[2–9] To our knowledge, the previous meta-analyses regarding the association between pre-existing asthma and the risk for ICU admission in COVID-19 patients had limited number of included studies (Sunjaya et al.'s paper has the most included studies, with 21).[2] Moreover, many studies on this topic are emerging since then. Therefore, it is necessary to clarify the impact of pre-existing asthma on the risk for ICU admission among COVID-19 patients on the basis of the latest data.

This meta-analysis strictly abided by the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). An extensive search of the literature was performed in PubMed, Springer Link, Web of Science, Wiley Library, EMBASE, Scopus, Elsevier ScienceDirect and Cochrane Library to find all compliant articles published from January 1, 2020 to October 30, 2021. The following keywords were exerted on the search strategy: "COVID-19", "2019-nCoV", "SARS-CoV-2", "2019 novel coronavirus", "coronavirus disease 2019", "severe acute respiratory syndrome coronavirus 2", "asthma", "asthmatic", "ICU", "intensive care unit admission" and "ICU admission". The reference lists, cited by the included studies and relevant reviews, were eligible as an exploratory objective to identify extensive articles. The inclusion criteria included: (1) adult COVID-19 patients confirmed by reverse transcriptase-polymerase chain reaction (rt-PCR); (2) peer-reviewed original articles in English; (3) individual study populations being at least fifteen cases; (4) the key available data of the included studies, four-table data or effect (95% confidence interval (CI)), must be clearly stated. Case reports, repeated articles, review papers and preprints were eliminated.

The pooled risk ratio (RR) with corresponding 95% CI was utilized to evaluate the association between asthma and ICU admission among COVID-19 patients throughout a random-effects meta-analysis model. The heterogeneity of effect among the included studies was quantitatively presented by I^2 statistic. Sensitivity analysis was conducted to check whether the result was robust or not. The potential publication bias was evaluated by Begg's test. The package "meta" of R software (Version 4.1.1) was applied. Significant association was not admitted until two tailed $P < 0.05$.

Eventually, seventy and seven eligible articles encompassing 854,405 COVID-19 patients were included in our meta-analysis. The included studies stemmed from 26 countries distributed in five continents - North America ($n = 19$ studies), Europe ($n = 32$ studies), Asia ($n = 21$ studies), South America ($n = 4$ studies) and

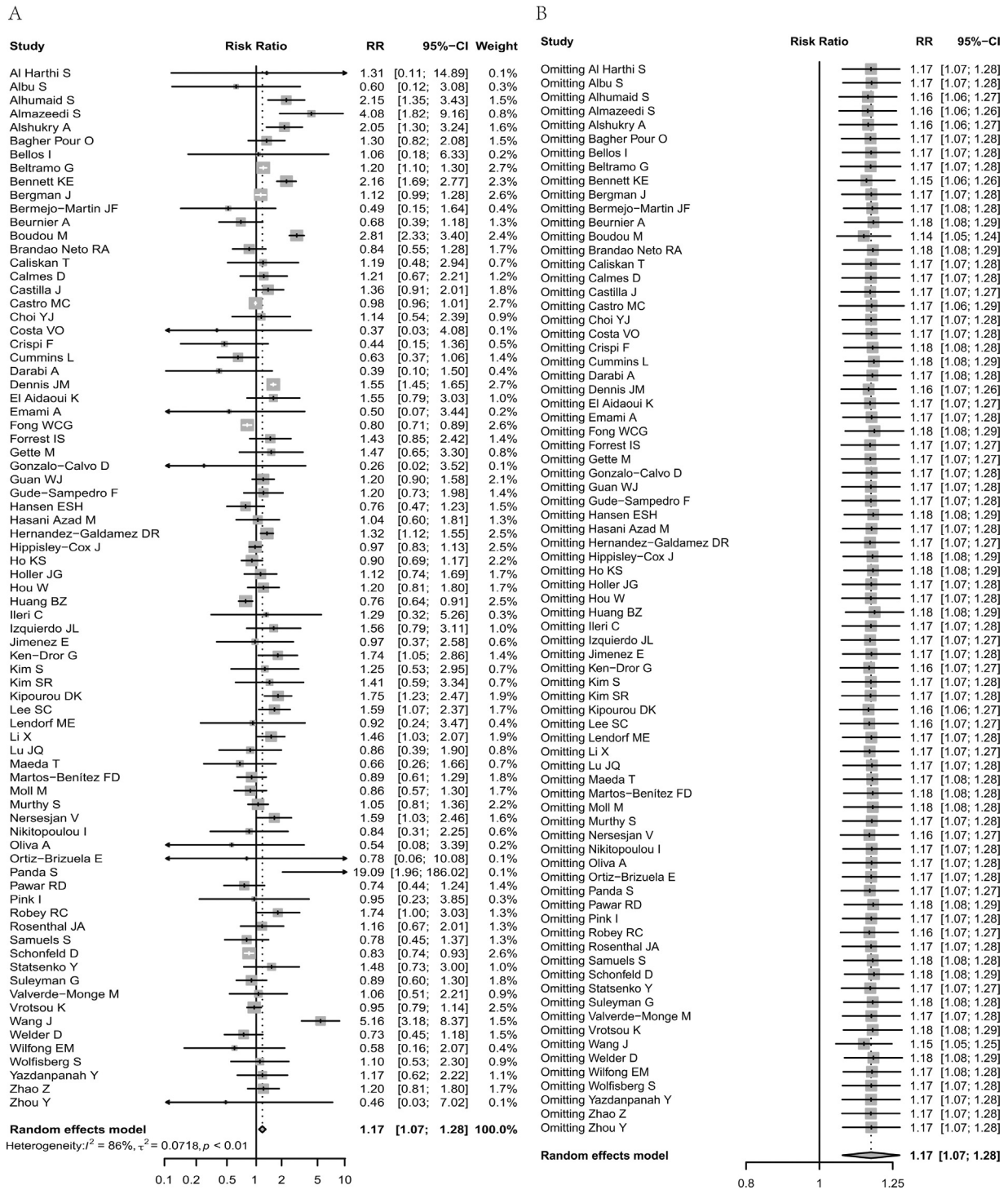

Fig. 1. (A) Forest plot indicated that coronavirus disease 2019 (COVID-19) patients with asthma had a significantly increased risk for admission to intensive care unit (ICU) compared to those without asthma: pooled risk ratio (RR) with its 95% confidence intervals (CI); (B) Sensitivity analysis for pooled RR and 95% CI by deleting one single study from overall pooled analysis each time showed that our results were robust.

Africa (n = 1 study). Seventy studies reported the association between asthma and ICU admission among hospitalized COVID-19 patients. The general information of included studies is summarized in Table 1. Overall, this present meta-analysis showed that there was a significant association between pre-existing asthma and the increased risk for ICU admission among COVID-19 patients (RR: 1.17, 95% CI: 1.07–1.28; I^2 = 86%, random-effects model) (Fig. 1A). In the further subgroup analysis by continents, we observed that COVID-19 patients with asthma were at higher risk for ICU admission compared with those without asthma in Asia (RR: 1.59, 95% CI: 1.26–2.00) and Europe (RR: 1.17, 95% CI: 1.01–1.36), rather than in South America (RR: 0.91, 95% CI: 0.78–1.04), North America (RR: 0.96, 95% CI: 0.84–1.11) and Africa (RR: 1.55, 95% CI: 0.79–3.02). When the setting of patients was restricted to hospitalization, the significant association between asthma and the increased risk for ICU admission among COVID-19 patients still existed (RR: 1.19, 95% CI: 1.09–1.31). Subsequently stratified analyses based on age, sample size, study design and male percentage

Table 1
The general information of the eligible studies in the meta-analysis.

Author	Location	Study design	Cases	Male (%)	Age	ICU		Non-ICU		Setting
						Asthma	Non-asthma	Asthma	Non-asthma	
Lee SC (PMID: 33311519)	Korea	Retrospective study	6811	NR	NR	27	163	615	6006	Hospitalized
Bergman J (PMID: 33704634)	Sweden	Nationwide study	15,872	59.4	64.1 ± 18.4	211	2283	997	12,381	Hospitalized
Castilla J (PMID: 34199198)	Spain	Prospective study	2080	51.92	NR	23	223	124	1710	Hospitalized
Choi YJ (PMID: 32978309)	Korea	Retrospective study	7590	40.8	44.5	7	208	211	7164	All patients
Gude-Sampedro F (PMID: 33349845)	Spain	Retrospective study	2492	53.13	70.2 ± 15.4	14	270	89	2119	Hospitalized
Hansen ESH (PMID: 33527079)	Denmark	Retrospective study	5104	47	54.8 (40.5–72.3)	17	299	337	4451	All patients
Martos-Benítez FD (PMID: 33411264)	Mexico	Retrospective study	38,324	58.3	46.9 ± 15.7	Effect (95% CI): 0.89 (0.61–1.28)				Hospitalized
Schonfeld D (PMID: 33571300)	Argentina	National database	41,703	53.2	55 (37–72)	269	5383	2090	33,961	Hospitalized
Dennis JM (PMID: 33097559)	UK	Retrospective study	19,256	60.1	67 ± 16.88	669	4778	929	12,880	Hospitalized
Wang J (PMID: 33332437)	China	Retrospective study	562	51.6	47 (35.0–57.0)	22	31	46	463	Hospitalized
Almazeedi S (PMID: 32766546)	Kuwait	Retrospective study	1096	81	41 (25–75)	6	36	37	1017	Hospitalized
Beurnier A (PMID: 32732333)	France	Prospective study	112	53.6	60	11	33	26	42	Hospitalized
Calmes D (PMID: 33038592)	Belgium	Retrospective study	596	50.7	58.8	10	78	47	461	Hospitalized
Emami A (PMID: 32835530)	Iran	Retrospective study	1239	55.9	51.48 ± 19.54	1	97	24	1117	Hospitalized
Fong WCG (PMID: 33626216)	UK	Retrospective study	617	NR	NR	78	495	24	20	Hospitalized
Guan WJ (PMID: 33684635)	China	Retrospective study	39,420	49.9	55.7	41	5507	203	33,669	Hospitalized
Ho KS (PMID: 33647451)	USA	Retrospective study	4902	55.9	64.99 ± 16.92	45	1005	188	3664	Hospitalized
Kim S (PMID: 33012003)	Korea	Retrospective study	2043	35	56.1	5	120	61	1857	Hospitalized
Kipourou DK (PMID: 33902520)	Kuwait	Prospective study	3995	70.4	NR	31	284	204	3476	Hospitalized
Rosenthal JA (PMID: 33059035)	USA	Retrospective study	274	NR	NR	11	57	28	178	Hospitalized
Valverde-Monge M (PMID: 34149705)	Spain	Retrospective study	2539	50.2	62.7	7	142	106	2284	Hospitalized
Ortiz-Brizuela E (PMID: 32584326)	Mexico	Prospective study	140	60.7	49.0 (39.0–61.3)	0	29	2	109	Hospitalized
Zhao Z (PMID: 32730358)	China	Retrospective study	593	60.4	58.88 ± 17.49	16	179	25	373	Hospitalized
El Aidaoui K (PMID: 33033687)	Morocco	Retrospective study	134	54.5	53 (36–64)	5	40	5	84	Hospitalized
Yazdanpanah Y (PMID: 33058220)	France	Prospective study	246	57	62 (50–73)	7	64	14	161	Hospitalized
Hippisley-Cox J (PMID: 32737124)	UK	Prospective study	19,486	48.12	62.18 ± 20.84	178	1108	2586	15,614	All patients
Ken-Dror G (PMID: 33199428)	UK	Prospective study	429	56.4	70 ± 18	13	69	29	318	Hospitalized
Bermejo-Martin JF (PMID: 33317616)	Canada	NR	200	55.5	65 ± 19.5	2	98	6	94	Hospitalized
Caliskan T (PMID: 33331576)	Turkey	Retrospective study	565	NR	48 ± 19.664	4	87	17	457	Hospitalized
Samuels S (PMID: 33409769)	USA	Retrospective study	493	51.93	62.9 ± 18.3	10	137	32	314	Hospitalized
Holler JG (PMID: 33421989)	Denmark	Cohort study	2431	54.1	69 (53–80)	20	339	102	1970	Hospitalized
Crispi F (PMID: 33536488)	Spain	Prospective study	397	50.4	47 ± 12.2	3	57	39	298	Hospitalized
Bennett KE (PMID: 33880459)	Ireland	Retrospective study	2811	57.5	NR	50	388	108	2265	Hospitalized
Cummins L (PMID: 33942510)	UK	Retrospective study	1195	62	NR	14	138	152	891	Hospitalized
Castro MC (PMID: 33947740)	Brazil	Retrospective study	465,857	56.2	61 (47–73)	4947	167,526	8639	284,745	Hospitalized
Beltramo G (PMID: 34016619)	France	Retrospective study	89,530	53.05	65 ± 20	640	2633	14,464	71,793	Hospitalized
Wolfisberg S (PMID: 34375985)	Switzerland	Retrospective study	486	65	65.9 ± 14.7	6	86	23	371	Hospitalized
Panda S (PMID: 34468994)	China, India	Retrospective study	420	66.4	37 (24–50)	Effect (95% CI): 19.09 (1.55–147.19)				Hospitalized

(*continued on next page*)

Table 1 (*continued*)

Author	Location	Study design	Cases	Male (%)	Age	ICU		Non-ICU		Setting
						Asthma	Non-asthma	Asthma	Non-asthma	
Oliva A (PMID: 34501466)	Italy	Retrospective study	97	62	65 (58–78)	1	24	6	66	Hospitalized
Boudou M (PMID: 34531478)	Ireland	Retrospective study	3781	56.5	62.2	75	540	103	3063	Hospitalized
Murthy S (PMID: 33688026)	Canada	Cohort study	188	61.2	64 (53–75)	38	290	52	431	Hospitalized
Jimenez E (PMID: 33172949)	Spain	Retrospective study	572	60.2	53	4	46	43	479	Hospitalized
Gonzalo-Calvo D (PMID: 34048985)	Spain	Prospective study	79	72.22	68.0 (56.6–77.0)	0	36	3	40	Hospitalized
Alshukry A (PMID: 33216801)	Kuwait	Retrospective study	417	62.83	45.39 ± 17.064	15	67	26	309	Hospitalized
Alhumaid S (PMID: 34030733)	Saudi Arabia	Cohort study	1014	57	47.2 ± 19.3	11	194	15	794	Hospitalized
Li X (PMID: 33194455)	USA	Retrospective study	1108	57.3	61.94 ± 18.68	23	248	43	794	Hospitalized
Brandao Neto RA (PMID: 33411707)	Brazil	Prospective study	506	57.3	60.1 ± 15.1	11	289	11	195	Hospitalized
Statsenko Y (PMID: 33637550)	United Arab Emirates	Retrospective study	560	66.25	39.0 (33.0–49.0)	7	65	31	457	Hospitalized
Huang BZ (PMID: 34389242)	USA	Retrospective study	3404	NR	NR	107	845	377	2075	Hospitalized
Nersesjan V (PMID: 33438076)	Denmark	Prospective study	61	63	62.7	3	32	0	26	Hospitalized
Lendorf ME (PMID: 32800073)	Denmark	Retrospective study	111	60	68.7 (56–78)	2	18	10	81	Hospitalized
Bellos I (PMID: 33820751)	Greece	Cohort study	42	69	56.65 ± 14.12	1	9	3	29	Hospitalized
Hasani Azad M (PMID: 34196210)	Iran	Retrospective study	2351	52.5	47.02 ± 20.4	12	216	107	2016	Hospitalized
Suleyman G (PMID: 32543702)	USA	Case series	355	46.5	61.4	19	122	34	180	Hospitalized
Pink I (PMID: 34021897)	Germany	Retrospective study	99	73.7	57	1	51	1	46	Hospitalized
Ileri C (PMID: 33501850)	Turkey	NR	140	58.6	55 ± 16	2	12	14	112	Hospitalized
Zhou Y (PMID: 33109234)	China	Retrospective study	1087	48.3	61.94 ± 18.68	0	97	11	979	Hospitalized
Welder D (PMID: 34132393)	USA	Cohort study	658	52.7	61.4	15	124	79	440	Hospitalized
Hernandez-Galdamez DR.(PMID: 32747155)	Mexico	Cross-sectional study	23,084	NR	NR	143	1563	1358	20,020	Hospitalized
Darabi A (PMID: 34476916)	Iran	Case series	400	51.2	49.2	2	66	27	305	All patients
Hou W (PMID: 33746590)	USA	Retrospective study	593	60.4	58.3	16	179	25	373	Hospitalized
Lu JQ (PMID: 33976972)	USA	Retrospective study	1307	58.2	60.86 ± 17.72	6	98	81	1122	Hospitalized
Forrest IS (PMID: 34089483)	USA	Retrospective study	688	63.5	67.2	10	153	20	505	Hospitalized
Gette M (PMID: 34070021)	France	Retrospective study	292	63.7	68 (57–81)	5	44	16	227	Hospitalized
Izquierdo JL (PMID: 33090964)	Spain	Retrospective study	10,504	52.5	58.2 ± 19.7	9	74	750	9671	All patients
Robey RC (PMID: 34278556)	UK	Retrospective study	221	61	58	13	31	30	147	Hospitalized
Kim SR (PMID: 33260724)	Korea	Retrospective study	2959	39.8	53.15 (38.64–65.87)	5	128	75	2751	Hospitalized
Bagher Pour O (PMID: 34454118)	Iran	Prospective study	226	50.4	56.36 ± 18.54	7	105	4	110	Hospitalized
Wilfong EM (PMID: 34179689)	USA	Retrospective study	128	58.6	56.0 (45.4–67.8)	2	37	9	80	Hospitalized
Costa VO (PMID: 34411145)	Brazil	Retrospective study	58	22.1	34 ± 22.1	0	39	1	18	Hospitalized
Maeda T (PMID: 32720702)	USA	Retrospective study	224	56.7	63 ± 17	4	53	19	148	Hospitalized
Vrotsou K (PMID: 33795313)	Spain	Retrospective study	14,197	38.9	53.7 ± 17.4	88	3622	266	10,221	All patients
Moll M (PMID: 32710891)	USA	Retrospective study	210	48.1	62.21 ± 16.23	15	87	20	88	Hospitalized
Pawar RD (PMID: 34133005)	USA	Cohort study	396	54.3	64.8 ± 17.0	12	110	39	235	Hospitalized
Albu S (PMID: 33998551)	Spain	Cross-sectional study	30	63.3	54 (43.8–262)	1	15	2	12	Outpatient
Al Harthi S (PMID: 34567884)	Oman	Cross-sectional study	102	77.5	49.9 ± 14.7	0	19	1	82	Hospitalized
Nikitopoulou I (PMID: 34576169)	Greece	Cohort study	116	74.1	60.5	2	67	2	45	Hospitalized

Note: The age (years) was presented as mean ± standard deviation or median (interquartile range, IQR); CI, confidence interval; ICU, intensive care unit; NR, not clearly reported; UK, The United Kingdom; USA, the United States of America.

(%) showed that COVID-19 patients with asthma had a significantly higher risk for ICU admission compared to those without asthma among studies with < 60 years old (RR: 1.26, 95% CI: 1.06–1.51), studies with ≥ 1000 cases (RR: 1.21, 95% CI: 1.08–1.37), studies with male percentage ≥ 50% (RR: 1.22, 95% CI: 1.10–1.36) and retrospective studies (RR: 1.23, 95% CI: 1.09–1.38). The forest plot of sensitivity analysis demonstrated the robustness of our findings (Fig. 1B). There was no potential publication bias in Begg's test ($P = 0.0641$).

In conclusion, our study demonstrated that pre-existing asthma was significantly associated with an increased risk for ICU admission among COVID-19 patients. Thus, COVID-19 patients with asthma should receive greater medical attention to prevent illness progression. Further well-designed studies based on risk factors-adjusted estimates are warranted to confirm our findings.

Data availability statement

The data that support the findings of this study are included in this article and available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

All authors report that they have no potential conflicts of interest.

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Xueya Han, Jie Xu, Hongjie Hou, Haiyan Yang*
Department of Epidemiology, School of Public Health, Zhengzhou University, No. 100 of Science Avenue, Zhengzhou 450001, Henan, China

Yadong Wang*
Department of Toxicology, Henan Center for Disease Control and Prevention, No. 105 of South Nongye Road, Zhengzhou 450016, Henan, China

*Corresponding authors.
E-mail addresses: yhy@zzu.edu.cn (H. Yang), wangyd76@163.com (Y. Wang)

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Large increases in SARS-CoV-2 seropositivity in children in England: Effects of the delta wave and vaccination

Dear Editor,

Recently, Whitaker et al. reported changes in SARS-CoV-2 seroprevalence in adults after introduction of a vaccination programme. Here we describe the impact of the delta wave and initiation of vaccination on seroprevalence in children.[1] Sero-epidemiological surveys are important to monitor temporal and geographical distribution of SARS-CoV-2 and provide information on asymptomatic infections. Age-stratified surveys enable monitoring of prevalence estimates in different age groups and their contribution to transmission.

The UK Health Security Agency (UKHSA, formerly PHE) along with NHS partners and academic collaborators implemented a range of national sero-surveillance programmes to monitor antibody prevalence to COVID-19 in children and young adults, which included expansion of existing collections. Here we present results from residual serum samples collected from children aged one to 17 years in England from September 2020 to October 2021.

The UKHSA Sero-epidemiology Unit (SEU) coordinates a national collection across seven NHS regions of residual serum samples from routine microbiological testing which was enhanced at the start of the pandemic to increase sample numbers and geographic representation. Overall, a total of 26 hospital trusts have participated in the main SEU collection since the start of the pandemic with an average of 200 samples from children aged one

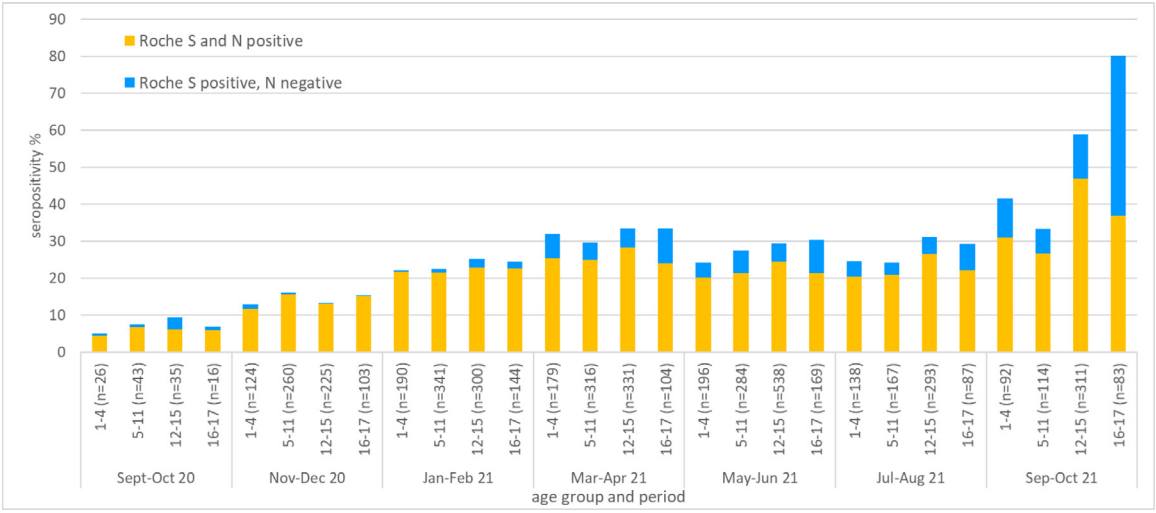

Fig. 1.. Population weighted seropositivity estimates (posterior median) of residual samples from the SEU and paediatric collections by period and age group, obtained from September 2020 to October 2021 using the Roche *S* and *N* assay. Stacked columns represent the proportion of samples testing positive with both assays (yellow) and the proportion testing positive with the Roche *S*, but negative with Roche *N* (blue).

to 17 years tested each month. In addition, a targeted paediatric collection from 18 hospitals across England providing paediatric services was established, with approximately 500 paediatric samples collected each month. Demographical data collected include age, sex and geographical region. The SEU has ethical approval for collection of anonymised samples for serosurveys of diseases for which a vaccine exists or is in active development (05/Q0505/45).

Samples were tested using two serological assays. The Roche Elecsys assay was used for detection of high avidity total antibody to SARS-CoV-2 nucleocapsid (N) protein, which informs on previous exposure to SARS-CoV-2. Sensitivity and specificity are 83.9% (95% CI 74.8–90.7) and 100% (95% CI 99.1–100), respectively in samples collected within 12 weeks of onset.[2] The Roche Elecsys assay was used to detect antibodies to SARS-CoV-2 spike (S) protein receptor binding domain with a sensitivity of 95.5% (95%CI 93.2–97.1) and specificity of 100% (95% CI 99.1–100).[3] This assay detects previous infection as well as vaccine induced immune response. As waning with assays based on S antibody detection are less pronounced than for N-based assays, analyses are focused on results from the S assay.[4]

Bayesian multilevel regression and poststratification (MRP) models[5] were used to estimate seropositivity, with poststratification by age group and NHS region based on Office for National Statistics population estimates. Analyses were carried using RStan within R.[6]

From 1st September 2020 to 31st October 2021, 5209 paediatric sera (age groups 1–4 years, $n = 945$; 5–11 years, $n = 1525$; 12–15 years, $n = 2033$; 16–17 years, $n = 706$) were obtained from the SEU and targeted paediatric collections.

The overall national prevalence estimate of seropositivity, weighted by age group and NHS region based on results from the Roche *S* assay, increased from 7.6% (95% CrI 3.2–18.2%) for the period September to October 2020 to 31.5% (25.3%–39.1%) in March and April 2021, and, after remaining stable over summer, increased to 46.1% (38.3–53.6%) in October 2021 (Fig. 1 and Table 1).

Roche *S* seropositivity varies by age with higher seropositivity persisting in children aged above 12 years at 59% (48.9–67.5%) for 12 to 15 year olds and 80.3% (69.2–88.4%) in those aged 16 to 17 in October.

Estimates based on the Roche *N* assay were largely comparable to results from the S assay from September to February 2021. However thereafter, N-based estimates were overall lower, with more pronounced increases of S-based estimates, particularly in those aged 16 to 17-years in recent months sowing an increase from 36.9% in August to 80.3% in October.

Seropositivity also varies by geographical region with the higher seropositivity in London and Northern regions compared to the South West throughout the surveillance period (see Table 1).

Our findings show large recent increases in seropositivity in children from September to October 2021 after a plateau which had persisted since the beginning of a phased exit out of national lockdown. Whilst increases of estimates in all age groups based on the Roche *N* assay indicate an increase in transmission following the start of the school academic year and is consistent with other surveillance data,[7] the more pronounced increases seen in S-based estimates during this time period in older children reflect the deployment of a vaccine programme for 16–17 year olds. Over 80% of this age group had detectable antibodies through infection and /or vaccination by October. The initial moderately higher estimates through S-based assays during the summer months is likely to reflect early waning of antibodies in the Roche *N*-assay. In comparison, in those aged 12 to 15 years for whom a vaccine has been made available at the end of the reported period there was a significant increase in N-based estimates (26.6–46.9%) in October.

Seroprevalence studies are required to understand transmission dynamics and inform on the amount of asymptomatic infection; in children, almost half of all COVID−19 infection have been shown to be asymptomatic.[8]

This study has limitations, residual samples are not collected at random but obtained from individuals undergoing diagnostic and screening tests. Individuals having to provide regular blood samples may be more vulnerable, using more precautions and thus are unlikely to be representative of the general population. However, these provide valuable information on trends over time and enable comparison with other surveillance data which show trends consistent with our findings; school based studies report large increases in the beginning of the year with a third of students seropositive by Ladhani[9] which then stabilized over summer.[10]

These findings highlight the importance of ongoing surveillance of paediatric seroprevalence to assess the extent of transmission in the paediatric population during the third wave and inform plans for future interventions, including the offer of a second dose to adolescents and expanding the paediatric programme with poten-

Table 1
Population weighted seropositivity estimates (posterior median with 95% credible interval) of residual SEU and paediatric collections samples collected September 2020 to October 2021 using the Roche *S* and *N* assays.

		Roche *S*			Roche *N*		
Period	Age, region	Pos	Total	Population weighted% pos (95% CI)	Pos	Total	Population weighted% pos (95% CI)
Sept-Oct 2020	All	8	119	7.6% (3.2% - 18.2%)	6	120	6.3% (2.2% - 17%)
	1–4	0	26	5.1% (0.4% - 16.4%)	0	26	4.5% (0.4% - 15.7%)
	5–11	3	42	7.5% (2.4% - 20.5%)	3	43	6.7% (2% - 20.3%)
	12–15	4	35	9.5% (3.3% - 25.5%)	2	35	6.1% (1.6% - 19.2%)
	16–17	1	16	7% (1.4% - 21.8%)	1	16	6% (1.3% - 20.4%)
	Lon	3	14	10.7% (3.3% - 32.4%)	3	14	10.5% (2.5% - 33.2%)
	NE	2	63	4.3% (1% - 10.2%)	1	64	2.7% (0.4% - 8%)
	NW	3	27	8.1% (2.7% - 20%)	2	27	5.6% (1.4% - 15.9%)
	SW	0	13	4.6% (0.3% - 14.6%)	0	13	3.3% (0.2% - 12.9%)
Nov-Dec 2020	All	94	711	14.8% (11.2% - 19.8%)	90	712	14.1% (10.6% - 19%)
	1–4	13	124	13% (7.6% - 19.2%)	11	124	11.7% (6.3% - 18%)
	5–11	42	259	16.2% (11.7% - 22.8%)	40	260	15.6% (11.2% - 22.1%)
	12–15	25	225	13.4% (9% - 19.1%)	25	225	13.1% (8.8% - 18.8%)
	16–17	14	103	15.5% (10.2% - 24.4%)	14	103	15.3% (9.9% - 24.4%)
	Lon	20	61	30.3% (19.8% - 42.6%)	20	61	30.3% (19.8% - 42.5%)
	Mid	3	20	13.3% (4.6% - 28.9%)	3	20	12.9% (4.3% - 28.6%)
	NE	30	206	14.5% (10.2% - 19.6%)	28	206	13.5% (9.4% - 18.5%)
	NW	32	199	15.6% (11.2% - 21%)	31	200	15% (10.6% - 20.2%)
	SW	3	35	9.2% (3.2% - 19.6%)	2	35	7% (1.9% - 16.5%)
Jan-Feb 2021	All	201	970	23.3% (18.4% - 29.5%)	182	975	22.1% (17.3% - 28.3%)
	1–4	32	190	22.2% (15.5% - 29.3%)	31	190	21.8% (15.6% - 28.7%)
	5–11	68	340	22.6% (17.1% - 29.4%)	62	341	21.6% (16.3% - 28.3%)
	12–15	70	298	25.2% (19.4% - 32.6%)	61	300	23% (17.6% - 30.2%)
	16–17	31	142	24.5% (18.3% - 32.6%)	28	144	22.8% (17% - 30.5%)
	Lon	32	88	34% (24.7% - 44.3%)	31	90	32.6% (23.6% - 42.7%)
	Mid	10	22	39.4% (21.8% - 59.6%)	10	22	39.5% (21.9% - 59.7%)
	NE	74	412	17.7% (14.3% - 21.6%)	67	413	16.1% (12.8% - 19.8%)
	NW	75	288	25.6% (20.9% - 30.8%)	66	288	22.6% (18.1% - 27.7%)
	SW	2	66	4.8% (1.3% - 11.3%)	2	68	4.5% (1.2% - 10.8%)
Mar-Apr 2021	All	288	925	31.5% (25.3% - 39.1%)	244	930	25.7% (20.1% - 33%)
	1–4	53	178	31.9% (24.3% - 40.6%)	43	179	25.4% (18.5% - 33.8%)
	5–11	87	315	29.7% (22.6% - 37.9%)	78	316	25% (18.7% - 32.8%)
	12–15	107	329	33.6% (26.4% - 42.3%)	97	331	28.3% (21.4% - 37.1%)
	16–17	41	103	33.5% (25.5% - 43.8%)	26	104	24.1% (16.4% - 32.7%)
	Lon	41	102	38% (29% - 47.7%)	35	102	33% (24.5% - 42.6%)
	Mid	9	20	39.4% (22.9% - 59.5%)	8	21	32.9% (17.8% - 52.6%)
	NE	114	489	23.1% (19.4% - 27%)	103	492	20.3% (16.9% - 24.1%)
	NW	118	267	43.3% (37.4% - 49.2%)	95	267	34.9% (29.4% - 40.7%)
	SW	4	35	15.9% (6.5% - 29%)	2	36	10.1% (2.8% - 22.2%)
May-Jun 2021	All	328	1186	27.5% (20.7% - 37.2%)	281	1187	21.8% (16.1% - 31.1%)
	1–4	43	196	24.3% (16.3% - 34.9%)	39	196	20.3% (13.6% - 30.1%)
	5–11	76	284	27.5% (19.9% - 37.9%)	63	284	21.4% (15.1% - 31.3%)
	12–15	152	537	29.4% (22% - 39.7%)	141	538	24.4% (17.7% - 34.7%)
	16–17	57	169	30.5% (22.1% - 42.2%)	38	169	21.3% (14.6% - 31.5%)
	Lon	96	211	42.6% (35.6% - 49.9%)	80	211	36.1% (29.6% - 43.1%)
	Mid	5	16	27.5% (12.4% - 48.7%)	3	16	17.4% (6% - 36.2%)
	NE	125	511	23.8% (20% - 27.9%)	120	512	22% (18.3% - 26.1%)
	NW	87	276	31.2% (25.9% - 36.9%)	69	276	24.7% (19.8% - 30.1%)
	SW	15	171	9% (5.4% - 13.9%)	9	171	5.8% (3% - 10%)
Jul-Aug 2021	All	202	685	26.4% (20.1% - 35.3%)	176	685	22.2% (16.5% - 31.4%)
	1–4	29	138	24.7% (16.8% - 34.9%)	24	138	20.5% (13.3% - 30.6%)
	5–11	39	167	24.2% (16.7% - 34.1%)	36	167	21% (14.3% - 30.9%)
	12–15	98	293	31.2% (23.1% - 41.8%)	89	293	26.6% (19% - 37.5%)
	16–17	36	87	29.2% (20.4% - 41.2%)	27	87	22.1% (14.6% - 33%)
	Lon	81	161	45.7% (37.1% - 54.4%)	72	161	41.4% (33.2% - 49.9%)
	Mid	35	111	30% (22.1% - 38.8%)	24	111	20.8% (14.1% - 28.9%)
	NE	80	354	21.3% (17.1% - 26%)	76	354	20% (15.9% - 24.6%)
	NW	3	12	24.3% (8.7% - 47.6%)	3	12	22.5% (7.5% - 46.6%)
	SW	3	45	8.6% (2.8% - 18.6%)	1	45	4.7% (0.9% - 13.3%)
Sep-Oct 2021	All	327	600	46.1% (38.3% - 53.6%)	239	600	33.5% (26.8% - 40.9%)
	1–4	42	92	41.5% (30.2% - 53.6%)	30	92	31.1% (21.7% - 41.9%)
	5–11	37	114	33.3% (24% - 44.1%)	29	114	26.7% (18.3% - 36.8%)
	12–15	182	311	59% (48.9% - 67.5%)	152	311	46.9% (37.5% - 56%)
	16–17	66	83	80.3% (69.2% - 88.4%)	28	83	36.9% (26.5% - 48.9%)
	Lon	39	63	43.3% (31.8% - 55.4%)	31	63	40.2% (29.3% - 52.8%)
	Mid	52	87	55% (45.3% - 65.1%)	34	87	36.6% (27.7% - 46.5%)
	NE	159	316	44.1% (38.2% - 50.2%)	135	316	34.4% (28.6% - 40.8%)
	NW	37	55	59.5% (47% - 72.1%)	25	55	39.4% (28.5% - 52%)
	SW	39	76	34.9% (24.7% - 46.9%)	14	76	18.2% (10.4% - 29%)

tial future availability of vaccines approved for use in children from 5 years of age. Acknowledgement: We would like to thank all hospital trusts that made this surveillance possible by providing samples throughout the pandemic.

Acknowledgement

We would like to thank all hospital trusts that made this surveillance possible by providing samples throughout the pandemic.

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Clarissa Oeser*
Immunisation and Vaccine Preventable Diseases Division, UKHSA: UK Health Security Agency, 61 Colindale Avenue, London, United Kingdom

Heather Whitaker
Statistics, Modelling and Economics Department, UKHSA, 61 Colindale Avenue, London, United Kingdom

Ezra Linley, Ray Borrow, Simon Tonge
Public Health Laboratory Manchester, Manchester Royal Infirmary, Sero-epidemiology Unit, UKHSA, Oxford Road, Manchester, United Kingdom

Colin S. Brown
HCAI, Fungal, AMR, AMU and Sepsis Division, UKHSA, 61 Colindale Avenue, London, United Kingdom

Charlotte Gower
Immunisation and Vaccine Preventable Diseases Division, UKHSA: UK Health Security Agency, 61 Colindale Avenue, London, United Kingdom

Lenesha Warrener
Reference Laboratories, UKHSA, 61 Colindale Avenue, London, United Kingdom

Kevin E. Brown, Mary Ramsay, Gayatri Amirthalingam
Immunisation and Vaccine Preventable Diseases Division, UKHSA: UK Health Security Agency, 61 Colindale Avenue, London, United Kingdom

*Corresponding author.
E-mail address: clarissa.oeser@phe.gov.uk (C. Oeser)

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The real-life performance of metagenomic next-generation sequencing in sepsis

Dear Editor,

A recent paper by Peng and colleges highlights the potential of metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid as a front-line diagnostic for pneumonia.[1] Following various severe infections (including pneumonia), a life-threatening condition of sepsis may occur and affects a large proportion of the critically ill population. Culture-based diagnostic procedure still serves as the standard way to determine causative microorganism of sepsis,[2] although it is time-consuming and the overall positivity is low.[3] mNGS is quick, unbiased and untargeted, potentially delivering more information.[4,5] Until now, the clinical performance on the identification of sepsis causative microorganisms of mNGS by analyze plasma circulating cell-free DNA is still unknown.

Here, we conducted a prospective observational study to evaluate the real-life performance of mNGS for detecting the sepsis causative microorganisms using plasma sample, and provided new evidence on the detection of causative pathogen in sepsis using this novel method. It was performed in 2 mixed intensive care units (ICUs) in China and patients with presumed sepsis onset $<$ 1 h were enrolled from July 2019 to June 2020.

Blood samples were taken respectively for both traditional blood culture (BC) and mNGS before administering antibiotics (Details in Supplementary Materials). Sepsis was managed according to the Sepsis International Guideline.[6] Standard microbiologic cultures from sites considered potential sources of infection were obtained at day0 (before starting new antimicrobial therapy), day1 and day2 (before the first dose of antimicrobial agent on that day). In case of possible intravascular catheter-related infections, at least one BC set was obtained from the catheter (along with simultaneous peripheral blood cultures). Consecutive samples were taken if further microbiologic cultures and other traditional tests were deemed necessary because of the patient's clinical course. An independent multidisciplinary panel of senior experts, including one infectious disease specialist, an intensivist, and a microbiologist, independently reviewed the culture results, the patient clinical data, relevant medical events and response to antibiotics, defined the causative microorganisms for each patient. The results of mNGS were concealed to the panel and patients' physicians.

We excluded 8 patients and enrolled 62 (Fig. S1). The three primary infection sources were the lung (48.4%), intra-abdomina

Table 1
Comparison of clinical characteristics between NGS/BC negative and positive patients.

Variables	Total (n = 62)	NGS Negative (n = 28)	Positive (n = 34)	P value	BC Negative (n = 47)	Positive (n = 15)	P value
Male, n (%) [a]	30 (51.6)	11 (38.5)	19 (60.0)	0.256	22 (46.8)	8 (53.3)	0.660
Age (years, mean ± SD)	59.8 ± 15.2	56.0 ± 14.0	62.3 ± 15.0	0.253	59.7 ± 13.9	60.0 ± 19.4	0.948
Charlson Index	2.4 ± 1.7	2.2 ± 1.2	2.5 ± 2.0	0.468	2.3±.1.7	2.6±.1.9	0.530
Underlying diseases, n (%) [a]							
Solid tumor	9 (14.5)	3 (10.7)	6 (17.6)	0.683	6 (12.8)	3 (20.0)	0.786
Diabetes mellitus	19 (30.6)	6 (21.4)	13 (38.2)	0.153	14 (29.8)	5 (33.3)	0.950
Chronic renal failure	9 (14.5)	2 (7.1)	7 (20.6)	0.257	6 (12.8)	3 (20.0)	0.786
Liver cirrhosis	4 (6.5)	2 (7.1)	2 (5.9)	0.750	3 (6.4)	1 (6.7)	0.572
Primary infection sources, n (%) [a]							
Lung	30 (48.4)	12 (42.9)	18 (52.9)	0.429	22 (46.8)	8 (53.3)	0.660
Intra-abdomina	9 (14.5)	1 (3.6)	8 (23.5)	0.063	6 (12.8)	3 (20.0)	0.786
Skin and soft tissue	9 (14.5)	7 (25.0)	2 (5.9)	0.078	8 (17.0)	1 (6.7)	0.568
Intravascular devices	5 (8.1)	2 (7.1)	3 (8.8)	0.821	3 (6.4)	2 (13.3)	0.752
Others	9 (14.5)	5 (17.9)	2 (5.9)	0.280	8 (17.0)	1 (6.7)	0.322
Laboratory data							
Procalcitonin (ng/ml, mean ± SD)	7.9 ± 21.8	1.6 ± 1.7	13.1 ± 28.5	0.037	6.8 ± 20.5	11.4 ± 25.9	0.484
Procalcitonin (ng/ml, median and IQR) [b]	1.6 (0.5–4.4)	1.1 (0.3–2.2)	2.0 (0.5–8.6)	0.043	1.5 (0.4–2.6)	1.6 (0.5–8.6)	0.379
White blood cell count ($\times 10^9$/L, mean ± SD)	13.6 ± 7.5	10.7 ± 4.1	15.9 ± 8.8	0.006	12.6 ± 7.0	16.5 ± 8.5	0.085
Lactate (mmo/l, mean ± SD)	2.8 ± 2.1	2.3 ± 1.5	3.4 ± 2.3	0.035	2.6 ± 1.6	3.2 ± 3.1	0.059
Serum albumin (g/L, mean ± SD)	30.8 ± 5.5	32.9 ± 5.5	29.1 ± 5.0	0.006	31.2 ± 5.8	29.5 ± 4.4	0.298
Serum creatinine (μmol/L, mean ± SD)	106.0 ± 73.5	99.1 ± 64.4	111.4 ± 80.3	0.524	103.7 ± 68.7	112.9 ± 88.5	0.679
Severity evaluation and intensity of care							
APACHE II Score (mean ± SD)	18.1 ± 5.6	15.8 ± 4.2	19.9 ± 6.1	0.004	17.7 ± 5.2	19.1 ± 6.9	0.404
SOFA Score (mean ± SD)	6.3 ± 2.2	5.6 ± 2.3	6.9 ± 2.0	0.017	6.3 ± 2.3	6.6 ± 2.1	0.604
Septic shock, n (%) [a]	31 (50.0)	8 (28.6)	23 (67.6)	0.002	23 (48.9)	8 (53.3)	0.767
Invasive mechanical ventilation, n (%)	47 (75.8)	18 (64.3)	29 (85.3)	0.055	35 (74.5)	12 (80.0)	0.663
Renal replacement therapy, n (%)	19 (30.6)	6 (21.4)	13 (38.2)	0.153	14 (29.8)	5 (33.3)	0.950
Outcomes							
ICU days (mean ± SD) [b]	24.4 ± 12.2	22.2 ± 11.7	26.2 ± 12.5	0.199	23.7 ± 12.0	26.5 ± 11.9	0.439
28-d mortality, n (%)	21 (33.9)	7 (25.0)	14 (41.2)	0.180	16 (34.0)	5 (33.3)	0.960
90-d mortality, n (%)	34 (54.8)	10 (35.7)	24 (70.6)	0.006	25 (53.2)	9 (60.0)	0.645

NGS, next generation sequencing; BC, blood culture; ICU, intensive care unit; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.
[a] Chi-square test
[b] Mann-Whitney U test; Unspecified: Two-independent samples t-test.

(14.5%), skin and soft tissue (14.5%). The overall 28-day and 90-day mortality was 33.9 and 54.8% (Table 1).

Positivity rate was 24.2% for BC and 54.8% for mNGS, respectively. BC and mNGS detected 10 and 31 of the 44 causative microorganisms (Table S1). mNGS had a dramatically better performance in determining causative microorganisms, as demonstrated by significant higher sensitivity (81.3% vs 28.6%), Youden's index (0.546 vs 0.101), accuracy (77.4% vs 51.6%), negative predictive value (78.6% vs 46.8%) and positive likelihood ratio (3.047 vs 1.543), and lower negative likelihood ratio (0.256 vs 0.877) when compared to BC (all $P < 0.05$, Fig. 1A). The consistency test showed that mNGS, instead of BC results did have consistency with identified causative microorganisms ($\kappa = 0.093$, $P = 0.359$ and $\kappa = 0.547$, $P < 0.001$, respectively).

As compared with mNGS–negative patients, mNGS–positive patients showed significantly higher procalcitonin, C-reactive protein, white blood cell count and lactate levels, lower albumin level, and higher severity scores, incidence of septic shock and 90-day mortality (35.7% vs 70.6%, $P < 0.01$). No such difference was observed between BC–positive and –negative patients (Table 1). The 90-day overall survival rate was lower for mNGS-positive patients than for mNGS-negative patients ($P = 0.013$, Fig. 1B), but did not differ between BC-positive and BC-negative patients ($P = 0.962$, Fig. 1C).

In this study, we reported that in patients with presumed sepsis, mNGS test performed better than traditional BC in detecting causative microorganisms of sepsis. It is a good method to differentiate patients that were more severely infected and at higher death risk, as presented by a higher level of infectious indicators, higher severity scores and 90-day mortality in mNGS-positive patients.

Due to the distributed capillaries, the live or breakdown pieces of causative microorganisms would exist in the bloodstream when the microorganisms multiply in a local site and immune system activates in sepsis patients. Bloodstream infection (BSI) occurs when large numbers of live microorganisms release with infectious symptoms. BC is not only considered as the golden standard for diagnosis of BSI but also used as an important tool to determine the primary causative microorganisms of sepsis.[3,7] It is recommend to be obtained in all patients with suspected sepsis by the guidelines.[2,6] However, it is questioned by high volume requirements and prolonged incubation time, and most importantly, the low overall positivity rate (30–40%),[3] attributable to technical shortfalls in blood culture acquisition, fastidious organisms and very low rates of viable microorganisms

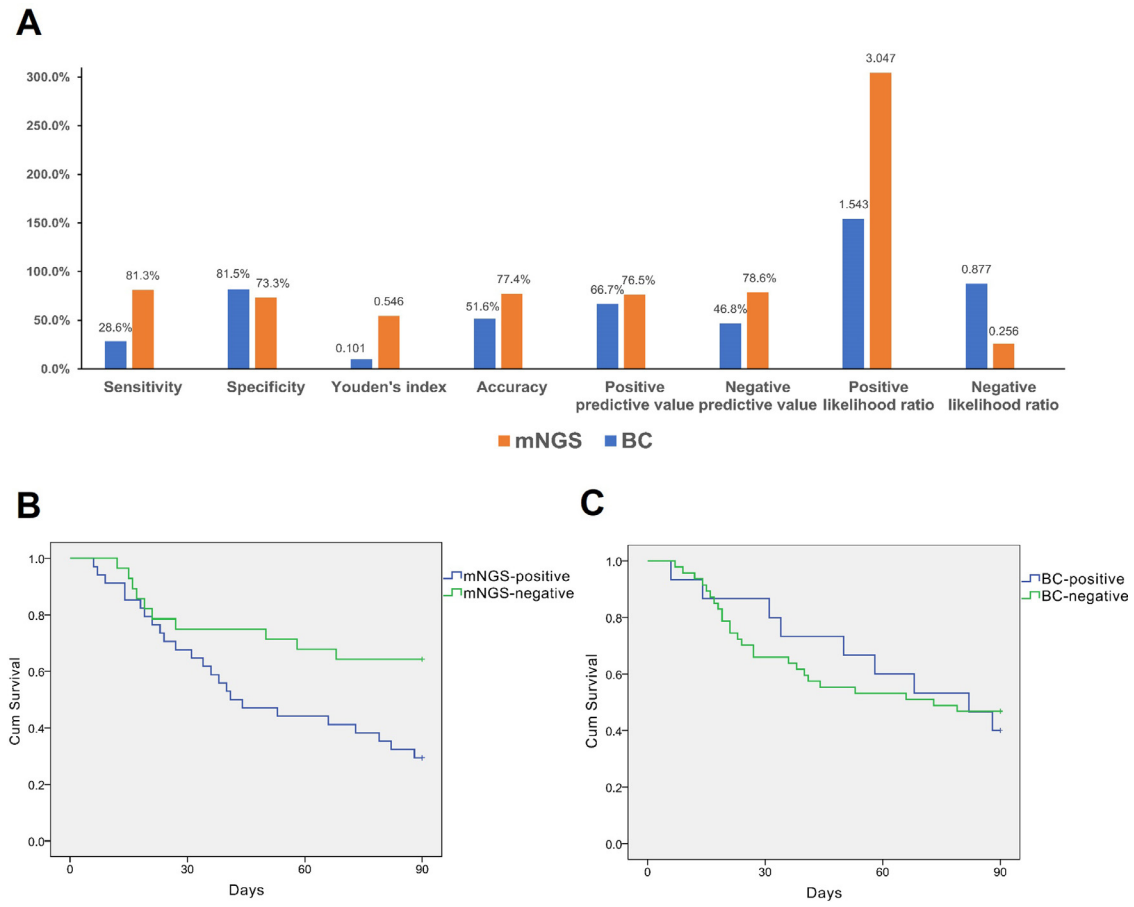

Fig. 1. The real-life performance and Kaplan-Meier survival analysis of mNGS and BC in patients with presumed sepsis.
Source: **(A)** The real-life performance of mNGS and BC to determine causative bacteria and fungi. **(B)** Kaplan-Meier analysis of survival in mNGS-positive and mNGS-negative patients, survival of patients was followed for 90 days. **(C)** Kaplan-Meier analysis of survival in BC-positive and BC-negative patients, survival of patients was followed for 90 days. ICU, intensive care unit; mNGS, metagenomic next generation sequencing; BC, blood culture.

in the blood stream.[8] In this study, we found that mNGS test showed better clinical performance in causative bacteria and fungi determination than traditional BC. Technically, even if the bacterium or fungus were largely killed under preexisting antibiotics, the remaining DNA remnants in the circulation might be sufficient for a positive mNGS result, other than a positive culture result. Grumaz and colleagues have demonstrated that mNGS results from plasma samples matches data from other specimens, such as tracheal secretion, swabs, catheter cultivation, or abdominal infection source.[5] mNGS test using plasma samples may provide more information about the sepsis than traditional cultures.

An ideal microbiological approach should have the ability to distinguish the inflammatory response to infection.[9] Furthermore, the microbiological approach is crucial for the launch of antimicrobial and prognosis evaluation. Similar to previous report,[10] BC showed an invalid prognostic value of sepsis. mNGS performed better than traditional BC: comparing with mNGS-negative patients, a more severe infectious status and a higher mortality were found in mNGS-positive patients.

In conclusion, mNGS test performed better than traditional BC in detecting causative microorganisms. mNGS-positive patients were more severe infected and at higher death risk. The implementation of the study findings will make mNGS a good tool in the sepsis microorganism determination, and may lead to an early optimized antimicrobial use which may improve patient survival.

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Ethics approval and consent to participate

This study was approved by the hospital ethics committees of Qilu Hospital of Shandong University and Second Hospital of Shandong University. Informed consent was obtained from the patient or patient's legal representative.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

Not applicable

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.018.

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Mei Yin
Shandong Key Laboratory of Cardiovascular Proteomics and Department of Geriatric Medicine, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250012, China

Yue Zheng
Department of Emergency Medicine, Shandong Provincial Clinical Research Center for Emergency and Critical Care Medicine, Institute of Emergency and Critical Care Medicine of Shandong University, China Key Laboratory of Emergency and Critical Care Medicine of Shandong Province, Chest Pain Center, Qilu Hospital of Shandong University, Jinan 250012, China

Lu Zhang
Department of Critical Care Medicine, The Second Hospital of Shandong University, Jinan 250000, China

Weidong Qin, Hui Han, Dawei Wu
Department of Critical Care Medicine, Qilu Hospital of Shandong University, 107 Wenhua-xi Road, Jinan, Shandong 250012, China

Hao Wang*
Department of Critical Care Medicine, Qilu Hospital of Shandong University, 107 Wenhua-xi Road, Jinan, Shandong 250012, China Department of Pharmacology, School of Basic Medical Sciences, Shandong University, Jinan 250012, China

*Corresponding author: Department of Critical Care Medicine, Qilu Hospital of Shandong University, 107 Wenhua-xi Road, Jinan, Shandong 250012, China.
E-mail address: wanghao34@126.com (H. Wang)

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Efficacy and safety of tocilizumab in hospitalized COVID-19 patients: A systematic review and meta-analysis

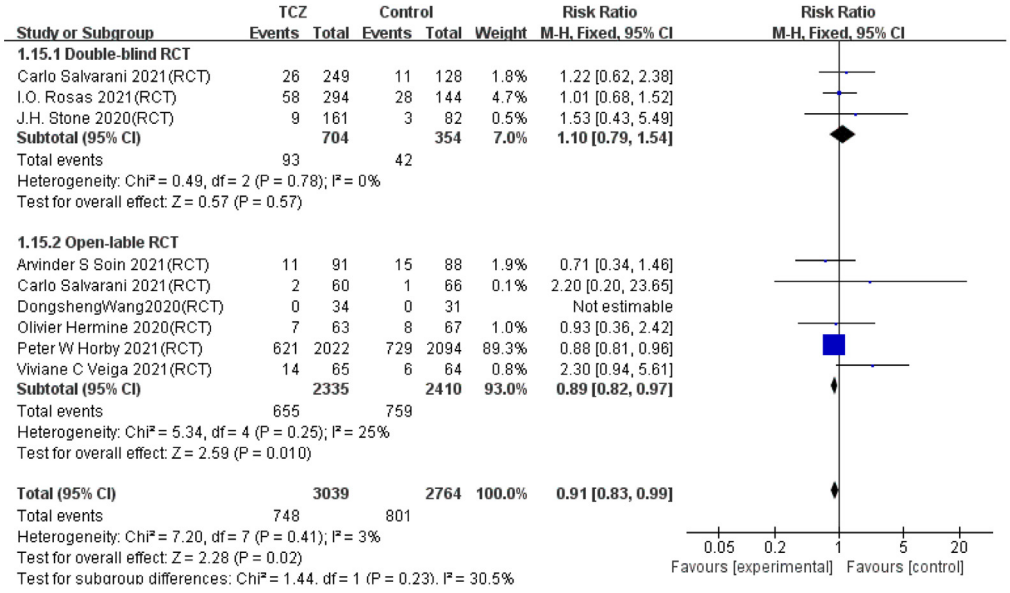
Dear Editor,

Highly infectious Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)-caused Coronavirus Disease 2019 (COVID-19) has brought about massive medical and economic burdens on communities worldwide. Accumulating evidence suggests that interleukin-6 (IL-6) are closely associated with the deteriorating health of COVID-19 patients and their deaths.[1] Tocilizumab (TCZ), an IL-6 receptor inhibitor, therefore, was proposed to be a promising candidate for COVID-19 therapy. Numerous randomized clinical trials (RCTs) and cohort studies on the efficacy and safety of TCZ in hospitalized COVID-19 patients have been published, and these, as would be expected, bear contradictory findings. Those early meta-analyses had limited value to the broader picture of the pandemic because they mostly assessed retrospective cohort studies, or scrutinized available published or preprinted RCTs alone or along with observational studies.[2–4] Given that more RCTs and cohort studies have been published recently, we conducted a updated meta-analysis, by systematically searching common databases between 2019 and August 11, 2021.

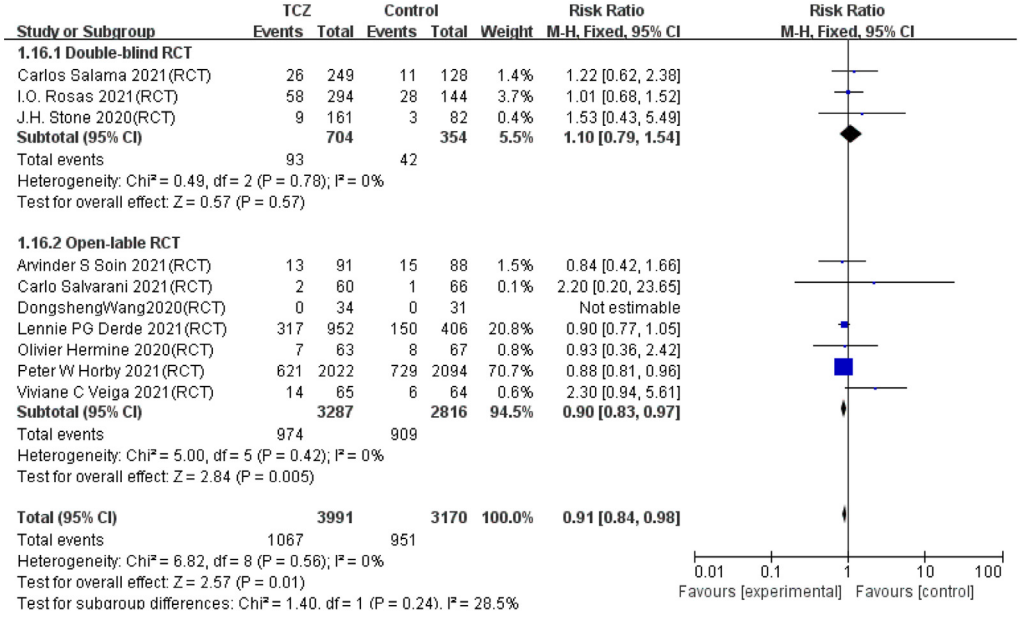
A total of 53 articles with 21,656 patients were identified, including 11 articles on 10 RCTs and 42 cohort studies. Detailed characteristics of the RCTs and cohort studies are described, respectively in Supplementary Table 1 and 2. Results from 9 RCTs showed that TCZ decreased 28–30 days mortality (Relative Risk [RR]=0.91; 95% confidence interval [CI], 0.83–0.99; $P = 0.02$; I^2=3%) (Fig. 1A). Additionally, TCZ administration in 9 RCTs instigated improved overall mortality (RR=0.91 [0.84–0.98]; $P = 0.01$; I^2=0%) (Fig. 1B). However, the largest RCT (RECOVERY NCT04381936)(5) greatly interfered with the pooled result on short-term and overall mortality, possibly because its sample size was much larger than those of other RCTs. Analyses of the 42 cohorts yielded consistent results that TCZ significantly reduced the short-term and overall mortality (Fig. 2A and B). Furthermore, TCZ decreased the risk of mechanical ventilation in RCTs (RR=0.81[0.70–0.95]; $P = 0.009$; I^2=0%) (Fig. 1C) but not in cohorts (RR= 1.17 [0.73–1.87]; $P = 0.51$; I^2=72%) (Fig. 2C). RCTs data revealed TCZ had no risk of increased secondary infection (RR=0.74 [0.50–1.08]; $P = 0.12$; I^2=42%) (Fig. 1D) and severe adverse events (SAE) (RR=0.96 [0.83–1.11]; $P = 0.59$; I^2=0%) (Fig. 1E), as did the cohorts with a pooled RR for secondary infection of 1.21 [0.90–1.61] ($P = 0.21$; I^2=84%) (Fig. 2D).

Our findings that TCZ was associated with a decreased risk of death in both RCTs and cohort studies were partly inconsistent with the conclusions of several other recent meta-analyses, possibly because our meta-analysis enrolled more RCTs and cohorts with larger sample sizes than those previous meta-analyses.[4–6]

A: 28-30 days mortality



B: overall mortality



C: progression to mechanical ventilation

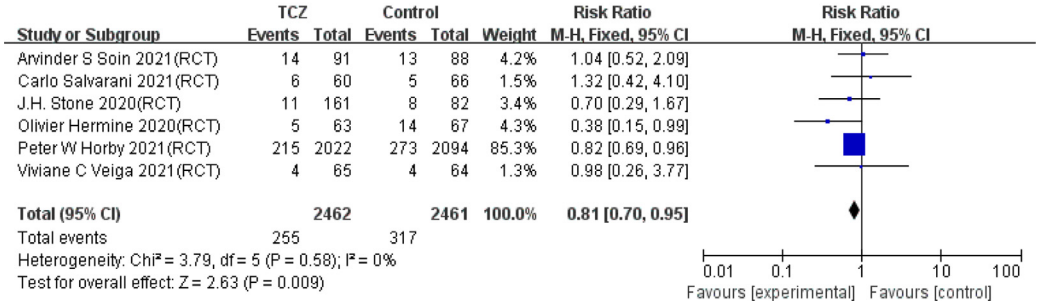


Fig. 1. Forest plot for the efficacy and safety of tocilizumab in randomized controlled trials. (A) 28–30 days mortality. (B) overall mortality. (C) Progression to mechanical ventilation. (D) Secondary infection. (E) Severe adverse events.

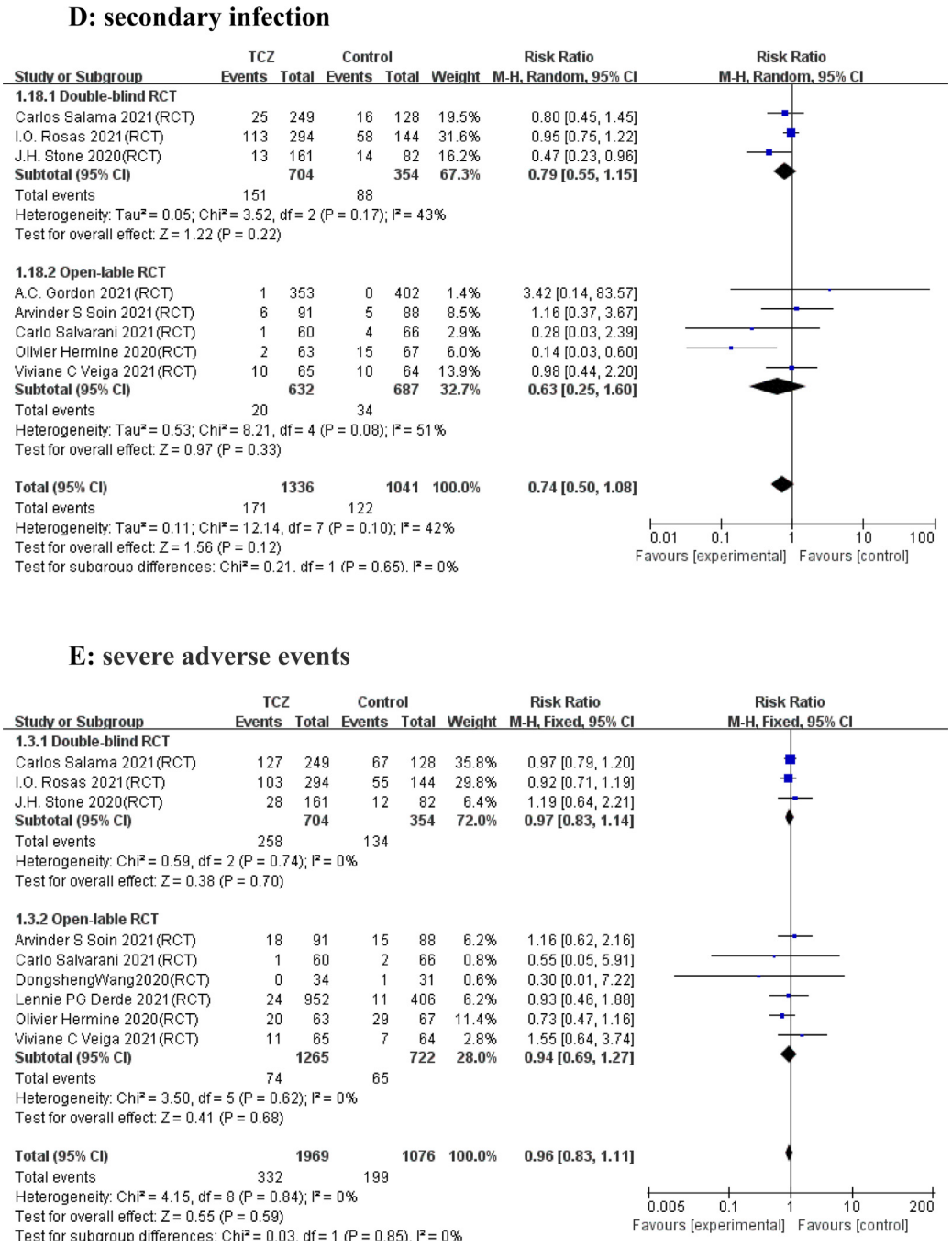


Fig. 1. Continued

A recent prospective meta-analysis with more unpublished data from ongoing RCTs, was consistent with our meta-analysis on the beneficial effect of TCZ on 28–30 days mortality.[7] The beneficial outcome of TCZ in critical COVID-19 patients can perhaps be attributed to its efficacy in interfering with the cytokine release syndrome. All the recent meta-analyses, including ours, have found no TCZ-induced increase in the risk of secondary infections,[4,6,7] for that TCZ possibly only inhibits IL-6-impacted immune responses and does not interfere with the functioning of immune processes that might help the body fight COVID-19.[8] In conclusion, TCZ improves COVID-19 patient outcomes without increasing SAE compared to usual care or placebo.

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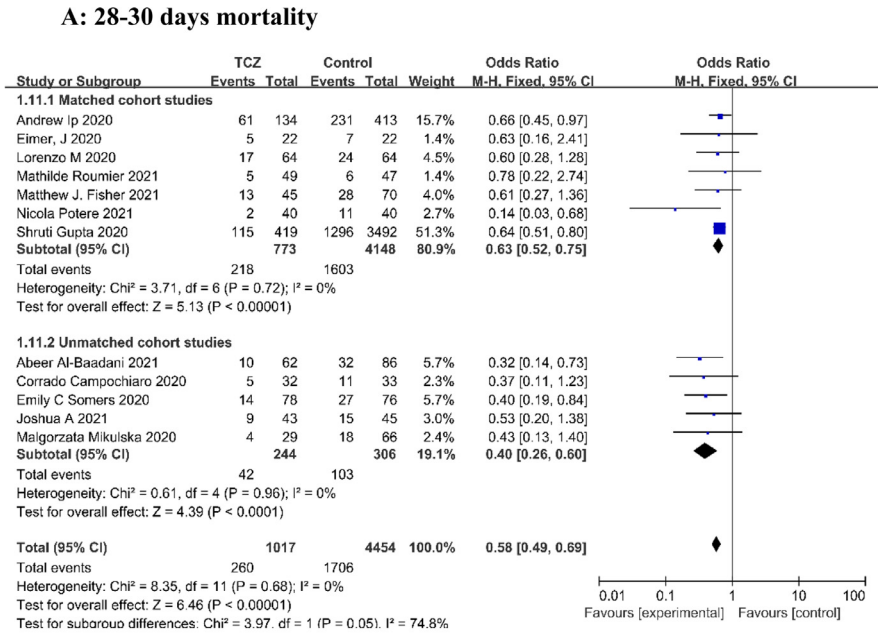


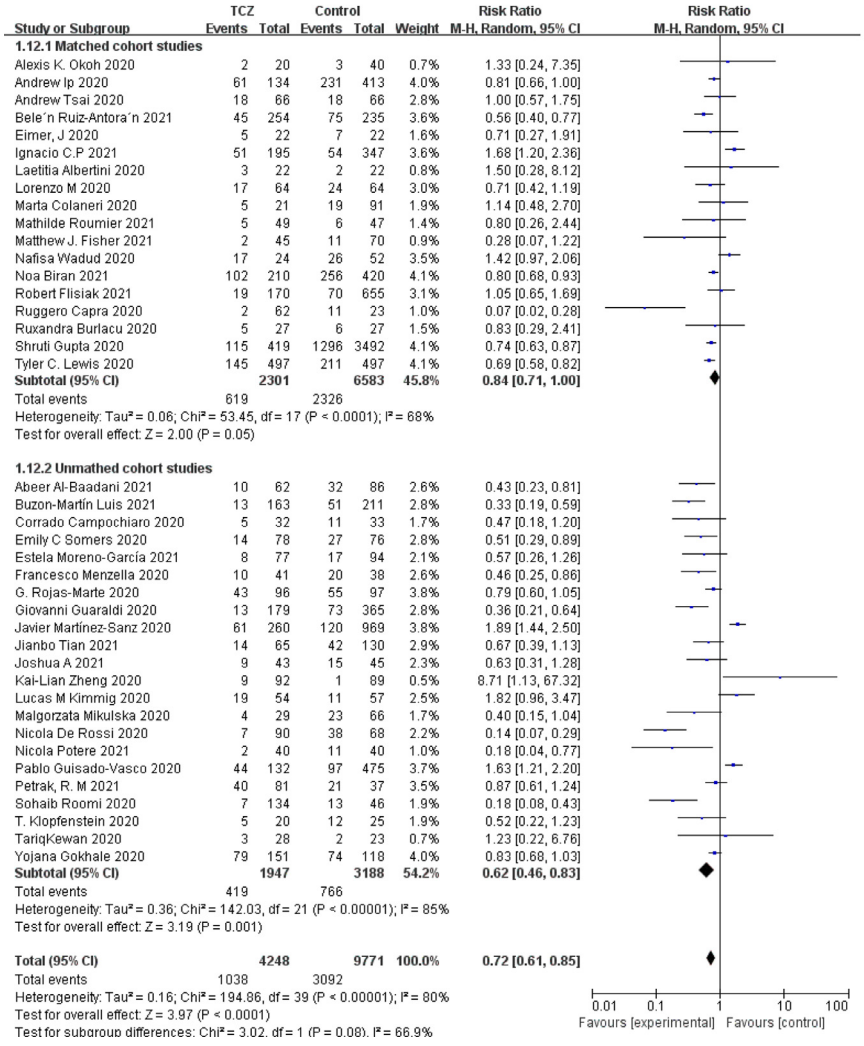
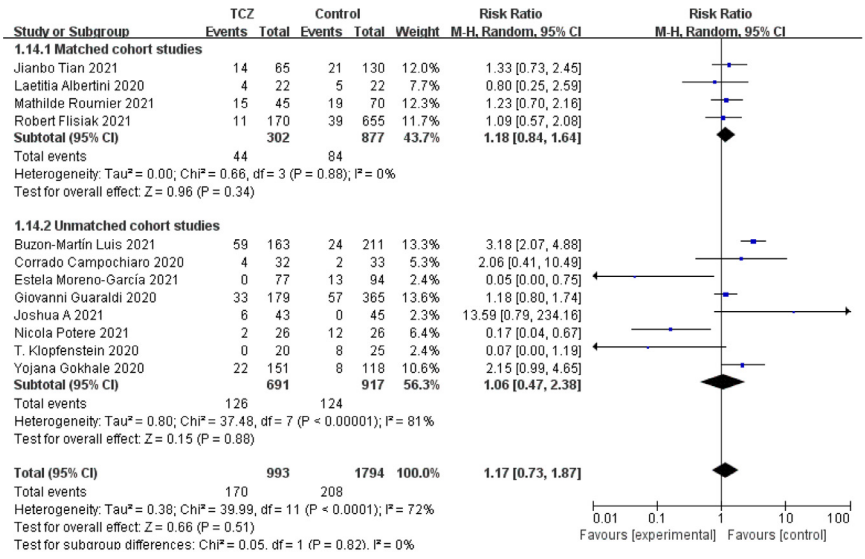


Fig. 2. Forest plot for the efficacy and safety of tocilizumab in cohort studies. (A) 28–30 days mortality. (B) overall mortality. (C) progression to mechanical ventilation. (D) secondary infection.

C: progression to mechanical ventilation



D: secondary infection

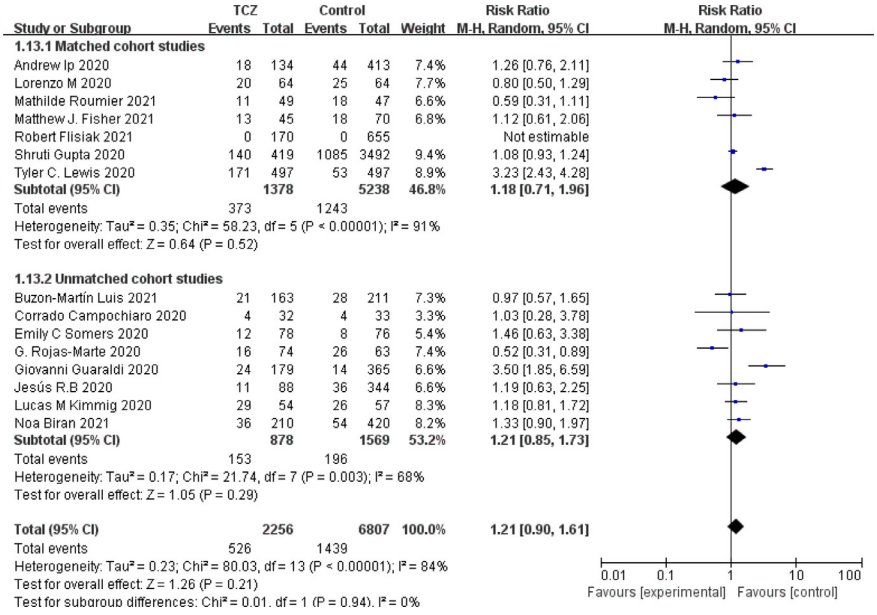


Fig. 2. Continued

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.013.

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Lili Luo[1]
Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Ting Luo[1]
Nephrology Division, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China

Mengyi Du, Heng Mei*, Yu Hu*
Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

*Corresponding authors.
E-mail addresses: hmei@hust.edu.cn (H. Mei), dr_huyu@126.com (Y. Hu)

[1] These authors contributed equally to this work.

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Alpha (B.1.1.7) and Delta (B.1.617.2 – AY.40) SARS-CoV-2 variants present strong neutralization decay at M4 post-vaccination and a faster replication rates than D614G (B.1) lineage

Dear Editor,

We read with interest the article by Dimeglio et al.,[1] showing the importance of the SARS-CoV-2 anti-S antibody response. Along with previous results showing a strong decrease of antibody and neutralization titers after vaccination,[2,3] especially for Gamma variant, their results highlight the usefulness of assessing antibody levels among HealthCare Workers (HCWs) and to monitor the effects of emerging SARS-CoV-2 variants. Likewise, the article by Douxfils et al.,[4] demonstrated post-vaccination decreasing neutralizing antibodies titers and highlighted the difficulty to obtain a definite protection threshold. They also could not verified the effects of Alpha and Delta variants. Recently, Goldberg et al.,[3] assessed Delta breakthrough infections among a large vaccinated population and demonstrated a decreasing protection in all age groups a few months after receiving a full vaccination scheme. To date, little data are still available regarding comparative neutralizing capability of antibodies against Alpha and Delta variants several months after vaccination schemes. Moreover, the infectious capacity of the Delta variant, that could explain in part its epidemiological success, is not characterized. In this study, we investigated the replicative cycle of Delta variant compared to the Alpha and B.1 variants. In addition, we also provided new elements and live virus neutralisation data that strengthen the analysis of vaccinated HCWs antibody responses up to 4 months after the second vaccine dose.

Replication kinetic was performed in triplicates on Vero E6 (ATCC, R CRL-1586) and A549 expressing ACE2 and TMPRSS2 receptors (InvivoGen, a549-hace2tpsa) by analyzing SARS-CoV-2 infectious titers, RNA and N antigen levels. The neutralizing titers of 45 sera from 9 BNT162b2 vaccinated HCWs, up to 5 months after the first vaccine dose, were analyzed by live virus neutralization assays with B.1 (EPI_ISL_4537783), Alpha (EPI_ISL_4536454) and Delta (EPI_ISL_4536228) strains. Decomplemented sera were subjected to serial two-fold dilutions (1:25 to 1:12800), incubated with 50μL of diluted virus at 2×10^3 PFU/mL in a 96-well plate at 37 °C, 5% CO_2 for 60 min. Then 3×10^4 cells Vero E6 cells were added to each well before being incubated at 37 °C, 5% CO_2.

Lower titers of infectious particles production were observed after 24 h for strain B.1 (titers comprised on VeroE6 from 2 to 220 PFU/mL for each replicate) than both Alpha and Delta strains (from 4000 to 40000 PFU/mL on VeroE6, $p = 0.04$) (Fig. 1). The viral RNA production in culture supernatants were also similar at 24h post-infection for Alpha and Delta variants but approximately 10 times higher than strain B.1 with both cell models (Vero E6: $p = 0.02$; A549: $p = 0.03$). Similar results were obtained with N antigen quantification but as soon as 15 h after cell infection with similar titers for Alpha and Delta variants and higher than with B.1 (Vero E6: $p = 0.02$; A549: $p = 0.02$). This difference last up to 48 h (Vero E6: $p = 0.02$; A549: $p = 0.02$) (Fig. 1). For all our measurements, a plateau with similar production levels was obtained for all variants since 72 hours.

We also evaluated the level of antibodies neutralization for the Delta strain after full BNT162b2 vaccination. Live virus neutralization showed a maximum neutralization titer one month after the second dose of vaccine for all 9 HCWs. The median dilution of neutralizing sera were similar or slightly lower with Delta and

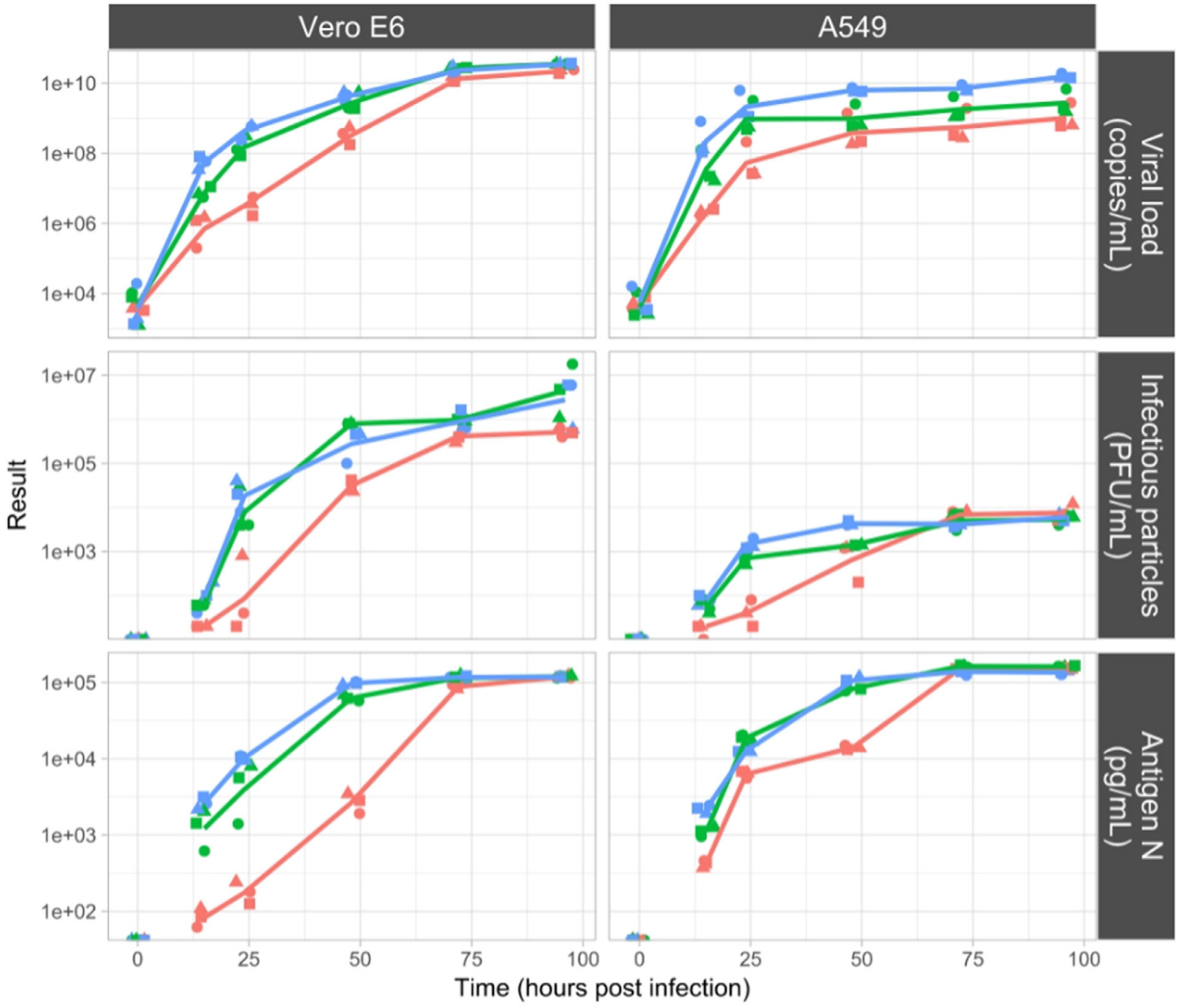

Fig. 1. Evaluation of viral production kinetics for Delta (B.1.617.2 – AY.40) strain, in blue, Alpha (B.1.1.7), in green and B.1, in red. Viral production has been assessed on two cell lines, Vero E6 and A549, and by RT-qPCR for RNA viral load, plaque assay for infectious particles and ELISA for N antigen production assessment. Results were obtained in triplicates (indicated by dot shapes).

Alpha, at 1:6400 [InterQuartile Range (IQR): 1:3200–1:6400] and 1:6400 [1:3200–1:12800], respectively, than with the B.1 strain, at 1:12800 [1:6400–1:12800]. Four months after the second dose of vaccine, all tested sera showed a decreasing neutralization activity, still slightly lower with Delta, at a median of 1:400 [IQR: 1:200–1:800], 1:100 [1:50–1:200] and 1:25 [1:25–1:50] for B.1, Alpha and Delta, respectively (Fig. 2).

Many efforts are focusing on vaccines efficacy against various variants,[5,6] a cornerstone for public health policies. However, live-virus neutralization follow-up data are still scarce today. A recent work demonstrated a 3–5-fold decreased Delta susceptibility 5 weeks after the second BNT162b2 vaccine dose compared to Alpha variant.[7] In the present study, we confirm and strengthen those results by observing strongly decreased neutralizing titers 4 months after the second BNT162b2 vaccine injection with Delta, but also Alpha and B.1 variants.

Other important concerns about Delta variant, especially regarding its epidemiological success, are the potential differences on the global viral fitness that has not been characterized to date. Here, we investigated the viral replication kinetics of B.1, Alpha and Delta variants. We evidenced a shorter replication cycle and a quicker production rate of infectious viral particles with both Alpha and Delta strains than with B.1. The 10-times higher *in vitro* replication levels from 24 to 48 h post-infection, observed with Alpha and Delta strains, are in line with its higher viral loads and 24 h earlier consultation observed among infected patients.[8–10] The Delta variant, which quickly replaced the Alpha and other variants, presents the same *in vitro* replication profile than the Alpha strain (Fig. 1). Thus, if a shorter replication rate could have helped the Alpha strain to successfully emerge over historical variants, and could be a pre-requisite for future variant expansion, other factors must have participated to the emergence of Delta over Alpha. The lower sensitivity to neutralizing antibody is probably a part of this equation.

In conclusion, we highlight a shorter replication cycle and a quicker production of viral materials and infectious particles with Alpha and Delta lineages compared to B.1 lineage. This is expected to play a role and explain in part the higher viral loads, higher transmissibility, and the large epidemiological success of Alpha and Delta variants. We also report a decreased neutralizing titers of

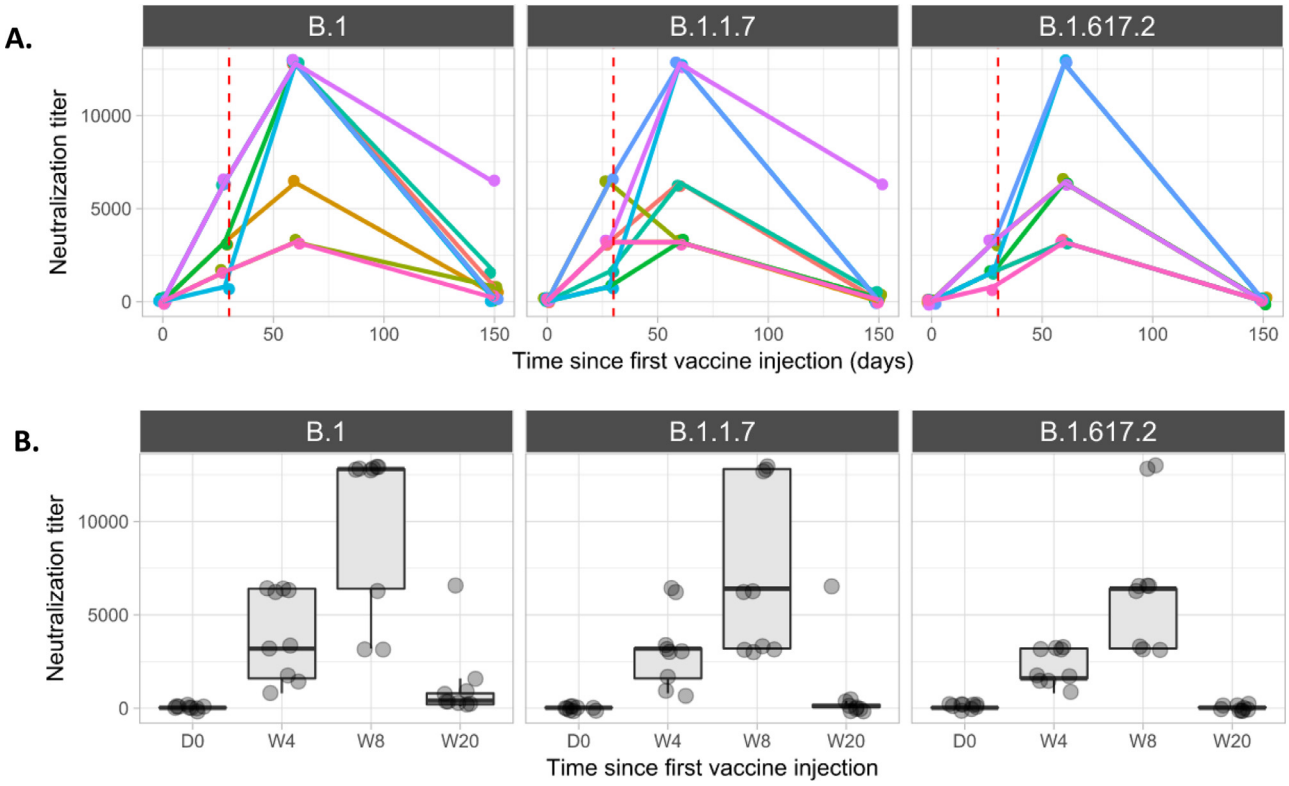

Fig. 2. Seroneutralization titers obtained among vaccinated healthcare workers for B.1, Alpha (B.1.1.7) and Delta (B.1.617.2 – AY.40) strains up to 4 months after the second vaccine dose. The A panel present the seroneutralization titers kinetics for each vaccinated HCWs. The administration of the second dose, 30 days after the first dose, is indicated by the vertical dashed line. The B panel represent median and interquartiles at each evaluated time point (*i.e.* day 0, week 4, week 8 and week 20 after the first vaccine dose).

BNT162b2 vaccine elicited antibodies against Delta correlated with a decreased sera neutralizing activity four months after complete vaccine scheme in HCWs for all tested strains. These observations highlight the question of vaccine humoral protection lasting and enhance the need for close cellular immunity evaluations against new variants.

Funding

None declared.

Declaration of Competing Interest

The authors have no relevant competing interest to disclose in relation to this work.

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Samuel Lebourgeois, Reyene Menidjel, Houssem Redha Chenane
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France

Valentine Marie Ferré, Gilles Collin, Florence Damond
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France
AP-HP, Service de Virologie, University Hospital Bichat-Claude Bernard, 16, rue Henri Huchard, Paris, France

Romain Coppée
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France

Yazdan Yazdanpanah
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France
AP-HP, Service des Maladies Infectieuses et Tropicales, University Hospital Bichat-Claude Bernard, Paris, France

Jean-François Timsit
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France
AP-HP, Service de Réanimation Médicale et Infectieuses, University Hospital Bichat-Claude Bernard, Paris, France

Nadhira Houhou-Fidouh
AP-HP, Service de Virologie, University Hospital Bichat-Claude Bernard, 16, rue Henri Huchard, Paris, France

Diane Descamps, Charlotte Charpentier, Benoit Visseaux
Service de Virologie, Hôpital Bichat Claude Bernard, Université de Paris, IAME, INSERM, 46, rue Henri Huchard, Paris F-75018, France
AP-HP, Service de Virologie, University Hospital Bichat-Claude Bernard, 16, rue Henri Huchard, Paris, France

*Corresponding author.
E-mail address: samuel.lebourgois@inserm.fr (S. Lebourgeois)

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Quantification and prognostic significance of interferon-γ secreting SARS-CoV-2 responsive T cells in hospitalized patients with acute COVID-19

Dear Editor,

We read with interest the article by Martin-Vicente and colleagues, who found no differences in SARS-CoV-2-specific immune humoral responses between patients with well controlled HIV and healthy controls. However, little remains known about the T-cell response during acute COVID-19. We therefore investigated the systemic T-cell response during acute SARS-CoV-2 infection in a hospitalised cohort of patients with COVID-19, using a functional T-cell assay developed to measure T-cell responses to four antigenic domains of SARS-CoV-2.

We conducted a prospective observational cohort study of hospitalised and nosocomially infected adult patients at University Hospitals of Leicester NHS Trust. Patients were eligible if they were 16 years or over, tested positive for SARS-COV-2 on nasopharyngeal RT-PCR using the hospital assay, with no previous history or record of infection and no existing conditions or treatments associated with T-cell immunodeficiency. One 10 ml lithium heparin anticoagulated blood for T-cell functional assay and one 6 ml blood anticoagulated using EDTA to measure antibody response was taken from each study participant within 24 h of a positive routine PCR test. Serology was performed using the commercially available SARS-CoV-2 Total Assay manufactured by Siemens, which detects IgG and IgM to the S1 RBD antigen and gave a qualitative result.[1] To measure T-cell responses, we used the T-SPOT® Discovery SARS-CoV-2 kit (T-SPOT), which uses an ELISpot technology to detect IFN-γ release from T-cells after exposure to four SARS-CoV-2 peptides antigens: Spike protein S1 and S2 domains, Membrane and Nucleoprotein peptides.[2] Routine clinical, radiological, laboratory and demographic data at the time of sampling was collected and prospective outcomes during admission including requirement for CPAP, invasive ventilation and 28 day mortality, were recorded.

Between 8th February and 8th March 2021, 114 participants were recruited into our study. Table 1 shows participant demographic data. The median age was 64 (IQR 52–78). Most participants were (91%) were symptomatic (fever, cough, breathlessness, anosmia) at time of sampling. 31% of patients had received one dose of either the Pfizer BioNTech or Oxford AstraZeneca vaccine (n = 36, 31%) in the weeks prior to acute infection; 29 had received their vaccine 2 weeks or longer prior to admission. The median duration of symptoms prior to sampling was 10 days (IQR 7 to 15). Almost all patients were antibody positive at time of sampling (n = 95, 93%). 84 (73%) participants received oxygen during hospitalisation; a fifth required continuous positive airway pressure (CPAP) in the days following blood sampling (n = 24, 21%) for progressive respiratory failure. None required mechanical ventilation. 7 (6%) study participants died within 28 days of hospital admission.

Of 87 participants who had a valid T-SPOT assay reading, the responses to the spike protein antigens S1 and S2 were most sensitive, being positive in the highest proportion of participants and at the greatest amplitude. The median T-SPOT for S1 was 5 spots (IQR 2 to 54); S2: 5 spots (IQR 2 to 22); Nucleocapsid: 3 spots (IQR 0 to 7); Membrane: 3 spots (IQR 1 to 10). Strong correlation was observed between the response to S1 and responses to the other three antigens (Pearson's correlation coefficient to S2: 0.54, $p < 0.001$; Nucleocapsid: 0.32, $p = 0.003$; Membrane: 0.51, $p < 0.001$.) However, there was little concordance between T-SPOT responses and the antibody assay (Pearson's correlation coefficient between S1 and Antibody: 0.06, $p = 0.27$). We observed no association of T-cell responses with either prior vaccination status or interval after symptoms onset. The T-SPOT assay was also positive in the 12 asymptomatic patients and could be detected within 3 days of a positive PCR test. Finally, we noted that patients with higher T-cell responses to S1 protein were more likely to receive CPAP prospectively during hospitalization, following sampling (Fig. 1).

Our study is the first to evaluate T-cell responses using the T-SPOT assay in hospitalized patients with acute COVID-19. We found that T-cell responses appeared as early as two days after symptom onset in early COVID-19, and can be positive in the context of a negative combined antibody assay. T-cell responses did not differ according to vaccination status and appeared to be related to a more severe disease phenotype. Previous studies have assessed the utility of the T-SPOT assay in convalescent patients and found that T-SPOT responses to the same proteins in this study were seen in the absence of anti-Spike IgG on long-term follow-up. We found similar levels of discordance in patients with acute COVID-19, highlighting the potential of the T-SPOT assay to pick up immunological responses in COVID-19 positive patients where antibody responses are negative. T-SPOT responses were also much higher in studies in convalescent individuals, highlighting clear differences in the

Table 1
Participant demographic, clinical, laboratory and radiological data and clinical outcomes.

Variables	Patients	Missing data
Demographic data		
Age – median years (IQR)	64 (52 to 78)	0
Male – n (%)	69 (61%)	0
White ethnicity – n (%)	89 (79%)	
Asian ethnicity – n (%)	23 (20%)	0
Black ethnicity - n (%)	2 (1%)	
Autoimmune disease – n (%)	20 (18%)	0
Hypertension – n (%)	42 (37%)	
Diabetes – n (%)	30 (26%)	
Ischaemic heart disease – n (%)	35 (31%)	
Chronic kidney disease – n (%)	9 (8%)	
Cancer – n (%)	5 (4%)	
Chronic lung disease – n (%)	23 (20%)	
Neurological disease – n (%)	13 (11%)	
Gastroenterological/liver disease – n (%)	10 (9%)	
Haematological- n (%)	5 (4%)	
Number of comorbidities – median (IQR)	1 (1-2)	
Clinical data		
Admission oxygen saturations – median % (IQR)	96% (94 to 97)	0
Any oxygen in hospital – n (%)	84 (73%)	
White cell count – median $x10^9$ cells/L (IQR)	8.0 (6.2-11.0)	13 (not performed on sampling)
Lymphocyte - median $x10^9$ cells/L (IQR)	1.27 (0.87-1.68)	13
Urea – median mmol/L (IQR)	7.1 (5.1-9.6)	13
Creatinine – median μmol/L (IQR)	68 (57-89)	13
CRP – median mg/L (IQR)	21 (5-60)	13
Haemoglobin – median g/L (IQR)	129 (113-141)	13
IL-6 – median pg/ml	23 (10-68)	86 (never performed)
Nosocomial acquired infection – n(%)	10 (8%)	0
Findings of COVID-19 pneumonia on CXR – n (%)	88 (79%)	2 (CXR never performed)
Duration of symptoms – median days (IQR)	10 (7 to 15)	0
Treatment with dexamethasone – n (%)	73 (64%)	0
Vaccinated - n (%)	36 (31%)	0
Pfizer – n (%)	24 (21%)	
Astra Zeneca – n (%)	12 (11%)	
Combined IgG/IgM positive– n (%)	95 (93%)	12 inconclusive
T cell responses – median spots (IQR)		
Panel 1/S1 protein	5 (2 to 54)	27 inconclusive
Panel 2/S2 protein	5 (2 to 22)	
Panel 3/Nucleocapsid protein	3 (0 to 7)	
Panel 4/Membrane protein	3 (1 to 10)	
Clinical outcomes		
Received CPAP following sampling – n (%)	24 (21%)	0
28 day mortality – n (%)	7 (6%)	0

kinetics of the T-cell response compared with antibody response over time after infection and vaccination.[3,4]

We provide real-world data on the T-cell response of patients who had received one dose of the Pfizer BioNTech or Oxford AstraZeneca vaccine prior to developing COVID-19 requiring hospitalization. We did not observe amplification of the T-cell response in this group, compared with the unvaccinated population. This could reflect the design of current vaccines, which focus on generating a neutralizing antibody response rather than T-cell response, measurement of T-cell responses in a primarily older cohort where there is immunosenescence or may reflect insufficient time for the vaccines to induce robust T-cell immunity, despite the fact that the majority of our patients had their first dose 2 weeks or longer prior to admission.

Finally, we found an association between higher T-SPOT responses (especially S1) and increasing disease severity at the time of sampling, as evidenced prospective need for CPAP. It is not clear whether these T-cell responses are protective or deleterious. A protective role for the higher T-cell responses in severe disease is supported by the low 28-day mortality rate in this cohort. However, it is possible that the elevated T-cell responses are a marker of immune hyperstimulation generating cytokine over-production and cell death.[5] Studies comparing T cell phenotype and cytokine levels are needed to resolve this question.

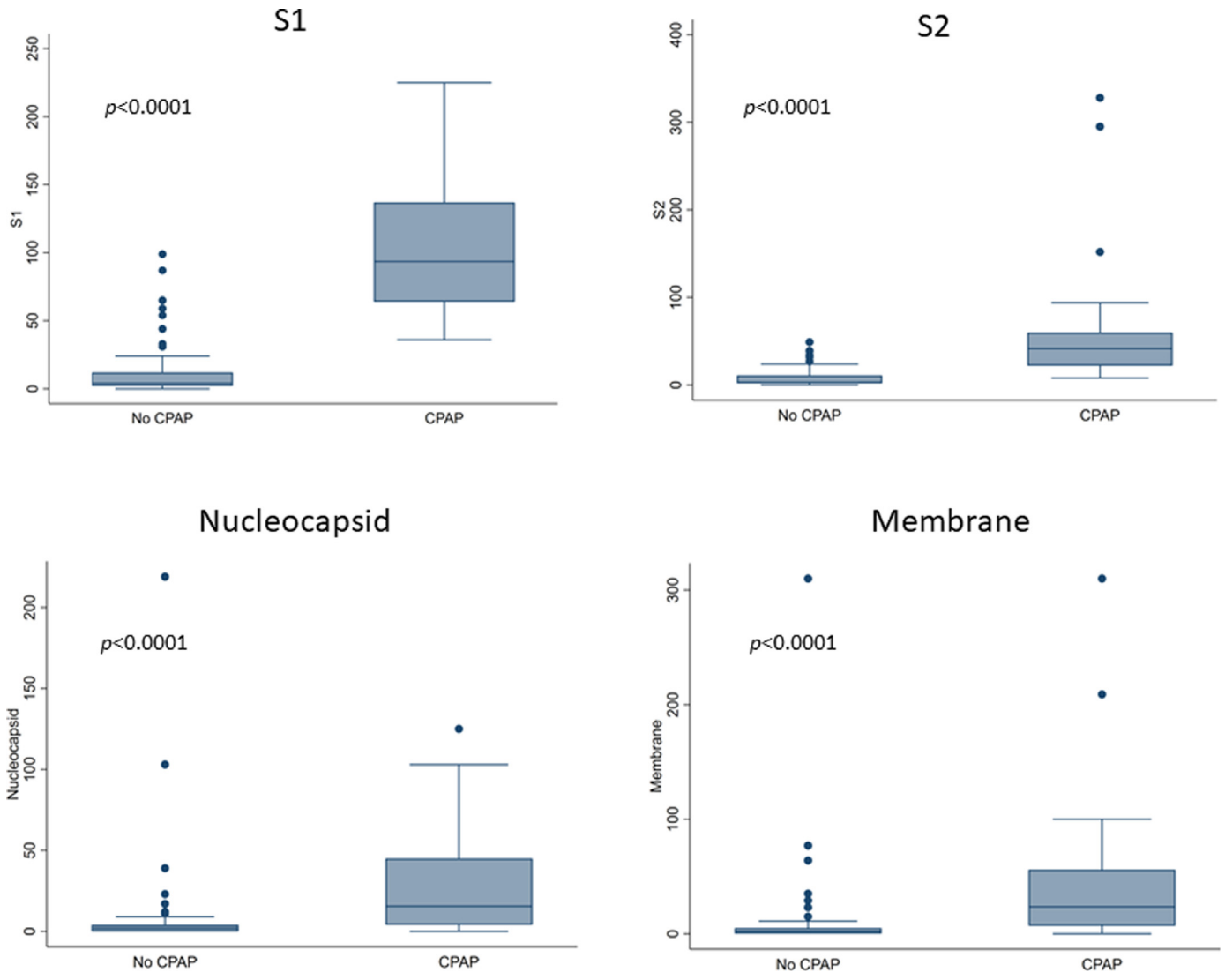

Fig. 1. Box plots illustrating T-SPOT values, stratified by whether they prospectively.

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Daniel Pan*[1]
Department of Respiratory Sciences, University of Leicester, United Kingdom
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Jee Whang Kim[1]
Department of Respiratory Sciences, University of Leicester, United Kingdom
Respiratory Biomedical Research Center, University of Leicester, United Kingdom

Joshua Nazareth[1]
Department of Respiratory Sciences, University of Leicester, United Kingdom
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Sara Assadi, Adam Bellass
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Jack Leach, James G Brosnan, Adam Ahmed
University of Leicester Medical School, United Kingdom

Fleur Starcevic
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Shirley Sze

Department of Cardiovascular Sciences, University of Leicester, United Kingdom

Christopher A Martin
Department of Respiratory Sciences, University of Leicester, United Kingdom
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Caroline M Williams, Michael R Barer
Department of Respiratory Sciences, University of Leicester, United Kingdom
Department of Microbiology, University Hospitals of Leicester NHS Trust, United Kingdom

Amandip Sahota
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Prashanth Patel
Department of Chemical and Metabolic Diseases, University Hospitals of Leicester NHS Trust, United Kingdom

Andrea Tattersall
Oxford Immunotec Ltd, United Kingdom

Andrea Cooper
Department of Respiratory Sciences, University of Leicester, United Kingdom
Trudeau Institute, Saranac Lake, New York, USA

Manish Pareek[2]
Department of Respiratory Sciences, University of Leicester, United Kingdom
Department of Infectious Diseases and HIV Medicine, University Hospitals of Leicester NHS Trust, United Kingdom

Pranabashis Haldar[2]
Department of Respiratory Sciences, University of Leicester, United Kingdom
Respiratory Biomedical Research Center, University of Leicester, United Kingdom

*Corresponding author at: Department of Respiratory Sciences, University of Leicester, United Kingdom.
E-mail address: daniel.pan@nhs.net (D. Pan)

[1] Daniel Pan, Jee Whang Kim and Joshua Nazareth are joint first authors.
[2] Manish Pareek and Pranabashis Haldar are joint senior authors.

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Similar humoral immune responses against the SARS-CoV-2 spike protein in HIV and non-HIV individuals after COVID-19

Dear Editor,

We have read with interest the article of Venturas et al., who found persons living with HIV (PWH) are not at higher risk of moderate or severe COVID-19 than the general population[1]. The immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in PWH is a matter of controversy and intense research, as HIV infection may impair the immune response to SARS-CoV-2[2]. High levels of neutralizing antibodies against SARS-CoV-2 spike (S) protein are associated with less severe disease and a good prognosis in COVID-19[3]. These antibodies against the SARS-CoV-2 S protein block the virus union to its cellular receptor, the angiotensin-converting enzyme 2 (ACE2) receptor[2].

Thus, it is critical to determine whether the anti-SARS-CoV-2 neutralizing antibody response is impaired in PWH[2]. This study aimed to characterize plasma antibodies against SARS-CoV-2 S protein in PWH and CTRLs recovered from COVID-19.

We performed a cross-sectional study in 91 PWH from the Cohort of the Spanish HIV Research Network (CoRIS) seropositive for SARS-CoV-2 and with plasma specimens collected from April 1, 2020, to September 30, 2020[4]. We also included HIV-uninfected CTRLs seropositive for SARS-CoV-2 with plasma specimens stored in the National center for Microbiology Instituto de Salud Carlos III. Both groups were matched for age and time since initiation of symptoms and were not vaccinated against SARS-CoV-2. The Ethics Committee of Hospital General Universitario Gregorio Marañón approved the study (Ref# 162/20).

Blood samples were collected by venipuncture in EDTA tubes and were sent the same day to the Spanish HIV BioBank, where plasma samples were obtained and stored at −80 °C. These samples were sent to the Instituto de Salud Carlos III for its analysis. We used immunoassays to evaluate the antibody titer against the SARS-CoV-2 S protein, which gives us the area under the curve (AUC) of IgG, IgM, and IgA titration curves. Besides, we assayed the capacity of the antibodies to inhibit the binding of the soluble ACE2 receptor to S protein (see Supplemental file 1).

The differences between groups were calculated by the Mann-Whitney U test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables. Generalized Linear Models (GLM) with a gamma distribution (log-link) adjusted by age, gender, and COVID-19 disease severity were used to evaluate the differences in plasma anti-SARS-CoV-2 S protein antibody levels (IgG, IgM, and IgA) between groups. The inhibition of ACE2 binding to the S protein (inhibition percentage, y-axis) and the titers of plasma anti-SARS-CoV-2 S protein antibodies (sum of AUCs of IgG, IgM, and IgA titration curves, x-axis) were plotted according to a semilog line, and Pearson's correlation coefficient (r) was calculated. Then, GLM tests were used to assess if regression slopes in PWH and CTRLs were differents by analyzing the interaction between the groups (PWH vs. CTRLs) with the sum of AUCs and inhibition percentages. Statistical analysis was performed with GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, CA, USA) and IBM SPSS Statistics 25.0 (SPSS INC, Armonk, NY, USA). The level of significance was two-tailed and defined as $p < 0.05$ (two-tailed).

The study population included 91 PWH – fully described elsewhere[4] – and 21 CTRLs, whose characteristics are shown in Table 1. Concerning COVID-19, 92.3% PWH had asymptomatic or mild COVID-19 disease, 7.7% were hospitalized, and the median time from symptoms to plasma collection was 11 weeks. CTRLs had similar characteristics to PWH, except for gender.

No significant differences were found between groups in plasma levels of different classes of immunoglobulins against SARS-CoV-2 S protein [IgG ($p = 0.414$; Fig. 1A), IgM ($p = 0.862$; Fig. 1B), and IgA ($p = 0.134$; Fig. 1C)], and percentages of inhibition of ACE2 binding to the S protein ($p = 0.237$; Fig. 1D). Adjusted regression analysis also found no significant differences (Supplemental Table 1). Furthermore, we found solid and similar correlations between total plasma antibody titers against SARS-CoV-2 S protein and the percentage of inhibition of ACE2 binding to the S protein

Table 1
Epidemiological and clinical characteristics of SARS-CoV-2 infected patients.

Variable	Control group	HIV group	*p*-value
No.	21	91	
Demographic data			
Male sex at birth – No./with data (%)	13 (61.9%)	85 (93.4%)	< 0.001
Age - Median (Q1; Q3) – yr.	42.3 (38.9; 48.8)	44.2 (36.8; 51.6)	0.902
COVID-19 data			
Severity status (asymptomatic or mild) – No./with data (%)	18 (85.7%)	84 (92.3%)	0.277
Hospital admission – No./with data (%)	3 (14.3%)	7 (7.7%)	0.340
Time from symptoms - Median (Q1; Q3) – wk.	12.3 (11.1; 19.7)	11 (8.1; 15.4)	0.106
Oxygen-therapy – No./with data (%)	3 (14.3%)	6 (6.6%)	0.340
HIV infection data			
Mechanism of HIV acquisition – No./with data (%)			
Men having sex with men	-	68 (74.7%)	-
Heterosexual	-	20 (22%)	-
Injection drug use	-	1 (1.1%)	-
Other	-	2 (2.2%)	-
Age of HIV diagnosis - Median (Q1; Q3) – yr.	-	36.4 (28.1; 43.6)	-
Time with HIV infection - Median (Q1; Q3) – yr.	-	6.2 (3.3; 11.5)	-
Prior AIDS-defining conditions – No./with data (%)	-	11 (12.1%)	-
Age - Median (Q1; Q3) – yr.	-	45 (36.9; 46.9)	-
Last CD4+ count			
Median (Q1; Q3) - cells/mm3	-	696.5 (491.5; 939)	-
Distribution – No./with data (%)			
< 350	-	9/84 (10.7%)	-
350–499	-	13/84 (15.5%)	-
≥ 500	-	62/84 (73.8%)	-
Last HIV-RNA load ≤ 50 copies/mm3 – No./with data (%)	-	80 (94.1%)	-
Antiretroviral therapy – No./with data (%)	-	88 (96.7%)	-
Antiretroviral therapy (N[t]RTI backbone) – No./with data (%)			
TAF/FTC	-	40 (44%)	-
ABC/3TC	-	25 (27.5%)	-
TDF/FTC	-	5 (5.5%)	-
Antiretroviral therapy (third drug)			
NNRTI	-	48 (52.7%)	-
Protease inhibitor	-	4 (4.4%)	-
Integrase inhibitor	-	51 (56%)	-

Abbreviations: PWH. People with HIV; Q1. 1st quartile; Q3. 3rd quartile; N(t)RTI. nucleoside/nucleotide reverse transcriptase inhibitors; TAF. tenofovir alafenamide; FTC. emtricitabine; ABC. abacavir; 3TC. lamivudine; TDF; tenofovir disoproxil fumarate; NNRTI. non-nucleoside reverse transcriptase inhibitors.

in CTRLs ($r = 0.580$; $p = 0.005$; Fig. 1E) and PWH ($r = 0.548$; $p < 0.001$; Fig. 1F). No differences were found between the regression slopes of the two study groups ($p = 0.849$).

Several studies have reported that PWH usually shows poor antibody response to other viruses or viral vaccines[5–7], raising concerns about whether they can mount an adequate humoral response against SARS-CoV-2. This issue is relevant since high antibody titers against the SARS-CoV-2 S protein correlate with virus neutralization and protection[3]. Our study shows that PWH and CTRLs who recovered from COVID-19 display a similar antibody response against the S protein. To detect neutralizing antibodies, we used a stabilized trimeric S protein in its native pre-fusion conformation. The suitability of our assay was confirmed by the strong correlation between the antibody titers and their capacity to inhibit the interaction S protein-ACE2 receptor.

Our data agree with recently published results showing comparable anti-SARS-CoV-2 neutralizing antibody levels between PWH under effective antiretroviral therapy (ART) and HIV-uninfected individuals[8,9]. Succesful HIV suppression seems to be crucial for developing an adequate humoral immune response. In our study, almost all HIV patients analyzed were on ART, with good clinical, virological, and immunological control, which may have contributed to similar anti-SARS-CoV-2 antibody titers between PWH and CTRLs. We analyzed the antibody titers against the SARS-CoV-2 S protein and percentages of inhibition of ACE2 binding to the S protein according to $CD4^+$ strata (< 350, 350–500, > 500 cells/mm^3), and we did not find significant differences (data not shown). In contrast, lower neutralizing antibody titers against SARS-CoV-2 were found in PWH than in HIV-uninfected individuals recovering from COVID-19 by Spinelli et al.[10], although its sample size was three times lower than in our study. Differences in the characteristics of the study cohorts (sample size, ethnicity, age, sex, COVID-19 severity, percentage of people with unsuppressed viral loads, among others), study design, or assays for antibody characterization may explain these conflicting results.

In conclusion, no differences in quantitative and qualitative SARS-CoV-2-specific immune humoral response were found between well-controlled PWH and CRTLs after recovery from COVID-19. This finding suggests that PWH are not an at-risk population for this infection and are potentially good vaccination responders.

Contribution

- Study conception and design: Salvador Resino, Juan Berenguer, and Isidoro Martínez.
- Acquisition of data: all authors.
- Laboratory procedures: María Martín-Vicente, and María José Muñoz-Gómez.
- Analyses and interpretation of data: Salvador Resino and Isidoro Martínez.
- Drafting the article: Salvador Resino and Isidoro Martínez.
- Critical revision of the article: Juan Berenguer.
- Funding acquisition: Salvador Resino and Juan Berenguer.
- All authors have read and approved the final manuscript.

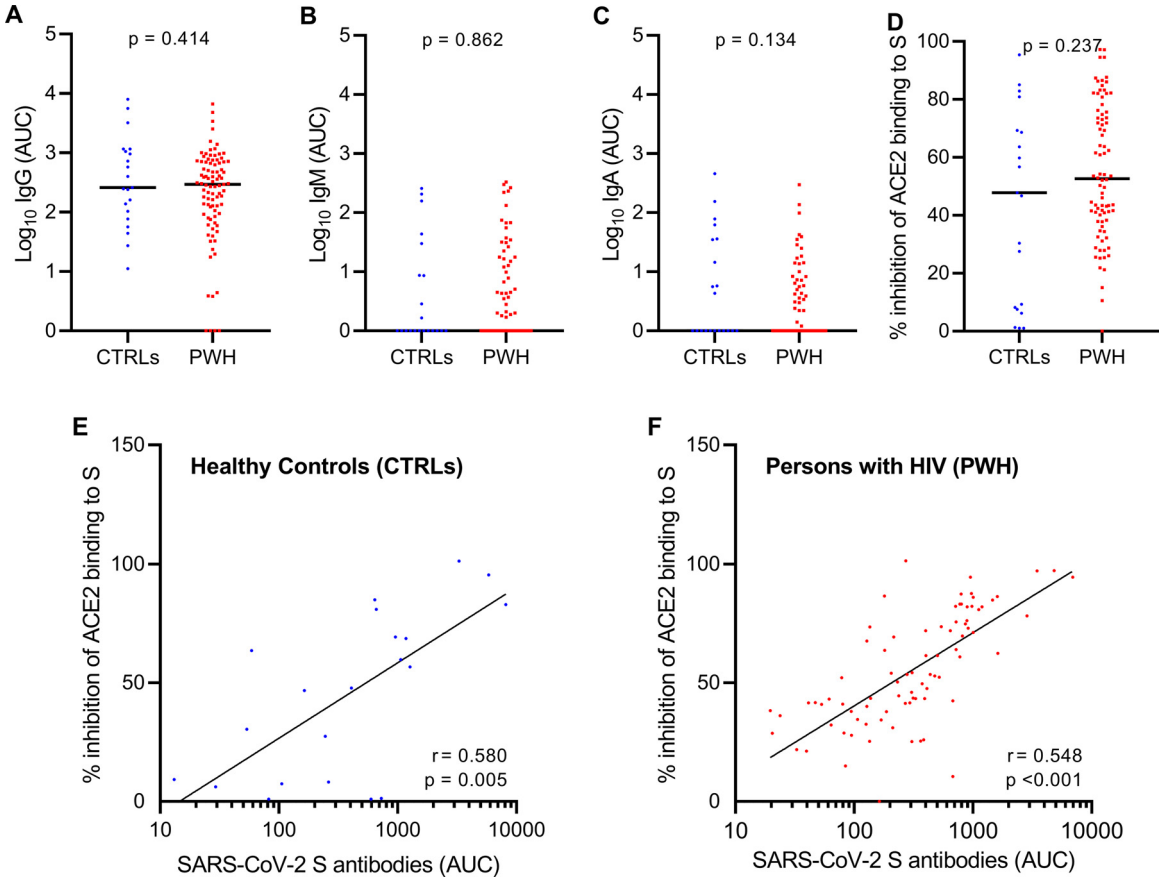

Fig. 1. Plasma levels of antibody against SARS-CoV-2 S protein (A–C) and percentages of inhibition of ACE2 receptor binding to the S protein (D). Correlation between antibody levels against SARS-CoV-2 S protein (sum of the AUC of IgG, IgM, and IgA) and percentages of inhibition of ACE2 receptor binding to the S protein (E and F).
Statistics: Differences were calculated by the Mann-Whitney U test, and medians were represented by a horizontal bar. Correlation analysis was performed using the Pearson test.
Abbreviations: AUC, the area under the curve; ACE2, angiotensin-converting enzyme 2; CTRLs, HIV-uninfected patients, PWH, persons living with human immunodeficiency virus; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus; IgG, anti-SARS-CoV-2 S IgG; IgM, anti-SARS-CoV-2 S IgM; IgA, anti-SARS-CoV-2 S IgA.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All participants gave their consent before enrollment. The Ethics Committee of Hospital General Universitario Gregorio Marañón approved the study (Ref# 162/20).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.002.

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María Martín-Vicente
Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain.
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.

Juan Berenguer*
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Unidad de Enfermedades Infecciosas/VIH, Hospital General Universitario Gregorio Marañón, IiSGM, Madrid, Spain

María José Muñoz-Gómez
Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain.
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.

Cristina Díez
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Unidad de Enfermedades Infecciosas/VIH, Hospital General Universitario Gregorio Marañón, IiSGM, Madrid, Spain

Rafael Micán
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Medicina Interna, Hospital Universitario La Paz, IdiPaz, Madrid, Spain

María Jesús Pérez-Elías
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Enfermedades Infecciosas, Hospital Ramón y Cajal, Universidad de Alcalá, IRYCIS, Madrid, Spain

Lucio Jesús García-Fraile
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Medicina Interna, Hospital Universitario de La Princesa, Madrid, Spain

Joaquin Peraire
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Medicina Interna, Hospital Universitari de Tarragona Joan XXIII, IISPV, Universitat Rovira i Virgili, Tarragona, Spain.

Inés Suárez-García
Servicio de Medicina Interna, Hospital Universitario Infanta Sofía, San Sebastián de los Reyes, Spain
Universidad Europea, Villaviciosa de Odón, Madrid, Spain.

María Ángeles Jiménez-Sousa, Amanda Fernández-Rodríguez
Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain.
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.

Mónica Vázquez
Unidad de Biología Viral, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain

Pablo Ryan
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Medicina Interna, Hospital Infanta Leonor, IiSGM, Madrid, Spain

Juan González-García
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Servicio de Medicina Interna, Hospital Universitario La Paz, IdiPaz, Madrid, Spain

Inmaculada Jarrín
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid, Spain

Vicente Mas
Unidad de Biología Viral, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain

Isidoro Martínez[1], Salvador Resino*[1]
Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain.
Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.

*Corresponding authors at: Centro de Investigación Biomédica en Red en Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain.
E-mail addresses: maria.mvicente92@gmail.com (M. Martín-Vicente), jbb4@me.com (J. Berenguer), mjose.munoz@isciii.es (M.J. Muñoz-Gómez), crispu82@gmail.com (C. Díez), micanrafael@hotmail.com (R. Micán),

mjperez90@gmail.com (M.J. Pérez-Elías), lucio.garciafraile@gmail.com (L.J. García-Fraile), jjperaire.hj23.ics@gencat.cat (J. Peraire), inessuarezgarcia@gmail.com (I. Suárez-García), jimenezsousa@isciii.es (M.Á. Jiménez-Sousa), amandafr@isciii.es (A. Fernández-Rodríguez), mvazquez@isciii.es (M. Vázquez), pabloryan@gmail.com (P. Ryan), juangonzalezgar@gmail.com (J. González-García), ijarrin@isciii.es (I. Jarrín), vmas@isciii.es (V. Mas), imago@isciii.es (I. Martínez), sresino@isciii.es (S. Resino)

[1] These authors contributed equally to this work.

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Divergent humoral responses in mild to moderate SARS-CoV-2 infection over time – indication of persistence of the virus?

Dear Editor,

Serum antibodies are an important pillar of the immune response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. With earlier data, we have shown that SARS-CoV-2 IgG and IgA antibody responses are both, gender dependent and characterized by a declining antibody concentration early on[1]. We can now show that antibodies, especially against spike protein (S), remain detectable for more than one year in most persons after PCR confirmed mild to moderate COVID-19, despite the fact that a relevant decline can be observed. We here present the extended longitudinal profile of IgG and IgA against S and of IgG against nucleocapsid protein (N) for more than one year (the cohort was initiated during the first infection wave in Switzerland in March 2020).

The study includes outpatients with a history of positive SARS-CoV-2 PCR, *i.e.* a mild to moderate disease course. The total cohort comprises 278 individuals (12.0–91.2 years, median = 51.2, IQR = 25.8; 59.5% females), of which 53 (24.8–91.2 years, median = 55.8, IQR = 13.9; 41% females) were followed for 14 months (supplementary Table 1). The study is registered in the Swiss COVID-19 database (https://swissethics.ch/covid-19/approved-projects; K2) and was approved by the regional ethics committee (ID2020–00,941). PCR analysis of stool and nasopharyngeal swabs were performed together with blood draws every week in a first month and then after another four weeks in the second month; this course was repeated if patients consented. All SARS-CoV-2 ELISA (anti-S IgG and IgA, Euroimmun, Lübeck, Germany; anti-N IgG, Epitope Diagnostics, San Diego, USA) were run on an automated DSX ELISA processor (Dynex Technologies) according to the recommendations of the manufacturers. We defined an OD ratio of 11 (anti-S IgG) or 9 (anti-S IgA) as the upper threshold of the dynamic range, since the assays saturate above these points[2]. Statistical definitions, analysis and visualizations were based on or performed with software R using the implemented statistical tests and the packages “tidyverse” and “ggplot2”[3].

During the initial 4 months after a positive PCR result, 94.2% of participants showed quantifiable evidence of seroconversion, while 5.8% did not (Fig. 1A–C). Upon their first visit (median 6 weeks after positive PCR; 95% CI 0.43 weeks) 11.9% (33 / 278), 21.6% (60 / 278) and 24.5% (68 / 278) had not developed measurable anti-S IgG, anti-S IgA or anti-N IgG, respectively. Furthermore, 66.9% of participants displayed quantifiable antibody concentrations for all three entities evaluated. Remarkably, all long-term sub cohort participants presented at least one quantifiable antibody entity at all time points until their last visit, while only 49% showed quantifiable antibody concentrations in all three entities. Note that study participants with no initially detectable antibodies against SARS-CoV-2 (5.8%) did not participate in the long-term sub cohort.

The statistically significant gender-associated difference in the antibody concentrations observed earlier persist for the first 3 months; thereafter, gender-associated differences are no longer observed[1]. In addition, a significant ($p < 2.1e{-}08$) age dependent difference in antibody concentrations becomes apparent at weeks 22 to 26 (Fig. 1D). Individuals younger than 54 years of age tend to show lower antibody concentrations than their older counterparts; this was also observed in other studies[4].

While antibody concentrations may have complex kinetics[5], we categorized the anti-S antibody longitudinal courses based on the slope of the robust regression line (Fig. 2). We identified two statistically distinctive patterns of antibody dynamics for anti-S IgG and IgA: declining antibody concentrations (decrease of anti-S IgG levels, average slope: −0.045 (±0.037) OD ratio/week, $n = 47$; decrease of anti-S IgA levels, average slope: −0.032 (±0.052) OD ratio/week, $n = 19$) and increasing antibody concentrations (increase of anti-S IgG levels, average slope: +0.029 (±0.020) OD ratio/week, $n = 6$; increase of anti-S IgA levels, average slope: +0.053 (±0.066) OD ratio/week, $n = 34$).

The majority (89%) of the long-term sub cohort showed declining anti-S IgG antibody concentrations, while a small subgroup (11%) showed increasing antibody concentrations over time (Fig. 2B). An even higher proportion of increasing antibody concentrations was observed with the individual courses of anti-S IgA antibodies (36% declining and 64% increasing antibody concentrations). As there are substantially more individual anti-S IgA increases than decreases, this might indicate an underlying mechanism of IgA stimulation. The detection of SARS-CoV-2 material in some stool samples early during the observation period might be hinting at such a stimulatory exposure (supplementary Table 2). Considering all results of our observation, one might therefore conclude that IgA antibodies might provide a more persistent and more stable defense against SARS-CoV-2 than IgG[6,7].

According to the current understanding, one would have to expect a continuous decrease in antibody concentration - in the absence of the antigen - after an initial increase[5]. Our current data, however, describe a secondary increase in anti-spike IgG in a few and in IgA in many more patients. If this increase was due to re-infection, a much steeper increase (*i.e.* a booster response) could be expected to be observed, at least temporarily[8]. In addition, nasopharyngeal swabs and stool samples for PCR testing were taken at every visit, but none of them were found to be positive in any of the individuals within the long-term sub-cohort; obviously, this observation does not allow to rule out a potential re-infection or re-exposure during the observation period with certainty. However, it seems at least to rule out persistence of a high viral load in the nasopharynx and the gut within this group. But even non-detectable persistence of virus particles might have provided sufficient antigen to induce the observed response, preventing waning of antibodies. This would be compatible with findings of coronavirus particles in the small bowel of covalescent study

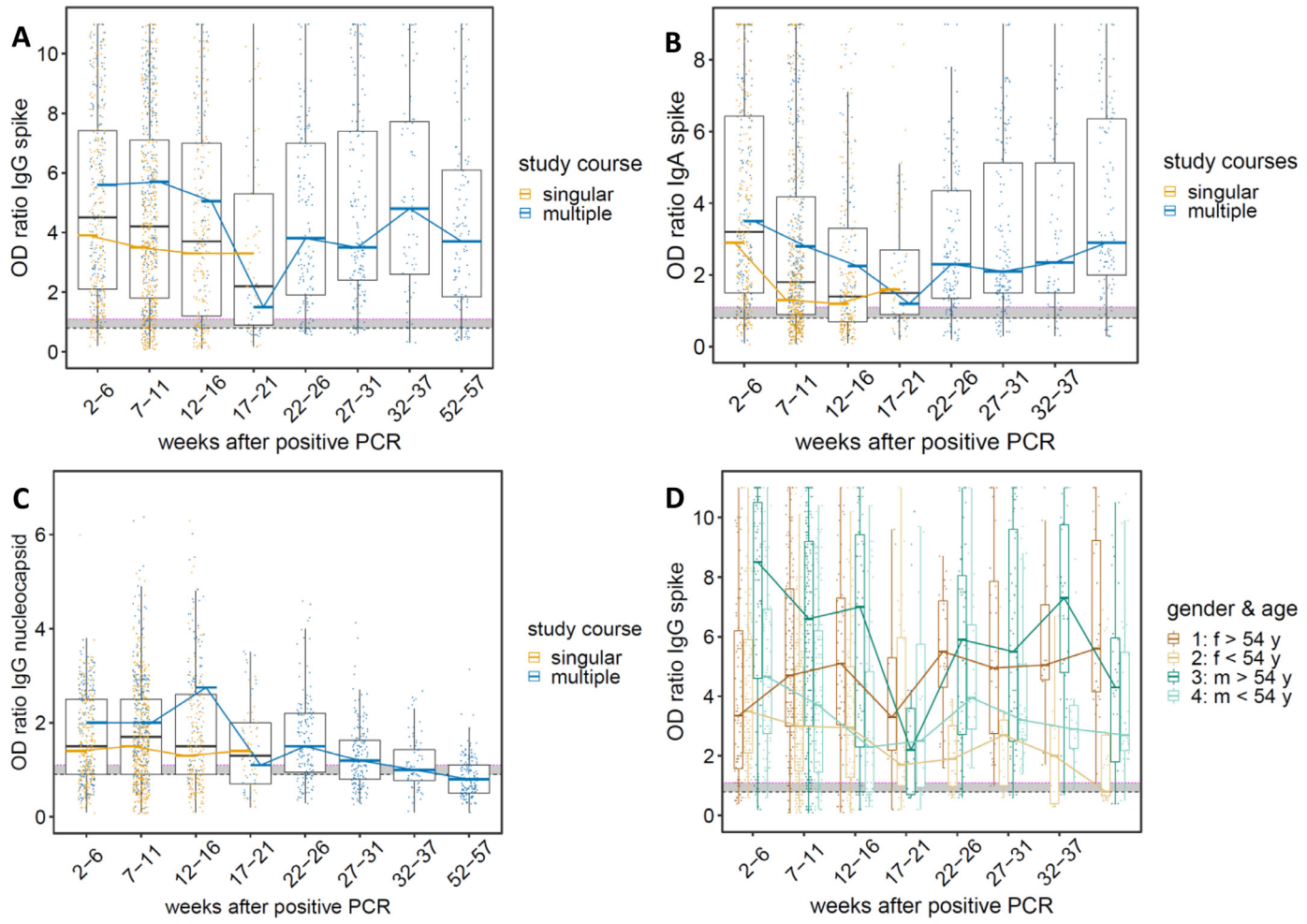

Fig. 1. *Overall dynamic changes in anti-SARS-CoV-2 antibody levels (IgG and IgA) over time*. Results were grouped according to the time after positive PCR diagnosis: 2–6 weeks (n = 408, 21.3%), 7–11 weeks (n = 690, 36.0%), 12–16 weeks (n = 257, 13.4%), 17–21 weeks (n = 73, 3.8%), 22–26 weeks (n = 143, 7.5%), 27–31 weeks (n = 156, 8.1%), 32–37 weeks (n = 72, 3.8%) and 52–57 weeks (n = 119, 6.2%). Horizontal bold lines indicate median values; boxes indicate quartiles 1 and 3; whiskers indicate 1.5 * IQR confidence intervals; dotted magenta line indicate optical density (OD) ratio at 1.1 (positive cut-off); dotted black line indicate OD ratio at 0.8 for anti-S antibodies, 0.9 for anti-N IgG (values below are considered negative); gray shaded region in-between OD ratio 0.8/0.9–1.1 contains borderline results. Black line represents all individuals, independent of the number of study courses. Yellow dots and line represent individuals performing a singular study course (1–5 longitudinal blood draws) and blue dots and line represent individuals with multiple study courses (1–15 longitudinal blood draws). Each point represents a single measurement. (**A**) anti-spike (S) IgG; **(B)** anti-S IgA; (**C**) anti-nucleocapsid (N) IgG; (**D**) Gender and age specific dynamic changes in anti-S IgG antibody levels over time. 1 = female individuals with age higher than 54 years; 2 = female individuals with age lower than 54 years; 3 = male individuals with age higher than 54 years; 4 = male individuals with age lower than 54 years.

participants or durable antigen presentation on follicular dendritic cells[9,10]. The observed IgA antibody increase over time might indicate a state of chronic infection[5] and may help to understand how our immune system copes with this virus.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.11.001.

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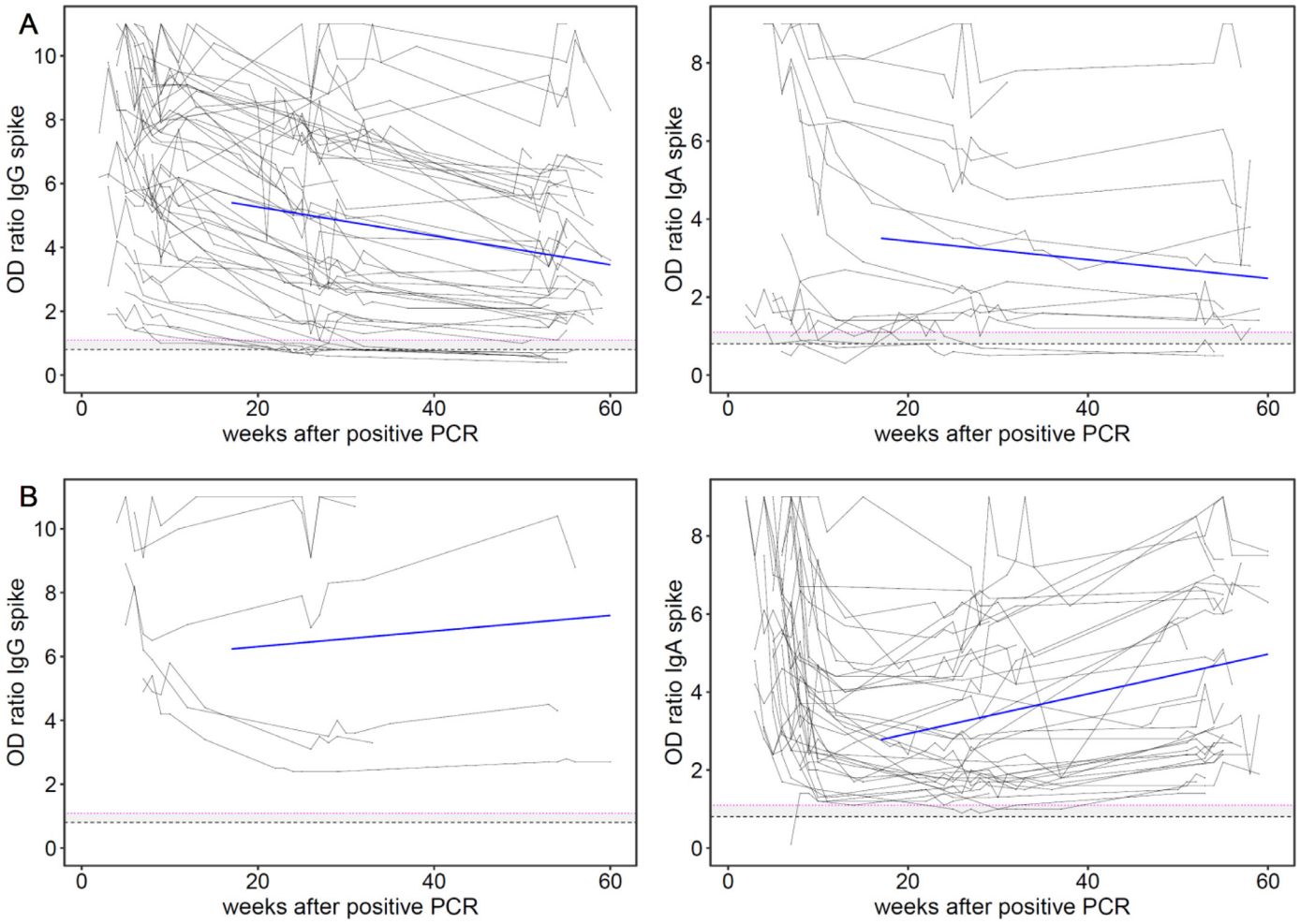

Fig. 2. *Longitudinal courses of individual anti-SARS-CoV-2 antibody levels (IgG and IgA) over time.* Individual longitudinal anti-spike IgG and anti-spike IgA courses were analyzed utilizing robust regression (period week 17–57) using an MM estimator, which is an M-estimation with Turkey's biweight initialized by a specific S-estimator. The corresponding slopes were categorized according to the following properties of their individual course: Declining antibody concentrations were defined by a negative slope value (slope < 0 OD ratio/week); increasing antibody concentrations were defined by a positive slope value (slope > 0 OD ratio/week). Blue line represents an illustrative line indicating the averaged present slope calculated by the robust regression of data from week 17 to 57; dotted magenta line indicate optical density (OD) ratio at 1.1 (positive cut-off); dotted black line indicate OD ratio of 0.8 (values below are considered negative); gray shaded region in-between OD ratio 0.8–1.1 contains borderline results. Each point represents a single measurement. (**A**) anti-spike IgG (n = 47, 89%) and IgA (n = 19, 36%) antibody levels with declining tendency. (**B**) anti-spike IgG (n = 6, 11%) and IgA (n = 34, 64%) antibody levels with increasing tendency.

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Justus J. Bürgi
Center for Laboratory Medicine, Frohbergstrasse 3, 9000 St. Gallen, Switzerland

Matthias Rösslein
Empa - Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland
Cantonal Corona Study Group, Oberer Graben 32, 9001 St. Gallen, Switzerland

Henry Hornung, Jana Jentsch, Viveka L. Boller, Günter Dollenmaier
Center for Laboratory Medicine, Frohbergstrasse 3, 9000 St. Gallen, Switzerland

Peter Wick
Empa - Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland
Cantonal Corona Study Group, Oberer Graben 32, 9001 St. Gallen, Switzerland

Karen Peier
Cantonal Corona Study Group, Oberer Graben 32, 9001 St. Gallen, Switzerland

Public Health Department, Oberer Graben 32, 9001 St. Gallen, Switzerland

Brigitte Nohynek, Aldo Fischer, Raphael Stolz, Michele Cettuzzi
Cantonal Corona Study Group, Oberer Graben 32, 9001 St. Gallen, Switzerland

Michael Reut, Regine Garcia Boy
Center for Laboratory Medicine, Frohbergstrasse 3, 9000 St. Gallen, Switzerland

Oliver Nolte, Wolfgang Korte*
Center for Laboratory Medicine, Frohbergstrasse 3, 9000 St. Gallen, Switzerland
Cantonal Corona Study Group, Oberer Graben 32, 9001 St. Gallen, Switzerland

*Corresponding author at: Center for Laboratory Medicine, Frohbergstrasse 3, St. Gallen 9000, Switzerland.
E-mail address: wolfgang.korte@zlmsg.ch (W. Korte)

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Evolution of SARS-CoV-2 immune responses in nursing home residents following full dose of the Comirnaty® COVID-19 vaccine

Dear Editor,

We read with interest the studies published by Tré-Hardy and colleagues [1,2] in the Journal of Infection showing a marked and significant decrease in serum SARS-CoV-2-Spike(S) antibody levels in healthcare workers at 3 and 6 months after complete vaccination with the mRNA-1273 vaccine (Spikevax). Real-world experience has shown mRNA COVID-19 vaccines to be effective in reducing incidence of both asymptomatic and symptomatic SARS-CoV-2 infections and related deaths in nursing home residents,[3] congruent with their ability to elicit robust virus-specific T and B cell immune responses in this population group.[4,5] Nevertheless, maintaining seemingly protective immune responses in these individuals over time may be compromised by the concurrence of older age, frailty and co-morbidities. To shed light on this issue, here we assessed SARS-CoV-2-Spike (S)-targeted antibody and functional T cell responses at around 6 months after vaccination with Comirnaty® (Pfizer–BioNTech) in a previously recruited cohort.[4]

Out of 53 nursing home residents enrolled in a previous study [4] with data on B and T cell immunity at a median of 17.5 days (range, 14−35 days) after second vaccine dose (baseline sample), 46 (44 females; median age, 89 years; range, 60−100; Supplementary Table 1) were reassessed (follow-up sample) at a median of 195 days (range, 179−195 days). The remaining 7 patients either died ($n = 4$; in no case attributable to COVID-19) or lacked the follow-up specimen ($n = 3$). Blood specimens were collected in sodium heparin tubes (Beckton Dickinson, U.K. Ltd., UK). Informed consent was obtained from participants. The study was approved by the Hospital Clínico Universitario INCLIVA Research Ethics Committee (February 2021). Total antibodies (IgG and IgM) against SARS-CoV-2-S protein receptor binding domain (RBD) and the nucleoprotein (N) were measured by Roche Elecsys® electrochemiluminescence sandwich immunoassays (Roche Diagnostics, Pleasanton, CA, USA). Antibody levels measured by the former assay correlate strongly with neutralizing antibody titers.[6] Cryopreserved plasma (-20 °C) specimens were thawed and assayed in singlets within 15 days after collection. Plasma specimens were diluted (1/10) for antibody quantitation when appropriate. SARS-CoV-2-S-reactive IFNγ-producing-CD8$^+$ and CD4$^+$ T cells were enumerated in whole blood by flow cytometry for ICS (BD Fastimmune, BD-Beckton Dickinson and Company-Biosciences, San Jose, CA) as previously described.[4]

Of the 46 residents, 10 (21.7%) had evidence of SARS-CoV-2 infection at baseline, as determined by both RT-PCR on nasopharyngeal specimens and detection of N-specific antibodies. No additional residents developed N-specific antibodies between sampling times. Data on SARS-CoV-2-RBD antibody levels were available for 45 participants. All 43 residents who tested positive at baseline also displayed detectable responses at follow-up, although overall, antibody levels were found to decrease significantly, by a median of 4.8 fold (range, 1.1−39) [median of 2249 IU/ml at baseline vs. median 307 IU/ml at follow-up, $P < 0.001$ (Fig. 1A)]. One of the two remaining residents developed SARS-CoV-2-S-specific antibodies (8 IU/ml) between sampling times. Antibodies waning was documented more frequently ($P < 0.001$) in SARS-CoV-2 naïve (29/35) than in recovered (1/10) residents (Fig. 1B). These observations were not unexpected as they have also been made in other population groups, including younger individuals seemingly with few or no comorbidities, at comparable timeframes [2,7,8] after full vaccination with mRNA vaccines.

Data on T cell responses were available for 46 participants. Overall, detectable SARS-CoV-2-S IFN-γ T cells (either CD8$^+$, CD4$^+$ or both) were documented in 82.6% (38/46) and 73.9% (34/46) of residents at baseline and follow-up, respectively ($P = 0.01$). The corresponding figures for SARS-CoV-2-S IFN-γ CD8$^+$ T cells were 72% (33/46) and 52.1% (24/46). As shown in Fig. 2A, 8 of 13 residents testing negative at baseline later acquired detectable responses, albeit at low frequencies (median, 0.08%; range, 0.01−0.21%), whereas SARS-CoV-2-S IFN-γ CD8$^+$ T cells were no longer detectable at follow-up in 16 out of 33 residents who tested positive at baseline. SARS-CoV-2-S IFN-γ CD4$^+$ T cells were detected in 26% (12/46) and 65.2% (30/46) of residents at baseline and follow-up, respectively. Nineteen participants developed CD4$^+$ T cell responses between testing time points (median, 0.1%; range, 0.03–1.14%), whereas one out of 12 with detectable responses at baseline had lost this at follow-up (Fig. 2B). The likelihood of having detectable SARS-CoV-2 IFN-γ CD8$^+$ and CD4$^+$ T at follow-up was higher ($P = 0.03$ and $P = 0.5$) in SARS-CoV-2 recovered (8/10 and 7/10, respectively) than in naïve residents (9/36 and 25/36, respectively). For those with detectable responses at both time points, overall, SARS-CoV-2-S IFN-γ CD8$^+$ T cell frequencies decreased significantly ($P = 0.001$) over time whereas the opposite ($P = 0.01$) was seen for CD4$^+$ T cells (Fig. 2C). Interestingly, the resident lacking anti-RBD antibodies at follow-up had detectable SARS-CoV-2-S CD4$^+$ T cell responses. In this regard, collectively, the above data suggested that SARS-CoV-2-S IFN-γ CD4$^+$ T cells may develop later than CD8$^+$ T cells in nursing home residents.

Supplementary Table 2 shows the combined results for all immunological parameters. No correlation, as evaluated by the Spearman rank test, was found between anti-RBD antibody levels and SARS-CoV-2-S IFN-γ CD4$^+$ (Rho=-0.015; $P = 0.94$) and CD8$^+$ (Rho:-0.18; $P = 0.87$) T cells.

Limitations of the current study are the relatively small sample size and lack of a control group; regarding the latter, most of the 17 controls included in our previous study [4] were unfortunately not available for follow-up sampling. Secondly, neutralization assays were not carried out. In summary, our data revealed

that a large percentage of nursing home residents displayed detectable SARS-CoV-2-S-reactive antibodies and T cell responses, respectively, by around 6 months after complete vaccination with Comirnaty® COVID-19 vaccine, although these generally declined over time. Whether these mid-term immune responses suffice to prevent COVID-19 remains to be determined. Our data also suggested that a booster (third) dose, which has been proposed for elderly people [9] may be delayed beyond 6 months in fully vaccinated COVID-19 recovered residents.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.10.026.

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Estela Giménez[1]
Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Av. Blasco Ibáñez 17, Valencia 46010, Spain

Juan Alberola[1]
Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain

Ignacio Torres, Eliseo Albert, María Jesús Alcaraz
Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Av. Blasco Ibáñez 17, Valencia 46010, Spain

Pilar Botija
Departamento de Salud Clínico-Malvarrosa, Dirección de Atención Primaria, Hospital Clínico Universitario de Valencia, Valencia, Spain

Paula Amat, María José Remigia
Hematology Service Clinic University Hospital, INCLIVA Health Research Institute, Valencia, Spain

María José Beltrán
Departamento de Salud Clínico-Malvarrosa, Dirección de Enfermería, Hospital Clínico Universitario de Valencia, Valencia, Spain

Celia Rodado
Departamento de Salud València Clínico Malvarrosa, Comisión Departamental de control de Residencias, Valencia, Spain

Dixie Huntley, Beatriz Olea
Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Av. Blasco Ibáñez 17, Valencia 46010, Spain

David Navarro*
Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Av. Blasco Ibáñez 17, Valencia 46010, Spain
Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain

*Corresponding author at: Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Av. Blasco Ibáñez 17, Valencia 46010, Spain.
E-mail address: david.navarro@uv.es (D. Navarro)

[1] These authors contributed equally to this work.

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Non-pharmaceutical interventions reduced the incidence and exacerbation of allergic diseases in children during the COVID-19 pandemic

Dear Editor,

Recently, a report entitled “Incident changes of rotavirus enteritis among children during the coronavirus disease-2019 pandemic in Hangzhou, China” has aroused our strong concern[1]. Fang et al.[1] found a significant reduction in the incidence of rotavirus enteritis among children by 84.8% in the COVID-19 pandemic period in Hangzhou, China, compared to that of the last two years by the generalized linear model with the Poisson distribution. Here, we observed that changes in human lifestyle and the living environment caused by non-pharmaceutical interventions (NPIs) in COVID-19 reduced children’s incidence and exacerbation of allergic diseases.

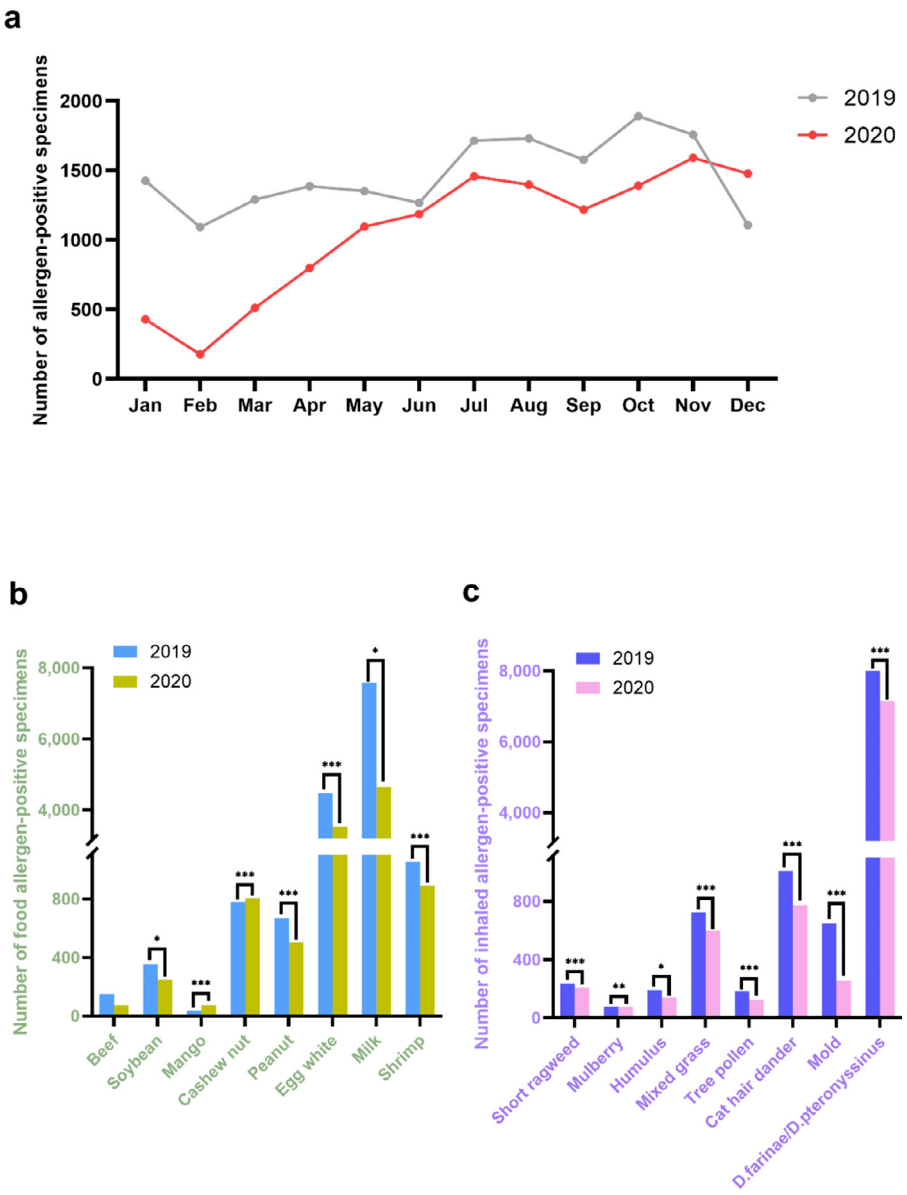

Fig. 1. The number of allergen-positive specimens before and during the COVID-19 pandemic. (a) The number of allergen-positive specimens each month before and during the COVID-19 pandemic. (b) The number of food allergen-positive specimens before and during the COVID-19 pandemic. (c) The number of inhaled allergen-positive specimens before and during the COVID-19 pandemic.

Allergic diseases, such as asthma, eczema, hay fever, and food and drug allergies, affect approximately one billion people worldwide and are the most common and economically expensive noncommunicable and chronic diseases among children[2]. The International Study of Asthma and Allergies in Childhood has indicated that the environment impacts the occurrence and development of allergic diseases, and the worse the socioeconomic environment, the higher the prevalence of allergic diseases may be[3]. Allergic diseases are usually mediated by immunoglobulin E (IgE), and allergic patients are prone to produce IgE antibodies to allergy-related environmental allergens[4]. Allergen-specific IgE (sIgE) levels of variable intensity reflect the likelihood of an allergic reaction[5]. Due to the enhanced awareness of infection prevention and control during the COVID-19 epidemic, human social behaviors and health habits have significantly changed in a short period. A series of NPIs have been undertaken worldwide, including keeping social distance, wearing masks, hand hygiene, controlling crowd gathering, reducing going out, business suspension et al. We compared the incidence of allergy and concentrations of various allergen-sIgE before and during the COVID-19 pandemic to explore the impact of COVID-19 on allergic diseases. The present study enrolled children who came to the children's hospital of Zhejiang University between January 2019 and December 2020 for allergen detection. Children infected with COVID-19 were excluded. Inhaled and food allergen-sIgE antibodies were detected by the allergen detection kit of Hangzhou Zheda Dixun Biological Gene Engineering Co., Ltd. Data were analyzed using Mann-Whitney *U* test for continuous variables and Chi-squared test for categorical variables. $P < 0.05$ was defined as statistically significant. All statistical analyses were processed with PASW 22.0 statistical software (IBM Corporation).

A total of 41,648 specimens were collected in 2019, of which 17,590 (42.23%) were allergen positive. However, a total of 24,714 specimens were collected in 2020, of which 12,731 (51.51%) were allergen positive. The number of allergen-positive specimens in 2020 was significantly lower than in 2019 ($P < 0.05$). The number of allergen-positive specimens in the first ten months of 2020

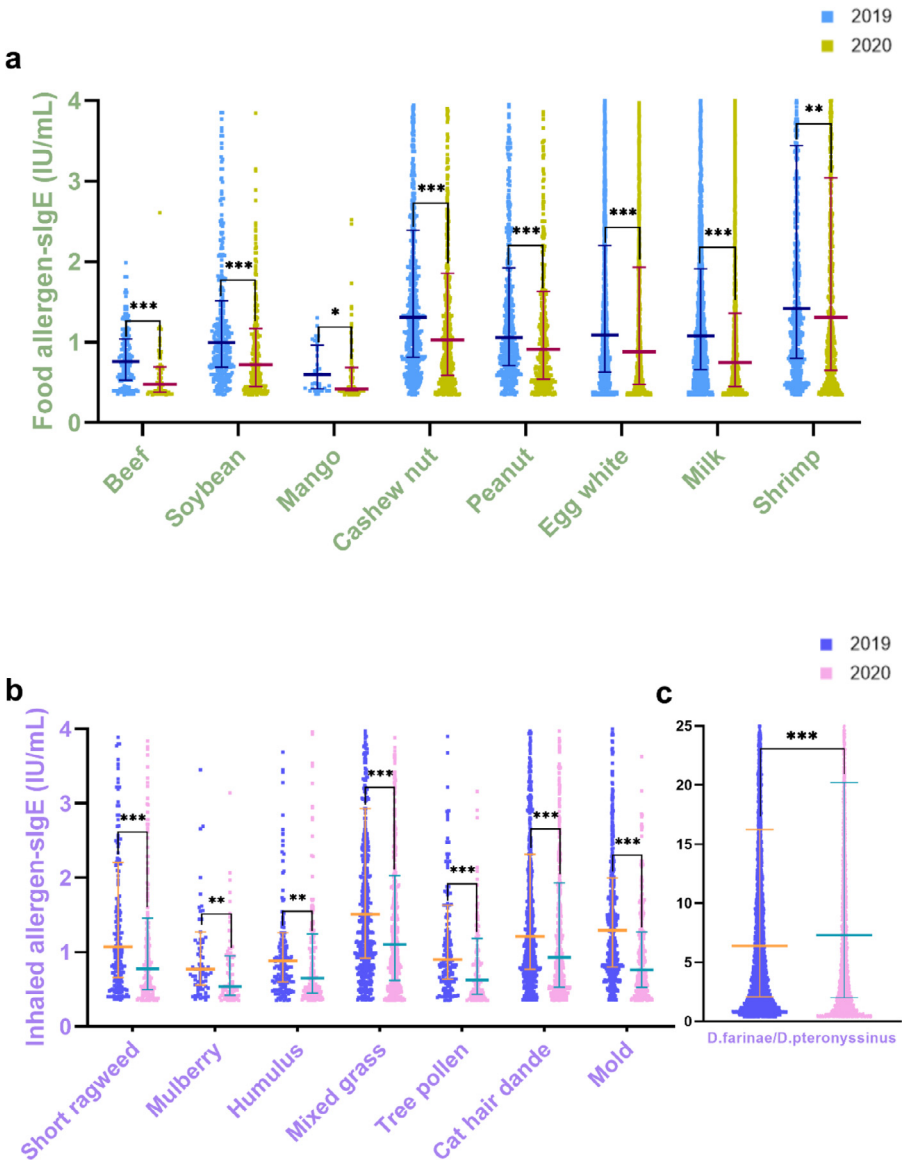

Fig. 2. The concentrations of allergen-sIgE antibodies before and during the COVID-19 pandemic. (a) The concentrations of food allergen-sIgE antibodies before and during the COVID-19 pandemic. (b) The concentrations of inhaled allergen-sIgE antibodies before and during the COVID-19 pandemic. (c) The concentrations of D.farinae/D.pteronyssinus sIgE antibodies before and during the COVID-19 pandemic. Abbreviation: sIgE, specific IgE.

was lower than that in the same period of 2019, especially in January and February. After that, the number of allergen-positive specimens tended to be the same in November, and the number of allergen-positive specimens in December 2020 was higher than that in 2019 (Fig. 1a). Except for Beef, Mango, and Cashew nut, the number of positive specimens of various allergens in 2020 was significantly lower than that in 2019 ($P < 0.05$) (Fig. 1b, 1c). It can be concluded that NPIs during the COVID-19 outbreak significantly reduced the incidence of allergic diseases among children.

The concentrations of food allergen-sIgE antibodies in 2020 significantly decreased compared with that of 2019 ($P < 0.05$) (Fig. 2a). A similar result was also obtained in inhaled allergens. The concentrations of their allergen-sIgE antibodies significantly decreased compared with that of 2019 ($P < 0.05$) (Fig. 2b). However, D.farinae/D.pteronyssinus, an indoor allergen, was an exception. The concentrations of its sIgE antibodies presented a significant increase in 2020 compared with that of 2019 ($P < 0.05$) (Fig. 2c). The decrease of antigen-sIgE concentrations may be attributed to the protective role of NPIs in avoiding exposure to allergens. NPIs reduce the risk of re-exposure to various allergens in children, thus alleviating the exacerbation of diseases. At the same time, staying at home for a long time increase the risk of exposure to the indoor allergen and prolong children's exposure time, which negatively impacts the prevention control of some allergic diseases[6]. The increased sIgE concentration of D.farinae/D.pteronyssinus supported this conclusion in the present study.

To sum up, our study found that, in general, the incidence of allergic diseases in children during the COVID-19 epidemic was reduced, and the exacerbation of diseases in allergic patients was also reduced. A recent meta-analysis encompassing 22,159 subjects demonstrated by random effect model that compared to the same period before the COVID-19 pandemic, pediatric asthma control during the pandemic was characterized by the lower incidence of asthma exacerbation (OR=0.26, 95%CI: 0.14,0.48), and lower emergency department visits (OR=0.11, 95%CI: 0.04,0.26)[7]. The control of allergic diseases has been significantly improved during the pandemic. Therefore, it is reasonable to specify that changes in hu-

man life and the living environment caused by NPIS in COVID-19 have a critical influence on the prevalence and control of allergic diseases. First, owing to businesses suspension, parents spend more time on their children's diets, which unquestionably reduces their exposure to food allergens to a great extent[8]. Second, measures such as wearing masks, washing hands frequently, strengthening indoor ventilation, and maintaining social distance not only hindered the spread of SARS-Cov-2 but avoided children's contact with inhaled allergens[9]. Third, air quality was significantly improved during the blockade, and air pollutants were significantly reduced, which also avoided children's exposure to inhaled allergens[10]. To a certain extent, NPIs during the COVID-19 pandemic have played a protective role in reducing children's exposure to allergens. The specific measures controlling the occurrence and development of allergic diseases should be further studied.

In conclusion, during the COVID-19 pandemic, non-pharmaceutical interventions reduced children's incidence and exacerbation of allergic diseases.

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Declaration of Competing Interest

All authors have declared that there is no conflict of interest.

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Qing Ye[1], Huihui Liu[1], Shiqiang Shang*
Department of Clinical Laboratory, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, National Children's Regional Medical Center, Hangzhou 310052, China

*Corresponding author.
E-mail address: shangsq@zju.edu.cn (S. Shang)

[1] These authors contributed equally to this work.

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Detection of human respiratory viruses among hospitalized children aged ≤ 5 years in Wuhan (China), from January to May 2020

Dear editor,

Respiratory virus infections can lead to influenza-like illnesses (ILIs), which may cause acute respiratory tract infections, and are a significant source of morbidity and mortality worldwide.[1,2] These kinds of infections occur mainly in infants and children, who can experience up to five or six episodes in any given year.[3] In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were reported in Wuhan, which caused coronavirus disease 2019 (COVID-19).[4] This novel coronavirus has spread across the world leading to a new global pandemic. The spread of SARS-CoV-2 has been curtailed by the implementation of various public health interventions, including lockdowns in Wuhan City, from January 23, 2020 to April 8, 2020. In this Journal, Mensah et al. reported that national lockdowns were associated with large declines in SARS-CoV-2 infection rates.[5] However, the impact of various public health interventions on the transmission of other respiratory viruses remains largely unknown. In this study, we present our findings from Wuhan during this same lockdown period.

Our study identified 1404 inpatient cases from Hubei Maternal and Child Health Hospital, who underwent testing for respiratory pathogens between January and May 2020. The samples were taken from infants and children aged ≤ 5 years, among whom 568 of them were female (40.46%) and 836 were male (59.54%). The mean age (± SD) of the patients was 1.21 ± 1.36 years (median: 0.83 years; interquartile range (IQR) 0.08–2.00 years) (Supplementary Table 1). We also tested for eight different respiratory viruses, including adenovirus (ADV), influenza A virus (Flu A), influenza B virus (Flu B), parainfluenza virus (PIV) 1–3, respiratory syncytial virus (RSV), and SARS-CoV-2. Analysis of the samples for each of these respiratory viruses, except for SARS-CoV-2, was performed using a rapid antigen detection kit (DIAGNOSTIC HYBRIDS, INC.). Detection of SARS-CoV-2 was performed using real-time RT-PCR detection of the N and RdRp genes, and positive samples were verified using an official approved clinical diagnostic kit (DAAN Gene Co., Ltd) as previously described.[6]

Of the 1404 hospitalized pediatric patients, 407 (407/1404, 28.99%) were positive for at least one pathogen, including 390 single infections and 17 co-infections (Supplementary Table 1). Among the single infections, RSV (292/1404, 20.80%) was the most common, followed by Flu A (51/1404, 3.63%), Flu B (21/1404, 1.50%), ADV (14/1404, 1.00%), PIV2 (4/1404, 0.28%), PIV1 (3/1404, 0.21%), SARS-CoV-2 (3/1404, 0.21%), and PIV3 (2/1404, 0.14%) (Supplementary Table 1). RSV was the predominant pathogen in all age groups (Supplementary Table 1). We also noted that the number of inpatients peaked in January and then decreased drastically in the following months, shifting from 813 cases in January to 60 cases in May 2020 (Fig. 1). The monthly detection rates for these respiratory viruses ranged from 0 to 46.37% for the patients

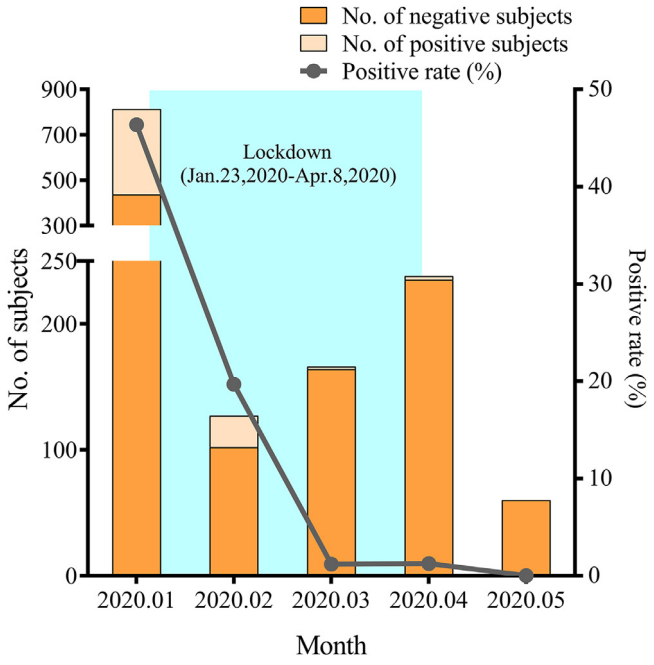

Fig. 1. Monthly distribution of respiratory virus positive subjects (light orange bars), negative subjects (orange bars) and positive rate (gray line) in Wuhan, from January to May 2020.

tested, and the peak detection rate was observed in January 2020 (377/813, 46.37%). The detection rate then decreased dramatically from 19.69% (25/127) in February 2020 to 0% (0/60) in May 2020 (Fig. 1). In addition, the number of different types of viruses detected decreased significantly, from eight in January, to four in February, to none in May 2020 (Supplementary Table 2). Most of the inpatients with a single respiratory infection were diagnosed with lower respiratory tract infections (339/390, 86.92%), and RSV accounted for these infections (272/339, 80.24%) (Supplementary Table 3, Supplementary Fig. 1).

Among the 1404 enrolled patients, seven (7/1404, 0.50%) tested positive for SARS-CoV-2, including three single infections and four co-infections. All seven patients were hospitalized before January 23, 2020, the day when the lockdown started (Supplementary Table 4). During the lockdown, the COVID-19 patients were centralized quarantined, and no more SARS-CoV-2 positive patients were hospitalized in the hospital over the course of this study. These seven patients were infants aged $\leq$ 2 years with two patients being $\leq$ 1 month old (Supplementary Table 4). All seven patients were diagnosed with respiratory tract infections, and five experienced high fever ($>$ 38.5 °C). All of these patients (except for patient 5 who was transferred) were treated with antibiotics and/or antivirals and recovered within 10 days.

This study describes the epidemiology of the respiratory virus infections of inpatients aged $\leq$ 5 years in Wuhan City during the Wuhan lockdown in 2020. We found that both the number of inpatients and the detection rates of respiratory viral infections decreased dramatically after COVID-19 lockdown measures were implemented. Our findings are consistent with those of other studies on the circulation of respiratory viruses during the COVID-19 pandemic.[7,8] These results strongly suggest that nonpharmaceutical interventions, including lockdowns, interrupt or reduce the spread of respiratory viruses. Our results also revealed that all seven SARS-Cov-2 positive infants contracted the virus before lockdown started. Additionally, in the early months of the pandemic, when the testing capacity was insufficient, SARS-CoV-2 infections in young children may have contributed to the spread of the virus. As reported in other similar cases, all six infants recovered within 10 days of hospitalization, suggesting that the clinical manifestations of COVID-19 in children may be less severe than that of adult patients.[9,10] The findings of this study were subject to at least three limitations. First, only a single center was enrolled in the study. Second, patients preferred to treat themselves at home during the lockdown period, reducing the number of patients seeking professional treatment, which may also have led to a reduction in inpatient admissions. Third, other common respiratory viruses, including rhinoviruses and common human coronaviruses, were not evaluated or enrolled in this study. Nevertheless, our results highlight the impact of nonpharmaceutical interventions, including lockdowns, on the spread of respiratory viruses.

Declaration of Competing Interest

None.

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Supplementary materials

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Mengchan Hao[1]
CAS Key Laboratory of Special Pathogens, Center for Biosafety Mega Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
University of Chinese Academy of Sciences, Beijing 100049, China

Yaqi Zhu[1]
Department of Laboratory Medicine, Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430070, China

Sanling Fan[1]
CAS Key Laboratory of Special Pathogens, Center for Biosafety Mega Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

Xiaoxue Wu[1]
Department of Laboratory Medicine, Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430070, China

Juefu Hu
CAS Key Laboratory of Special Pathogens, Center for Biosafety Mega Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

Ke Wu
CAS Key Laboratory of Special Pathogens, Center for Biosafety Mega Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
University of Chinese Academy of Sciences, Beijing 100049, China

Zhi Liu, Jinou Xi, Yaoyao Zhao, Tianzhu Zhang, Jianbo Xia, Chunchen Wu*
Department of Laboratory Medicine, Maternal and Child Health Hospital of Hubei Province, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430070, China

Jianjun Chen*
CAS Key Laboratory of Special Pathogens, Center for Biosafety Mega Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

*Corresponding authors.
E-mail addresses: chunchen_wu@126.com (C. Wu), chenjj@wh.iov.cn (J. Chen)

[1] These authors contributed equally to this work.
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High compliance to infection control measures prevented guest-to-staff transmission in COVID-19 quarantine hotels

Dear Editor,

We read with interest the article by Bou-Karroum et al. on the public health effects of travel-related policies on the COVID-19 pandemic, in which the authors demonstrated that early border closure and quarantine of travellers contributed positively to the control of the pandemic.[1] In particular, the authors identified 4 observational and 2 modelling studies on quarantine of travellers, which showed that the effectiveness of quarantine increased with increasing rates of compliance with quarantine.

Hotel quarantine for incoming travellers have been implemented in many places, such as the United Kingdom, Australia, New Zealand, Canada, and mainland China. In response to the emergence of SARS-CoV-2 variants of concern (VOCs), the Hong Kong SAR government imposed mandatory quarantine at designated hotels for all persons returning from places outside mainland China since December 2020.[2] However, quarantine hotels may serve as a hotspot for viral spread if there is lapse in infection control. There have been reports of COVID-19 transmission in quarantine hotels which involved transmission from returned travellers to staff.[3,4] A previous study has shown that SARS-CoV-2 RNA can be detected in surface swabs, pillow cover, sheet and duvet cover in guest rooms of a quarantine hotel where presymptomatic COVID-19 patients stayed,[5] although the infectivity may be low for indirect fomite transmission.[6]

In April 2021, two returning travellers infected with SARS-CoV-2 were diagnosed after checking out from quarantine hotels in Hong Kong, which triggered extensive contact tracing and mass testing.[7] There were no epidemiological links to other COVID-19 cases except that they stayed on the same floors with confirmed COVID-19 cases in the two quarantine hotels. Intra-hotel transmission was suspected. Our previous investigation suggested possible airborne transmission through ingression of air from the doorway when the doors of the guest rooms were opened, and that there was a lack of fresh air supply and absence of exhaust fan in the corridors of the implicated hotels.[8] As part of the investigation into this incident, we conducted a seroepidemiological survey of hotel staff members to assess whether silent transmission has occurred. Institutional review board approval was exempted since this is an emergency public health response.

A total of 136 individuals participated in the survey between 15th and 20th May 2021, including 90 staff members from the 2 implicated hotels (Hotels A and B), and 46 from hotel C, a third hotel under the same hotel chain which also served as a quarantine hotel but did not have any known intra-hotel transmission (Table 1). The questionnaire included basic demographics, COVID-19 vaccination status, work nature, exposure to quarantined guests or their belongings, personal protective equipment (PPE) usage and training on infection control.

Seventy three (53.7%) individuals had exposure to the guests or their belongings. Amongst them, all wore face masks during work, 93.2% (68/73) wore protective gowns, 78.1% (57/73) wore face shields, 94.5% (69/73) wore gloves, and 90.4% (66/73) wore goggles. One hundred and four (76.5%) individuals, including 90.4% (66/73) of the exposed, reported having received training on infection control and prevention, most commonly in the form of face-to-face teaching sessions (74/104, 71.2%) and self-reading materials (73/104, 70.2%), while 11.5% (12/104) individuals also attended online training class.

Fifty seven (41.9%) of the staff members had received at least one dose of COVID-19 vaccine before participating in the study. Amongst them, 43.9% (25/57) received the BNT162b2 mRNA vaccine (Pfizer-BioNTech), and 56.1% (32/57) received the CoronaVac inactivated virus vaccine (Sinovac Life Sciences). There was no statistically significant difference in the demographics and presence of underlying diseases between the vaccinated and non-vaccinated groups (Table 2). The vaccination rates were not significantly different between those with and without exposure to quarantined guests. However, individuals who received the BNT162b2 vaccine were significantly younger (median age 44.5 vs 52 years; $p = 0.01$) and were less likely to have underlying diseases than those who received the CoronaVac vaccine (proportion with underlying disease 16.0% vs 46.9%; $p = 0.02$).

Table 1
Demographics of hotel staff members in this study.

Characteristics	No. (%) unless otherwise specified			
	Total (n = 136)	Hotels A + B (n = 90)	Hotel C (n = 46)	p value (Hotels A + B vs. C)
Age – median years (range)	49.5 (24–70)	50 (24–66)	48 (27–70)	0.12
Sex – male (%)	76 (55.9)	52 (57.8)	24 (52.2)	0.59
Smoker (%)	45 (33.1)	28 (31.1)	17 (37.0)	0.56
Comorbidity:				
No comorbidites	86 (63.2)	54 (60.0)	32 (69.6)	0.35
Hypertension (%)	32 (23.5)	24 (26.7)	8 (17.4)	
Diabetes mellitus (%)	10 (7.4)	6 (6.7)	4 (8.7)	
Liver disease (%)	6 (4.4)	5 (5.6)	1 (2.2)	
Heart disease (%)	5 (3.7)	4 (4.4)	1 (2.2)	
Lung disease (%)	4 (2.9)	4 (4.4)	0 (0)	
Renal disease (%)	1 (0.7)	1 (1.1)	0 (0)	
Others (%)	11 (8.1)	10 (11.1)	1 (2.2)	
Work nature:				
Housekeeping	42	30	12	
Clerical work	30	17	13	
Engineering	20	11	9	
Concierge	13	8	5	
Security	12	11	1	
Kitchen	6	5	1	
Cleaning	5	3	2	
Linen room	3	2	1	
Management	2	1	1	
Meal delivery	1	1	0	
Restaurant	1	1	0	
Accounting	1	0	1	
Exposure to guests under quarantine:				
No exposure	63 (46.3)	36 (40.0)	27 (58.7)	0.04*
Face-to-face exposure (within 2 m)	32 (23.5)	24 (26.7)	8 (17.4)	
Stayed in the same room	8 (5.9)	7 (7.8)	1 (2.2)	
Contact with items which have been touched/used by quarantined guests	58 (42.6)	42 (46.7)	16 (34.8)	
Vaccination status				
Any COVID-19 vaccine (≥one dose)	57 (41.9)	31 (34.4)	26 (56.5)	0.02*
BNT162b2 (≥one dose)	25 (18.4)	11 (12.2)	14 (30.4)	
CoronaVac (≥one dose)	32 (23.5)	20 (22.2)	12 (26.1)	
Any COVID-19 vaccine (2 doses with last dose at least 14 days before joining study)	40 (29.4)	23 (25.6)	17 (37.0)	
BNT162b2 (2 doses with last dose at least 14 days before joining study)	13 (9.6)	6 (6.7)	7 (15.2)	
CoronaVac (2 doses with last dose at least 14 days before joining study)	27 (19.9)	17 (18.9)	10 (21.7)	

Table 2
Characteristics of the participating staff from 3 quarantine hotels based on vaccination status.

Group	Unvaccinated (n = 79)	Vaccinated (n = 57)	BNT162b2 (n = 25)	CoronaVac (n = 32)	Unvaccinated vs. Vaccinated	BNT162b2 vs. CoronaVac
Median age (years)	49.5	49	44.5	52	p = 0.66	p = 0.01*
Sex (male%)	58.2%	52.6%	64.0%	43.8%	p = 0.60	p = 0.18
Smoking	36.7%	28.1%	40.0%	18.8%	p = 0.36	p = 0.14
Underlying disease	39.2%	33.3%	16.0%	46.9%	p = 0.59	p = 0.02*
Exposure to guests or their belongings	54.4%	52.6%	40.0%	62.5%	p = 0.86	p = 0.11
Perceived knowledge (mean, standard error)	7.56, 0.22	7.33, 0.35	7.56, 0.43	7.16, 0.53	p = 0.86	p = 0.96

We performed both anti-nucleocapsid (N) IgG test and surrogate virus neutralisation antibody test (sVNT) for all participants (See Supplementary Methods). Since our ongoing COVID-19 serosurveillance in Hong Kong showed a very low seropositive rate (Supplementary Table), a positive test in the anti-N IgG assay or sVNT would be compatible with natural infection for non-vaccinated individuals. For BNT162b2 mRNA vaccine recipients, only a positive anti-N IgG test would signify natural infection. Since CoronaVac is an inactivated whole virus vaccine, antibody test is not useful in differentiating natural infection from vaccine-induced immunity. Amongst the 104 non-vaccinated or BNT162b2 mRNA vaccine recipients, all tested negative for anti-N IgG. For the 79 non-vaccinated individuals, sVNT was positive for 1 staff and indeterminate for another. However, the sera from these two individuals tested negative by both anti-S1 IgG assay (Euroimmun) and conventional live virus microneutralisation assay. Hence, there was no serological evidence of COVID-19 infection amongst hotel staff members.

The absence of transmission from hotel guests to staff members is likely related to the adequate training and compliance of staff members to different preventive measures. Furthermore, the hotel staff in this study had higher vaccination rate than the general population in Hong Kong (41.9% amongst hotel staff in this study vs 28.2% of the Hong Kong population as of 19 June 2021),[9] which likely contributed to the absence of transmission to the hotel staff despite possible airborne transmission inside the hotels.

There are some limitations in this study. First, some hotel staff members did not reply to the questionnaire or join the serosurveillance. Second, some vaccinated individuals have not completed the course of COVID-19 vaccination for 14 days before blood taking, which may have affected the interpretation of serology results.

In summary, we demonstrated that infection control training and strict compliance amongst hotel staff members, especially those with direct contact with quarantined persons, may have prevented guest-to-staff transmission of SARS-CoV-2, thus preventing secondary spread to other guests and in the community.

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Supplementary materials

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Xin Li
State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China
Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China

Hong Chen
The Department of Health, Centre for Health Protection, Hong Kong Special Administrative Region, China

Lu Lu, Lin-Lei Chen, Brian Pui-Chun Chan
State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China

Shuk-Ching Wong
Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China

Vincent Chi-Chung Cheng, Kwok-Yung Yuen
State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China
Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China

Kwok-Hung Chan
State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China

Kelvin Kai-Wang To*
State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China
Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China

*Corresponding author at: State Key Laboratory for Emerging Infectious Diseases, Carol Yu Centre for Infection, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China.
E-mail address: kelvinto@hku.hk (K.K.-W. To)

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Contrasting specific antibody response to BNT162b2 mRNA vaccination in SARS-CoV-2-naive and previously infected nursing home residents

Dear Editor,

We have read the article by Lim et al. with great interest.[1] The authors found rapid and robust antibody responses after adenovirus vector-based SARS-CoV-2 vaccination in previously infected individuals. So far, antibody responses induced by SARS-CoV-2 mRNA vaccination have been intensively investigated.[2] No requirement for the second SARS-CoV-2 vaccine has been discussed in previously infected individuals because of sufficient antibody responses elicited by only one dose.[3] However, the details of antibody responses after SARS-CoV-2 mRNA vaccination in nursing home residents have not been fully characterized, although a few studies have been briefly reported.[4–6] In this study, we evaluated the antibody response to BNT162b2 mRNA vaccination in SARS-CoV-2-naive and previously infected nursing home residents, with

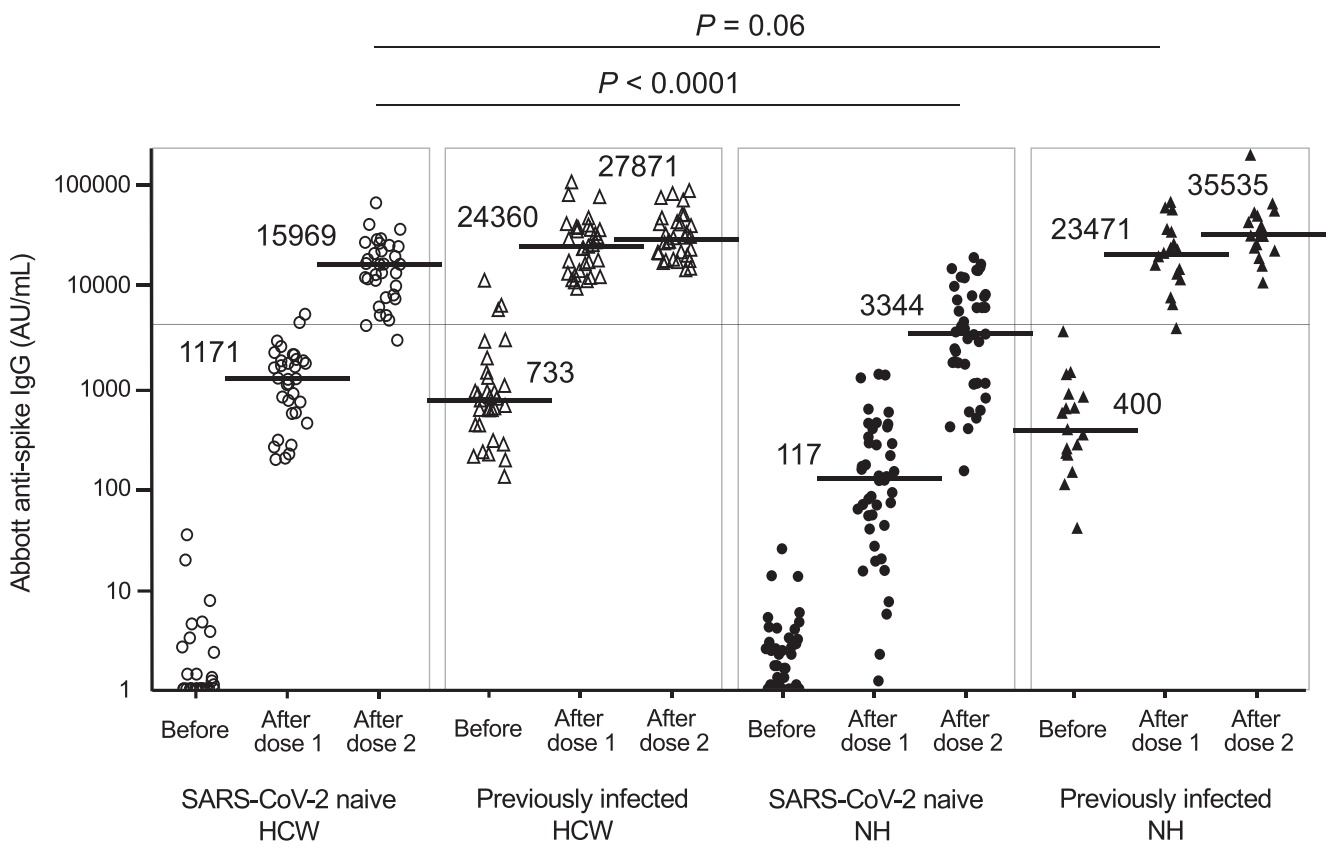

Fig. 1. Abbott anti-spike IgG antibody levels before and after BNT162b2 mRNA vaccination in SARS-CoV-2-naive and previously infected healthcare workers and nursing home residents. The white circles indicate data of SARS-CoV-2-naive healthcare workers ($n = 34$). The white triangles indicate data of previously infected healthcare workers ($n = 32$). The black circles indicate data of SARS-CoV-2-naive residents ($n = 43$). The black triangles indicate data of previously infected residents ($n = 17$). The horizontal solid bars and numbers in each group indicate the median values. The horizontal line indicates the value of 4,160 AU/mL, a threshold level indicating highly effective antibody neutralization. 50 AU/mL is the cut-off value. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HCW, healthcare workers; NH, nursing home residents.

healthcare workers as a control. COVID-19 outbreaks have severely affected nursing home residents.[7] Infection control during the outbreaks in nursing facilities is a critical public health issue.

This study was conducted as a serological follow-up evaluation after reporting a COVID-19 outbreak in a nursing facility in April 2020.[8] There was an outbreak in the hospital adjacent to the facility in January 2021. SARS-CoV-2-infected healthcare workers in the hospital were also included in the study control. BNT162b2 mRNA vaccination was performed twice at a 21-day interval from May to July, 2021. Serum samples after the first and second dose were collected on the scheduled day 21. Serological testing was performed using the serum samples collected before and after vaccination. The quantitative levels of IgG antibodies for the spike antigen of SARS-CoV-2 were examined using the Abbott Architect immunoassays (SARS-CoV-2 IgG II Quant, Abbott, Park, IL, USA). The anti-spike IgG levels of $\geq$ 4,160 AU/mL were used as a surrogate marker of highly effective antibody neutralization, based on the manufacturer's instruction. Anti-spike antibody levels were also measured using the Roche immunoassays (Elecsys Anti-SARS-CoV-2 S, Roche, Burgess Hill, UK). The details of the methods are shown in Supplementary methods.

This study included 126 individuals: 60 nursing home residents (mean age, 84.0 years; 43 SARS-CoV-2-naive and 17 previously infected) and 66 healthcare workers (mean age, 46.7 years; 34 SARS-CoV-2-naive and 32 previously infected). The baseline clinical characteristics of the 126 individuals are shown in Supplementary Table 1. Fig. 1 shows Abbott anti-spike IgG antibody levels before and after vaccination. The median IgG level in SARS-CoV-2-naive residents after the second dose was approximately five-fold lower than that in SARS-CoV-2-naive healthcare workers after the second dose (3,344vs 15,969 AU/mL, $P < 0.0001$). The frequency of IgG levels of $\geq$ 4,160 AU/mL in residents (41.9%, 18/43) was significantly lower than that in healthcare workers (94.1%, 32/34) ($P < 0.0001$). IgG levels in previously infected residents after the first dose were comparable to those in SARS-CoV-2-naive healthcare workers after the second dose. The results of Roche anti-spike antibody levels were similar to those of Abbott antibody levels (Supplementary Fig. 1). The basic data of Abbott and Roche antibody levels are shown in Supplementary Table 2. The relationship between age and post-vaccination anti-spike IgG levels is shown in Fig. 2. In SARS-CoV-2-naive healthcare workers and residents, increasing age significantly correlated with a decrease in IgG levels after both doses. In contrast, in previously infected healthcare workers and residents, a decline in antibody levels with increasing age was not shown. Next, IgG levels in the previously infected individuals were compared between two groups based on the duration from infection to vaccination (Fig. 2B). Post-vaccination IgG levels in the group with 13 to 15 months after infection appeared to be higher than in the group with 3 to 4 months, regardless of healthcare workers or residents. It was particularly significant in the comparison after the second dose ($P = 0.0002$).

In this study, we showed that after the second dose, anti-spike IgG levels in SARS-CoV-2-naive residents were extremely lower than those in SARS-CoV-2-naive healthcare workers. When using Abbott anti-spike IgG levels of $\geq$ 4,160 AU/mL as a threshold level indicating highly effective antibody neutralization, our results suggested that approximately 60% of SARS-CoV-2-naive residents after vaccination could not achieve antibody levels required to pro-

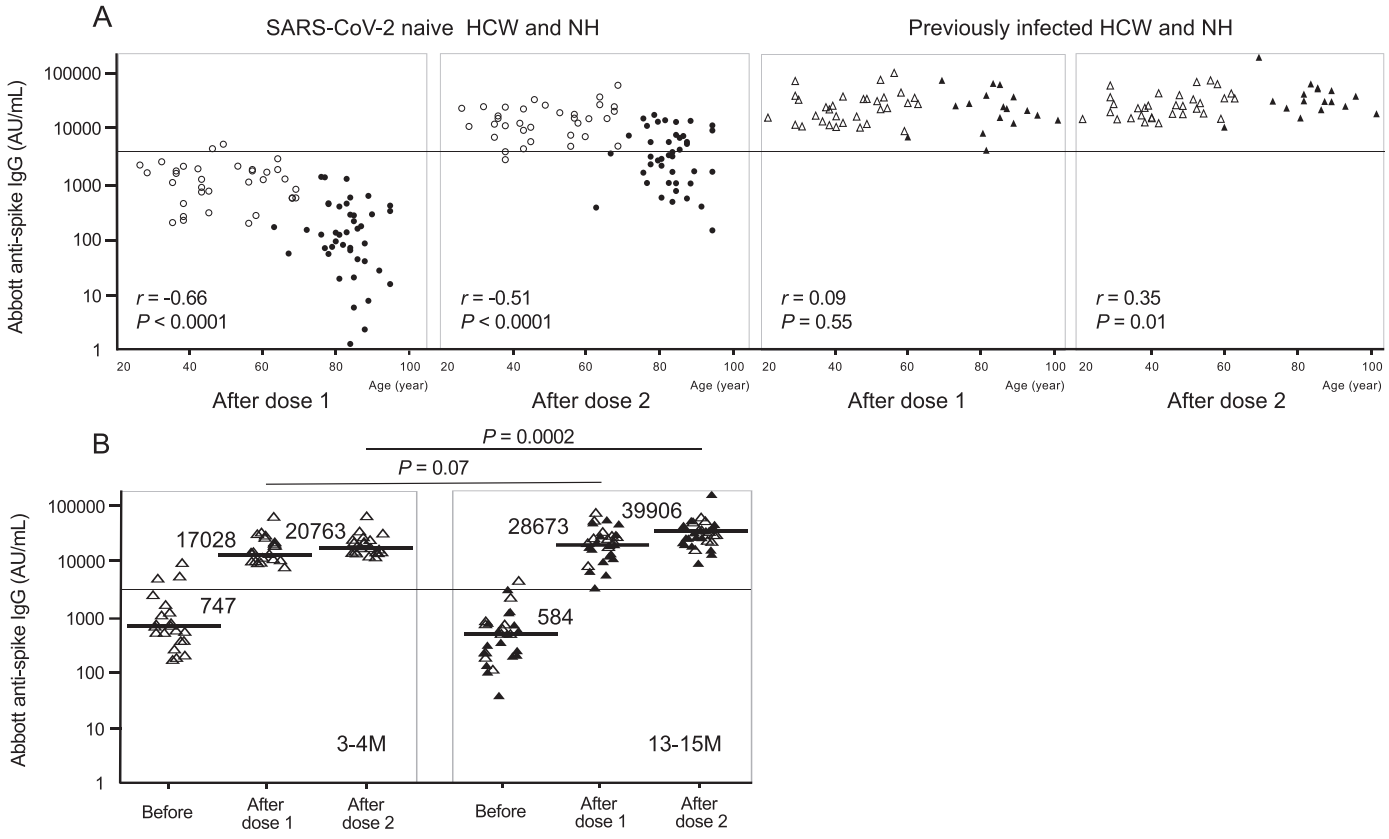

Fig. 2. A. Association of age with Abbott anti-spike IgG antibody levels after BNT162b2 mRNA vaccination. The white circles indicate data of SARS-CoV-2-naive healthcare workers. The white triangles indicate data of previously infected healthcare workers. The black circles indicate data of SARS-CoV-2-naive nursing home residents. The black triangles indicate data of previously infected residents. The horizontal line indicates the value of 4,160 AU/mL. **B.** Comparison of pre- and post-vaccination anti-spike IgG antibody levels by the duration from infection to vaccination in previously infected healthcare workers and residents. The group with 3 to 4 months from infection to vaccination (3-4 M) included 21 previously infected healthcare workers. The group with 13 to 15 months from infection to vaccination (13-15 M) included 11 previously infected healthcare workers and 17 previously infected residents. The white triangles indicate data of previously infected healthcare workers. The black triangles indicate data of previously infected residents. The horizontal solid bars and numbers in each group indicate the median values. The horizontal line indicates the value of 4,160 AU/mL. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; HCW, healthcare workers; NH, nursing home residents.

tect them against infection. SARS-CoV-2-naive nursing home residents may remain much more vulnerable to breakthrough infection than the general adult population. The clinical efficacy of the third (booster) dose of BNT162b2 vaccination in the elderly has just been reported.[9] The requirement of a high application order of re-vaccination may be reasonable in SARS-CoV-2-naive nursing home residents. The dynamics of antibody levels after vaccination in SARS-CoV-2 previously infected nursing home residents was completely different from that of SARS-CoV-2-naive residents. In this study, we showed that in previously infected residents after the first dose, antibody levels comparable to SARS-CoV-2-naive healthcare workers after the second dose were induced. Our findings suggest that even advanced aged nursing home residents only require one vaccine dose within approximately one year of their SARS-CoV-2 infection. The study on the third vaccination in the elderly did not include individuals previously infected with SARS-CoV-2.[9] It is likely that there is currently little discussion on the necessity of the third dose for previously infected individuals, including nursing home residents.

There has been little information on the factors related to rapidly increasing antibody responses after the vaccination of SARS-CoV-2 previously infected nursing home residents. It would be evident in this study that aging is not associated with an increase in post-vaccination antibody levels in SARS-CoV-2 previously infected individuals, at least within 15 months after infection. We obtained a finding that post-vaccination antibody levels were significantly higher in individuals with the longer duration after infection. Antibody responses after SARS-CoV-2 vaccination were more pronounced in adults with >3 months after infection than in those with 1to 2 months.[10] Intriguingly, SARS-CoV-2-specific memory function to demonstrate booster responses after vaccination might be maintained more effectively in individuals with the period of one year after infection than in those with three months, even in advanced aged nursing home residents.

Abbott anti-spike IgG levels of ≥ 4,160 AU/mL, which were used as a threshold of highly efficient antibody neutralization, could not necessarily reflect the standard levels required to protect against clinical SARS-CoV-2 infection. Post-vaccination antibody levels of SARS-CoV-2-naive residents were frequently below the threshold. However, lower antibody levels may work to protect against the infection. On the other hand, antibody levels required to protect against current SARS-CoV-2-variant infection may exceed the threshold, because current vaccines are derived from wild strains. A further observation will be needed regarding how much antibody levels after current vaccination could result in a breakthrough infection with circulating variant viruses, not only in SARS-CoV-2-naive residents but also in previously infected ones.

In conclusion, SARS-CoV-2-naive nursing home residents may not achieve sufficient antibody responses against SARS-CoV-2 infection, despite complete vaccination. In contrast, previously infected residents could maintain rapid and robust antibody responses to vaccination even more than one year after infection. We believe that our serological data of nursing home residents could

be of significant use to many healthcare professionals for future control measures for COVID-19 outbreaks.

Declaration of Competing Interest

There are no competing interests to declare for any of the authors.

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Supplementary materials

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Yong Chong*, Naoki Tani, Takeyuki Goto, Akiko Yonekawa
Medicine and Biosystemic Science, (The First Department of Internal Medicine), Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan

Hideyuki Ikematsu
Japan Physicians Association, Tokyo, Japan

Nobuyuki Shimono
Center for the Study of Global Infection, Kyushu University Hospital, Fukuoka, Japan

Yosuke Tanaka
Medical Corporation SOUSEIKAI, Kanenokuma Hospital, Fukuoka, Japan

Koichi Akashi
Medicine and Biosystemic Science, (The First Department of Internal Medicine), Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan

*Corresponding author.
E-mail address: ychong@intmed1.med.kyushu-u.ac.jp (Y. Chong)

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Persistence at one year of neutralizing antibodies after SARS-CoV-2 infection: Influence of initial severity and steroid use

Dear Editor,

Studies analyzing the persistence of protective immunity after SARS-CoV-2 infection are crucial to better understand the future dynamics of Covid-19 pandemic. We read with interest the results of Thangaraj et al.[1] regarding the evolution over time of anti-SARS-CoV-2 antibodies up to 7 months after an infection. Following 755 individuals, they observed a clear waning of anti-nucleocapside and anti-spike antibodies, but the persistence of neutralizing, anti-receptor binding domain (RBD) antibodies (NAb) in 86.2% of participants 181–232 days after RT-PCR diagnosis; those with more severe Covid-19 had higher NAb titres.

We conducted a follow-up of NAb titres 6 months (217 ± 19 days) and up to 1 year (377 ± 12 days) after a RT-PCR proven infection in 67 patients, infected between March and April 2020. Quantitative detection of SARS-CoV-2 antibodies targeting S1-RBD was determined by the Siemens SARS-CoV-2 IgG (sCOVG) assay on the Atellica IM platform (Siemens, Munich, Germany). Neutralizing antibody quantification was performed according to the previously published protocol,[2] based on a pseudotyped virus entry assay using a luciferase reporter gene. Pseudo-virus displaying full-length SARS-CoV-2 spike protein (derived from USA-WA1/2020 strain) was produced in HEK293T cells and used to infect HeLa-ACE2 cells. The result from this assay is expressed as the serum dilution required to reduce infection by 50% (ID50). The study was approved by the Comité de Protection des Personnes Sud-Est I on 20 August 2020 (Ref. 2020–84).

Mean age at positive RT-PCR was 59.8 ± 12 years; 42 (67.5%) of patients were males. Regarding Covid-19 severity, 17 (25.4%) individuals did not require oxygen supplementation, 17 (25.4%) required oxygen at a maximum of 2 L/min, and 33 (49.2%) required more than 2 L/min oxygen, among whom 29 were admitted to an intensive care unit. Dexamethasone was used in 20 of these 33 patients during the acute Covid-19 phase, all in patients admitted to ICU.

At the first sample (N = 67), median Atellica serology titre was 11.0 U/mL [IQR: 5–27]. It was correlated with age (p< 0.001, rho= 0.411) and severity (suppl. Table 1). Among those who required oxygen supplementation > 2 L/min, there was no significant difference according to steroid use (suppl Table 1). At this same time, the median ID50 NAb titre was 166 [IQR: 87–372]; two patients had no detectable NAb activity. Neutralization titres were correlated with age (p = 0.014, rho= 0.302)

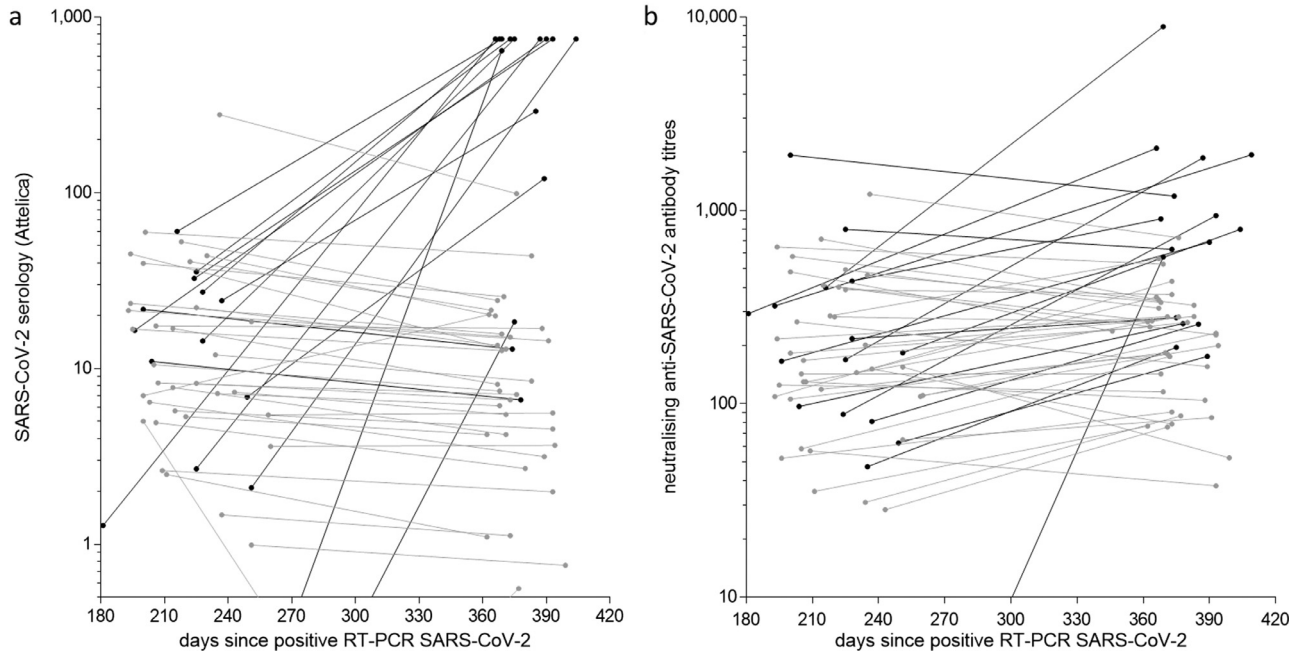

Fig. 1. Quantitative detection of SARS-CoV-2 IgG antibodies by Atellica serology (a) and neutralising antibody titres (b) at the two sample dates according to vaccination between the two titres (grey: no vaccination, black: vaccination). (The samples with no detectable antibodies are below the *X* axis).

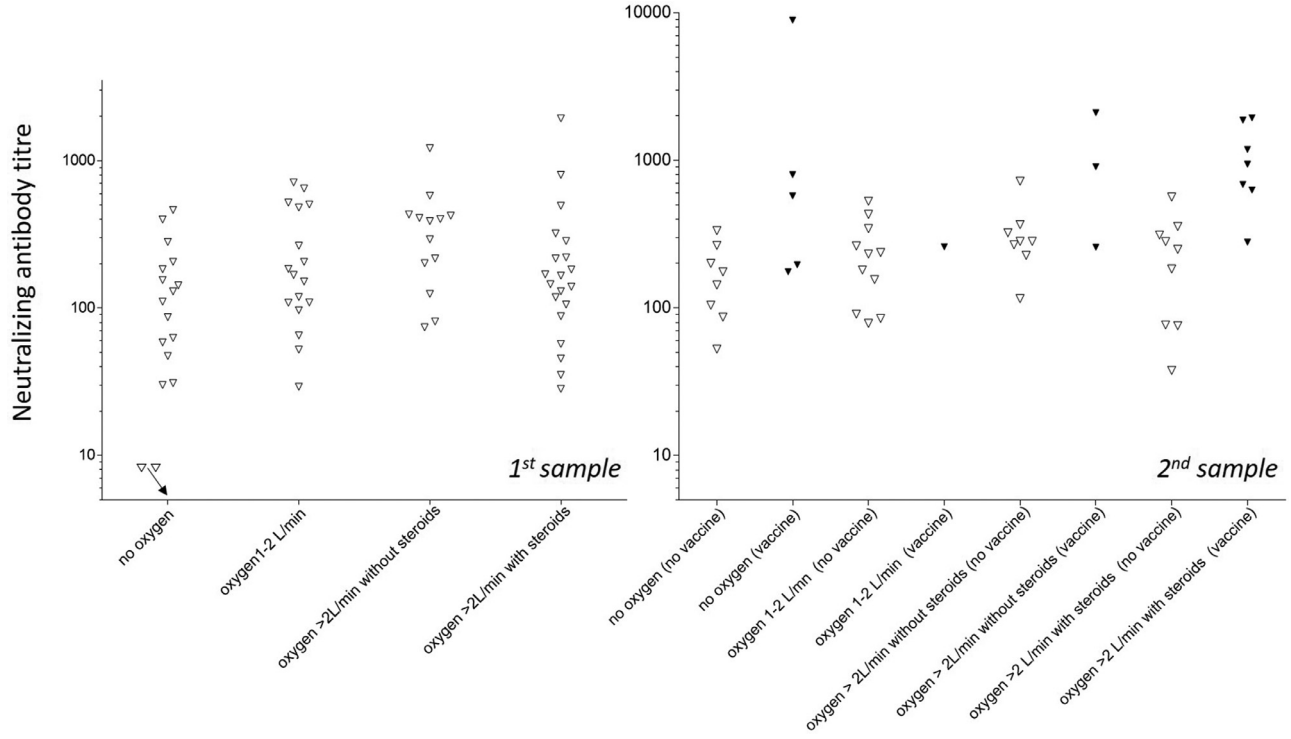

Fig. 2. Neutralizing antibody titres at the two sampling dates according to severity, steroid use, and (for the second date) vaccination (black triangles) or not (white triangles). (The two samples with no detectable NAbs are figured above the *X* axis with an arrow).

and severity (no oxygen vs. oxygen > 2 L/min: $p = 0.020$) (suppl. Table 1). Among individuals requiring oxygen supplementation > 2 L/min, there was no significant difference according to steroid use, although there was a trend toward lower titres for those who received steroids (suppl. Table 1). A positive correlation was observed between SARS-CoV-2 IgG antibodies as detected by the Atellica serology assay and the NAb titres ($p< 0.001$, rho= 0.455].

At the second sample ($N = 52$), 16 participants had received a first dose of the Covid-19 vaccine (Pfizer/BioNTech™, Moderna™, or AstraZeneca™) between the two samples. Median Atellica serology titre was 12.9 U/mL [4.8–85.1], with striking differences according to the vaccine status. Indeed, the median titre for those vaccinated before the second sample was 750.0 U/mL [16.2–750] vs. 6.9 U/mL [3.4–15.4] for unvaccinated subjects ($p< 0.001$) (Fig. 1); the Atellica serology titres remained stable between

the two dates for unvaccinated individuals but greatly increased among the vaccinated. Median ID50 neutralizing titres was 268 [177–545], with the same difference as above according to vaccine status. Indeed, the median ID50 titre for patients vaccinated between the two samples was 742 [269–1528] vs 237 [122–320] for unvaccinated subjects ($p< 0.001$) (Fig. 1). This difference was observed throughout the different severity groups (Fig. 2). There was no difference in titre according to the initial use of steroids (Suppl. Table 1).

Correlates of protection for Covid-19 are not completely established. However, the presence of NAb is associated with protection against many viral infections, and recent studies showed that the risk of SARS-CoV-2 reinfection was correlated with the NAb titres.[3] NAb response have therefore been particularly explored, mostly in the first months after SARS-CoV-2 infection, with somehow contrasting results.

We observed in our cohort that nearly all patients (65/67) had detectable NAb titres 7 months after their symptomatic SARS-CoV-2 infection, and that titres were stable between 6 months and 1 year (as measured by both EIA and neutralization assay). Relatively few studies have yet assessed NAb titers 1 year after infection; in a cohort of 73 subjects,[4] only 43% of individuals had detectable NAb titres after 1 year (vs 98% of 25 subjects sampled at months 5,6); in contrast, in a cohort of 620 individuals (58% inpatients and 42% outpatients),[5] the proportion with detectable NAb was high (80 to 90%) at 1 month and stable at 13 months (70–85%); in another recent study,[6] 97% of 367 patients had detectable NAb against initial SARS-CoV-2 strain at 13 months. In these different studies, those with more severe Covid-19 had higher NAb titres, as observed in our participants.

We did not observed a significant influence of steroid therapy at the acute phase on the long-term NAb titres; this had already been observed during earlier follow-up (< 1 month).[7]

Although unintended when we designed the study, we observed the expected booster effect of the vaccine dose. This so-called “hybrid immunity” has been observed in previous studies,[8] leading the French health authorities to recommend in early 2021 that subjects with a past SARS-CoV-2 infection should receive only one instead of two doses of the mRNA-based vaccine or AstraZeneca™ ChAd-based vaccine.[9]

Our study has several limitations, the first being its relatively small population size. Moreover, we did not assess the neutralizing potency of NAb against the Delta variant, which is less efficiently targeted by NAb induced by an infection with the viral strains circulating in 2020. Indeed, a recent pooled analysis.[10]concluded that the SARS-CoV-2 lineages Beta, Gamma, and Delta were less sensitive to NAb induced by a previous (2020) infection, with an average 4.1-fold (95% CI: 3.6–4.7), 1.8-fold (1.4–2.4), and 3.2-fold (2.4–4.1) reduction in IC50 titres.

Declaration of Competing Interest

Siemens Healthineers for Atellica sCOVG reagents offered free of charge.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.10.009.

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Olivier Epaulard*
Service des Maladies Infectieuses, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France
Groupe de Recherche en Infectiologie Clinique CIC-1406, Inserm – CHUGA – Université Grenoble Alpes, Grenoble, France
UMR 5075 – CEA-CNRS-UGA, Institut de Biologie Structurale, Grenoble, France

Marlyse Buisson
UMR 5075 – CEA-CNRS-UGA, Institut de Biologie Structurale, Grenoble, France
Laboratoire de Virologie, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

Benjamin Nemoz
Groupe de Recherche en Infectiologie Clinique CIC-1406, Inserm – CHUGA – Université Grenoble Alpes, Grenoble, France
UMR 5075 – CEA-CNRS-UGA, Institut de Biologie Structurale, Grenoble, France
Laboratoire de Virologie, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

Marion Le Maréchal
Service des Maladies Infectieuses, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France
Groupe de Recherche en Infectiologie Clinique CIC-1406, Inserm – CHUGA – Université Grenoble Alpes, Grenoble, France

Nicolas Terzi
Service de Médecine Intensive et Réanimation, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

Jean-François Payen
Département d’anesthésie et Réanimation, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

Marie Froidure, Myriam Blanc, Anne-Laure Mounayar, Fanny Quénard, Isabelle Pierre, Patricia Pavese
Service des Maladies Infectieuses, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France
Groupe de Recherche en Infectiologie Clinique CIC-1406, Inserm – CHUGA – Université Grenoble Alpes, Grenoble, France

Raphaele Germi, Laurence Grossi, Sylvie Larrat, Pascal Poignard, Julien Lupo
Groupe de Recherche en Infectiologie Clinique CIC-1406, Inserm – CHUGA – Université Grenoble Alpes, Grenoble, France
UMR 5075 – CEA-CNRS-UGA, Institut de Biologie Structurale, Grenoble, France
Laboratoire de Virologie, Center Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

*Corresponding author at: Service des Maladies Infectieuses, Center Hospitalier Universitaire Grenoble-Alpes, CS10217, 38043 Grenoble, France.
E-mail address: oepaulard@chu-grenoble.fr (O. Epaulard)

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SARS-CoV-2 vaccine breakthrough infection following a previous infection in a healthcare worker

Dear Editor,

Philomina et al. recently discussed in this journal breakthrough infections in healthcare workers from India.[1] SARS-CoV-2 infections and COVID-19 vaccines have been suggested to elicit immune response and reduce the predisposition to infections as well as severe disease. Reinfections[2] as well as vaccine breakthrough infections,[3] though rare, are now independently documented, but there is a paucity of literature on reinfections in fully vaccinated individuals. Here we describe a 28 year old male healthcare worker who was re-infected after being previously infected with SARS-CoV-2 and after completing the full course of Covishield/ChAdOx1 vaccine.[4]

The patient initially tested positive for SARS-CoV-2 on routine surveillance (TaqPath COVID-19 Combo kit by Thermofisher) with cycle threshold (C_T) values of 22. Subsequently, he developed fever for 2 days, breathlessness which lasted for seven days along with cough, bodyache and sore throat for ten days. On the seventh day of illness the patient suffered a brief drop in the oxygen saturation (SpO2) to 94% which recovered spontaneously on the next day. High-resolution Computed Tomography (HR-CT) revealed no abnormality and the patient tested negative for SARS-CoV-2 on reverse transcription-PCR (RT-PCR). Antibody titres two weeks after testing negative on RT-PCR, revealed a moderate level of antibodies (Elecsys Anti SARS-CoV-2, Roche Diagnostics) to spike protein (12). The patient then proceeded to take the first dose of Covishield/ChAdOx1 vaccine and subsequently the second dose four weeks later. He suffered mild post-vaccine effects including body aches and injection site pain lasting two days after both doses. A month after receiving the second dose, the patient again developed fever and tested positive on RT-PCR (C_T of 13) for SARS-CoV-2.

In the second episode of the infection, the patient had fever for four days, and cough, bodyache, headache, sore throat, loss of smell and taste for twelve days. The SpO2 level was around 94,95%, and the patient had mild difficulty in breathing throughout the symptomatic period. His HR-CT was normal and he had no evidence of primary or secondary immunodeficiencies. The clinical course and timelines are summarised in Fig. 1A and the clinical parameters are summarised in Table 1.

SARS-CoV-2 RNA isolated from the nasopharyngeal specimen of the patient during the post-vaccination episode of infection was taken up for genome sequencing following an amplicon-based COVIDSeq assay (Illumina Inc,) as per the previously described protocol.[5] The sequencing was performed on the Novaseq6000 platform (Illumina Inc.) to generate 100 × 2 base paired end reads. After quality checks, trimmed reads were aligned against the human reference genome (GRCh38). The unmapped reads were extracted and aligned to the SARS-CoV-2 reference genome NC_045512. Mu-

Table 1
Clinical and Biochemical investigations during the course described in the study.

Date	Lab Report	Results / Comments	Reference values
3/09/2020	RT-PCR	Positive	Negative
	Kit: TaqPath COVID-19Combo kit by Thermofisher	Ct value-22	
	C-Reactive Protein	22 mg/l	Upto 5.0 IU/mL
	D-Dimer	459 ng/ml	0–500 ng/ml
	LDH	200 units/l	85–227 U/lit
	High Resolution Computed Tomography (HR-CT)	Normal Score −0/25	0/25
18/09/2020	RT-PCR	Negative	Negative
	Kit: TaqPath COVID-19kit by Thermofisher		
	Neutralising Antibodies	12	
	Kit: Elecsys® Anti-SARS-CoV-2 by Roche		
13/04/2021	RT-PCR	Positive	Negative
	Kit: Covipath COVID-19 RT-PCR kit	Ct value ORF gene-13 Ngene-12 RNasePgene-24	
	C-Reactive Protein	40 mg/l	Upto 5.0 IU/mL
	D-dimer	570 ng/ml	0–500 ng/ml
	High Resolution Computed Tomography (HR-CT)	Normal Score 0/25	0/25

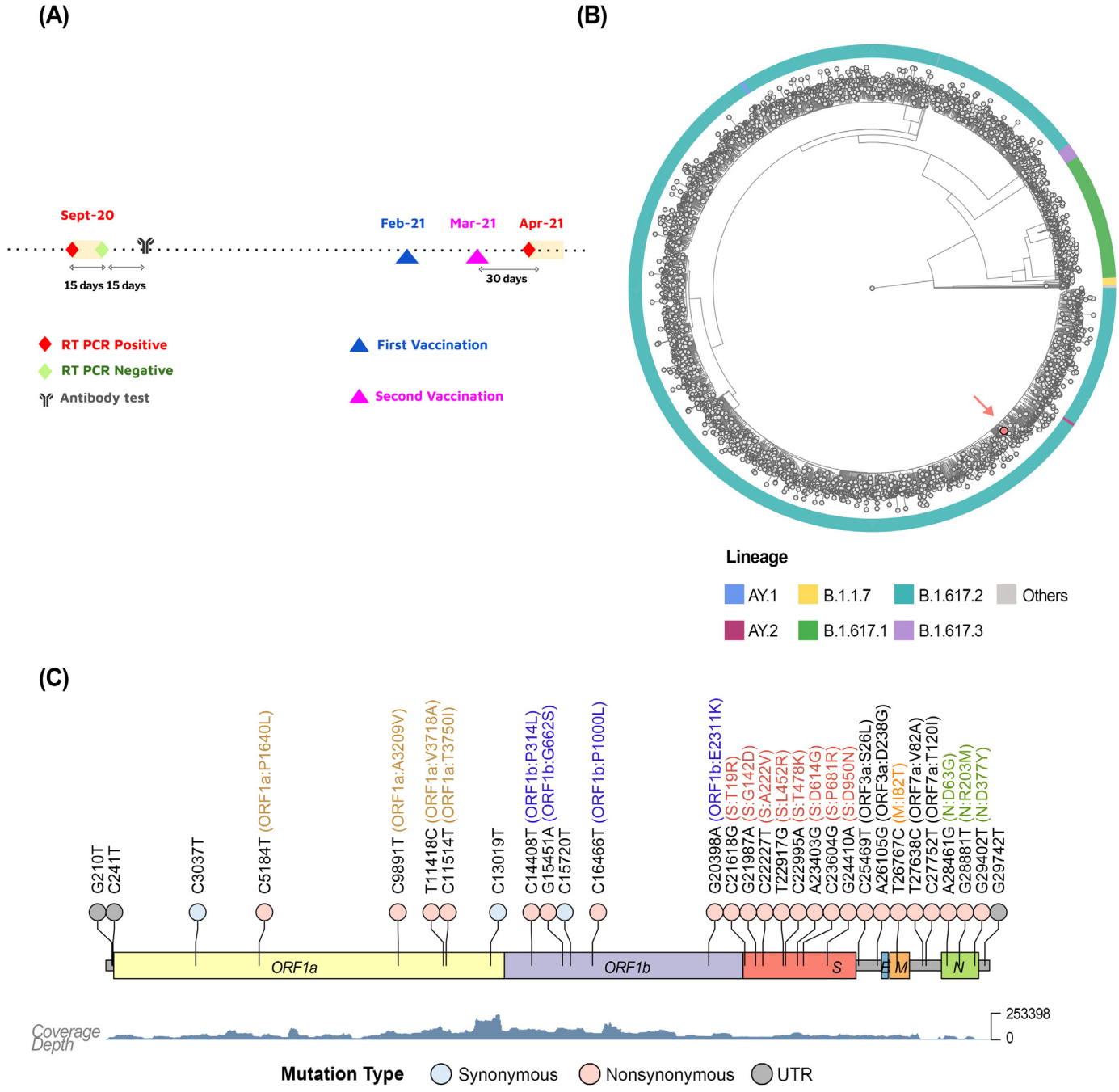

Fig. 1. (A) Summary of timelines and the clinical course for the patient. (B) Phylogenetic context of the genome isolate with other genomes sampled from the state of Maharashtra. (C) Genomic context of the mutations found in the genome isolate.

tations were filtered at a minimum coverage depth of 5 reads and a minimum frequency of 50%. Bases with quality lower than 20 were masked from the consensus sequence. Lineage assignment for the sequence was done using Pangolin (v3.1.11, pangoLEARN version 2021–08–09).[6]

Genome sequence for the viral isolate was assembled at a mean depth of coverage of 30460X, with genome coverage of 99.9%. Genomic analysis suggests the infection was caused by a virus belonging to the lineage B.1.617.2 (Delta) of SARS-CoV-2 (Fig. 1B). The sequence had a total of 30 distinct genetic mutations, 8 of which were in the Spike protein of the virus (Fig. 1C).

While a number of cases of reinfections and vaccine breakthrough infections have been reported, including in healthcare workers, infections following a previous infection and complete course of SARS-CoV-2 vaccines have previously not been documented. We also highlight that variants of concern, especially B.1.617.2 (Delta), have been previously suggested to escape immunity due to previous infections as well as vaccination.[7,8] Both reinfections and vaccine breakthrough infections seem to be enriched in healthcare workers potentially due to their high exposure.[9,10] To the best of our knowledge this is the first report on a combination of both reinfection as well as vaccine breakthrough infection in an individual. This report therefore highlights the need for close follow-up of rare and unusual cases of vaccine breakthroughs as well as reinfections especially in high-risk frontline workers.

Ethics

RNA extracted from nasopharyngeal swab samples were collected as part of routine COVID-19 testing after informed consent as per the institutional ethical committee guidelines (IHEC-CSIR-IGIB/IHEC/2020–21/01).

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Declaration of Competing Interest

Authors declare no conflicts of interest. The funders had no role in the preparation of the manuscript or decision to publish.

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Aayush Gupta*[1]
Department of Dermatology, Medical College, Hospital and Research Centre, Pimpri, Pune 411018, India

Rahul Bhoyar[1]
CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India

Shahzad Mirza
Department of Dermatology, Medical College, Hospital and Research Centre, Pimpri, Pune 411018, India

Bani Jolly, Vigneshwar Senthivel
CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India
Academy for Scientific and Innovative Research, Human Resource Development Centre, Campus, Ghaziabad, Uttar Pradesh 201002, India

Afra Shamnath
CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India

Mohit Kumar Divakar, Mohamed Imran, Vishu Gupta, Srashti Jyoti Agrawal
CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India
Academy for Scientific and Innovative Research, Human Resource Development Centre, Campus, Ghaziabad, Uttar Pradesh 201002, India

Sahjid Mukhida
Department of Dermatology, Medical College, Hospital and Research Centre, Pimpri, Pune 411018, India

Sridhar Sivasubbu, Vinod Scaria**
CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India
Academy for Scientific and Innovative Research, Human Resource Development Centre, Campus, Ghaziabad, Uttar Pradesh 201002, India

*Corresponding author. **Corresponding authors at: Knowledge Centre for Genome Informatics, CSIR- Institute of Genomics and Integrative Biology, Sukhdev Vihar Mathura Road, New Delhi 110025, India.
E-mail addresses: aayush.gupta@dpu.edu.in (A. Gupta), vinods@igib.in (V. Scaria)

[1] These authors contributed equally to this work.

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