WP6 Deliverable 6.13

Report on serology analysis including long-term sequelae and natural immunity

Universiteit Antwerpen (UANTWERPEN)

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1.0	03/07/2023	Angelina Konnova, Matilda Berkell, Surbhi Malhotra-Kumar, Samir Kumar-Singh (Uantwerpen)

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Executive summary

WP and deliverable context

The present report is part of ORCHESTRA project, a three-year international research project aimed at tackling the coronavirus pandemic. ORCHESTRA provides an innovative approach to learn from the pandemic SARS-CoV-2 crisis, derive recommendations to further management of COVID-19 and be prepared for possible future pandemic waves. The ORCHESTRA project aims at delivering sound scientific evidence for the prevention and treatment of the infections caused by SARS-CoV-2 assessing epidemiological, clinical, microbiological, and genotypic aspects of population, environment, and socio-economic features. The project builds upon existing, and new large scale population cohorts in Europe (France, Germany, Spain, Italy, Belgium, Romania, Netherlands, Luxemburg, and Slovakia) and non-European countries (India, Perú, Ecuador, Colombia, Venezuela, Argentina, Brazil, Democratic Republic of Congo, and Gabon) including SARS-CoV-2 infected and non-infected individuals of all ages and conditions. The primary aim of ORCHESTRA is the creation of a new pan-European cohort applying homogenous protocols for data collection, data sharing, sampling, and follow-up, which can rapidly advance the knowledge on the control and management of the COVID-19. Within ORCHESTRA project, Work Package 6 (WP6) aims at providing innovative laboratory capabilities combining serology, immunology, viral and human genomes, microbiota, and epigenetic analysis. It aims to describe markers and physiopathology of various COVID-19 outcomes including severe cases, long COVID and vaccine efficiency across various patient populations gathered within ORCHESTRA cohorts. The objectives of WP6 are distributed in two parts: (1) a retrospective part on frozen samples obtained during 2020 and (2) a prospective part starting in 2021.

Content of the document

The present report describes serum anti-Spike (S), anti-Nucleocapsid (N), and anti-RBD (ribosomal binding domain) titres in patients with varying degrees of COVID-19 disease severity in the context of the prospective studies within ORCHESTRA. Specifically, this report focuses on the longitudinal analysis of serological responses in patients up to 18 months following SARS-CoV-2 infection. Presently, the report is focused on the responses experienced shortly after diagnosis. A final report on viral variants/serology for breakthrough, primary versus incidental SARS-CoV-2 infections will be provided at the end of the project, where serological responses during primary infection compared to those experiencing a breakthrough infection (defined as patients having received at least two doses of an anti-SARS-CoV-2 mRNA vaccine ≥ 14 days prior to COVID-19 diagnosis) and correlates with SARS-CoV-2 variants within the two infection groups will be provided.

Dissemination level: Public

Core content

Detailed description of the conducted tests

Sample collection

Patient cohorts in which samples were collected for this deliverable include patients with a microbiologically confirmed SARS-CoV-2 infection, including patients with mild, moderate, and severe COVID-19. Nasopharyngeal swab (NPS) and serum samples were collected in Verona (UNIVR, Italy), Bologna (UNIBO, Italy), and Seville (SAS, Spain) during the initial stages of COVID-19 infection and 3 (M3), 6 (M6), 12 (M12), and 18 months after infection (M18). Collected samples were then transferred to UAntwerpen (Belgium) for longitudinal assessment of humoral immune responses to infection for this deliverable.

Serology

IgG titres were measured in serum samples using V-PLEX SARS-CoV-2 Panels 2, 6, 23, 25, 27, 29 or 32 Kits (IgG) from Meso Scale Discovery (MSD, MD, USA) according to the manufacturer's instructions. Briefly, 96-well plates were blocked with MSD blocking buffer A for 30 minutes and washed three times with PBS-Tween (0.05%). Samples were diluted 1:10 000 or 1:25 000 in Diluent 100 (MSD), incubated for two hours in the prepared plates, and subsequently washed three times. Detection antibody with a sulfo-tag was added and after a one-hour incubation, plates were washed and read with MSD Gold Read Buffer B on the QuickPlex SQ 120 (MSD). Quantitative IgG results were measured in Antibody Units (AU)/mL, converted to WHO Binding Antibody Units (BAU)/mL using a conversion factor provided by MSD.

Stratification of IgG responses

Antibody responses were stratified into the groups based on the quantitative IgG measurements as described in **Table 1**.

Table 1. Stratification of quantitative IgG results.

	Negative	Inconclusive	Low	Medium	High	Units
anti-Spike	<4.76	4.76 - <53	53 - <241	241 - <832	>832	BAU/mL
anti-RBD	<5.58	5.58 - <45	45 - <205	205 - <817	>817	BAU/mL
anti-N	<8.20	8.20 - <12	12 - <295	295 - <713	>713	BAU/mL

The upper limit for "Negative" was determined as the average plus one standard deviation of anti-Spike IgG measurements in 50 serum samples collected before 2019. The lower limits for "Low", "Medium" and "High" were based on the BAU/mL concentrations of "Low" (NIBSC code 20/140), "Mid" (NIBSC code 20/148) and "High" (NIBSC code 20/150) WHO International Standards for anti-SARS-CoV-2 immunoglobulins. Of note, while we still use these titers for "high IgG serology" as originally defined by WHO, currently, we achieve much higher IgG titers against SARS-CoV-2.

Key findings

Patient characteristics and sampling timepoints

Overall, 3669 samples were collected from patients and analysed by WP6 for assessment of longitudinal serological responses. As expected, the majority of analysed samples were collected during the acute phase of COVID-19 (T0) in this study (39.8%), whereas 60.2% constituted follow-up samples (**Table 2**).

Table 2. The number of patients available for analysis for each timepoint.

Timepoint	N (%)
T0	1460 (39.8)
M3	455 (12.4)
M6	743 (20.3)
M12	677 (18.5)
M18	334 (9.1)
Total	**3669**

Serology results for the Wuhan variant

The number of patients with high anti-spike and anti-RBD titres increased continuously up to 12 months after COVID-19. In total, 95% (95% CI [94, 97]) and 96% (95% CI [94, 97]) of patients develop high anti-spike and anti-RBD titres 12 months after COVID-19, respectively (**Figures 1 and 2, Table 3**). The number of patients with high titres decreases slightly by month 18, reaching 92% (95% CI [89, 95]) and 92% (95% CI [89, 95]) for anti-spike and anti-RBD titres, respectively.

In respect to anti-N titres, a temporary increase in titres was observed 3 months after COVID-19, followed by fast weaning of anti-N titres (Figures 1 and 2, Table 3). In total, 27% (95% CI [23, 31]) of patients develop high anti-N titres 3 months after COVID-19. The number of patients with high titres decreases by month 18, reaching 7% (95% CI [5, 11]).

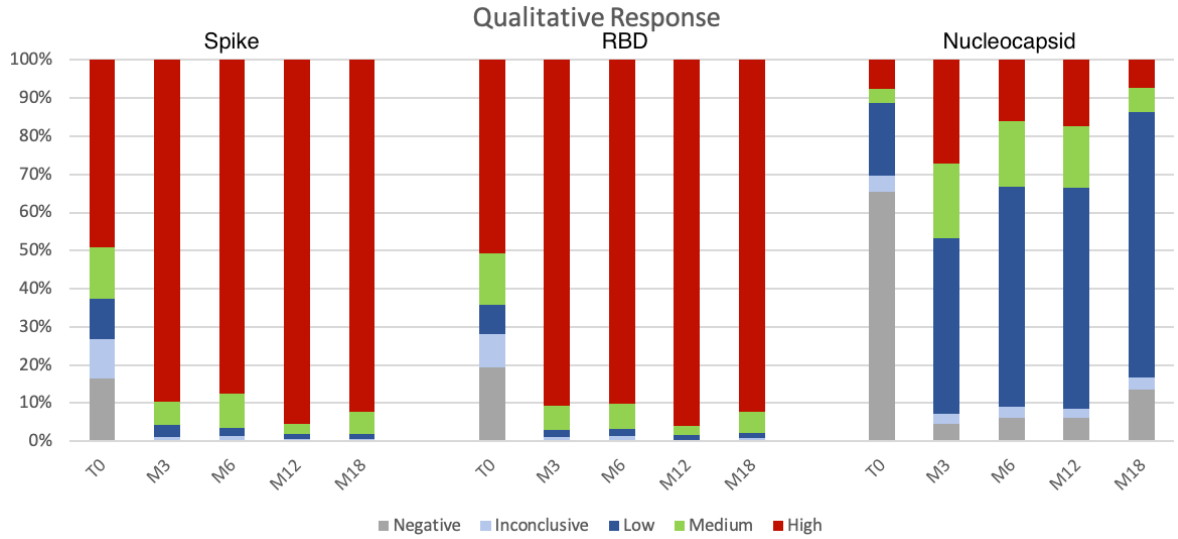

Figure 1. Qualitative serological results. Serological results were divided into “Negative”, “Inconclusive”; “Low”, “Medium”, and “High”, as described in the methodology section.

Table 3. Summary of qualitative serological results.

Anti-Spike	Negative n (%) [95% CI]	Inconclusive n (%) [95% CI]	Low n (%) [95% CI]	Medium n (%) [95% CI]	High n (%) [95% CI]	Total number of patients
T0	242 (17%) [15, 19%]	150 (10%) [9, 12%]	153 (10%) [9, 12%]	197 (13%) [12, 15%]	718 (49%) [47, 52%]	1460
M3	2 (0%) [0, 2%]	3 (1%) [0, 2%]	14 (3%) [2, 5%]	28 (6%) [4, 9%]	408 (90%) [87, 92%]	455
M6	3 (0%) [0, 1%]	8 (1%) [0, 2%]	15 (2%) [1, 3%]	67 (9%) [7, 11%]	650 (87%) [85, 90%]	743
M12	1 (0%) [0, 1%]	3 (0%) [0, 1%]	9 (1%) [1, 3%}	18 (3%) [2, 4%]	646 (95%) [94, 97%]	677
M18	1 (0%) [0, 2%]	1 (0%) [0, 2%]	4 (1%) [0, 3%]	20 (6%) [4, 9%]	308 (92%) [89, 95%]	334
Anti-RBD	**Negative n (%) [95% CI]**	**Inconclusive n (%) [95% CI]**	**Low n (%) [95% CI]**	**Medium n (%) [95% CI]**	**High n (%) [95% CI]**	**Total number of patients**
T0	283 (19%) [17, 22%]	126 (9%) [7, 10%]	115 (8%) [7, 9%]	195 (13%) [12, 15%]	741 (51%) [48, 53%]	1460
M3	2 (0%) [0, 2%]	3 (1%) [0, 2%]	8 (2%) [1, 3%]	29 (6%) [4, 9%]	413 (91%) [88, 93%]	455
M6	3 (0%) [0, 1%]	8 (1%) [0, 2%]	13 (2%) [1, 3%]	50 (7%) [5, 9%]	669 (90%) [88, 92%]	743
M12	1 (0%) [0, 1%]	2 (0%) [0, 1%]	9 (1%) [1, 3%]	15 (2%) [1, 4%]	650 (96%) [94, 97%]	677
M18	1 (0%) [0, 2%]	2 (1%) [0, 2%]	4 (1%) [0, 3%]	19 (6%) [3, 9%]	308 (92%) [89, 95%]	334
Anti-Nucleocapsid	**Negative n (%) [95% CI]**	**Inconclusive n (%) [95% CI]**	**Low n (%) [95% CI]**	**Medium n (%) [95% CI]**	**High n (%) [95% CI]**	**Total number of patients**
T0	955 (65%) [63, 68%]	64 (4%) [3, 6%]	277 (19%) [17, 21%]	55 (4%) [3, 5%]	109 (7%) [6, 9%]	1460
M3	21 (5%) [3, 7%]	12 (3%) [1, 5%]	209 (46%) [41, 51%]	90 (20%) [16, 24%]	123 (27%) [23, 31%]	455
M6	46 (6%) [5, 8%]	22 (3%) [2, 4%]	428 (58%) [54, 61%]	127 (17%) [14, 20%]	120 (16%) [14, 19%]	743
M12	42 (6%) [5, 8%]	16 (2%) [1, 4%]	393 (58%) [54, 62%]	108 (16%) [13, 19%]	118 (17%) [15, 21%]	677
M18	45 (13%) [10, 18%]	11 (3%) [2, 6%]	232 (69%) [64, 74%]	22 (7%) [4, 10%]	24 (7%) [5, 11%]	334

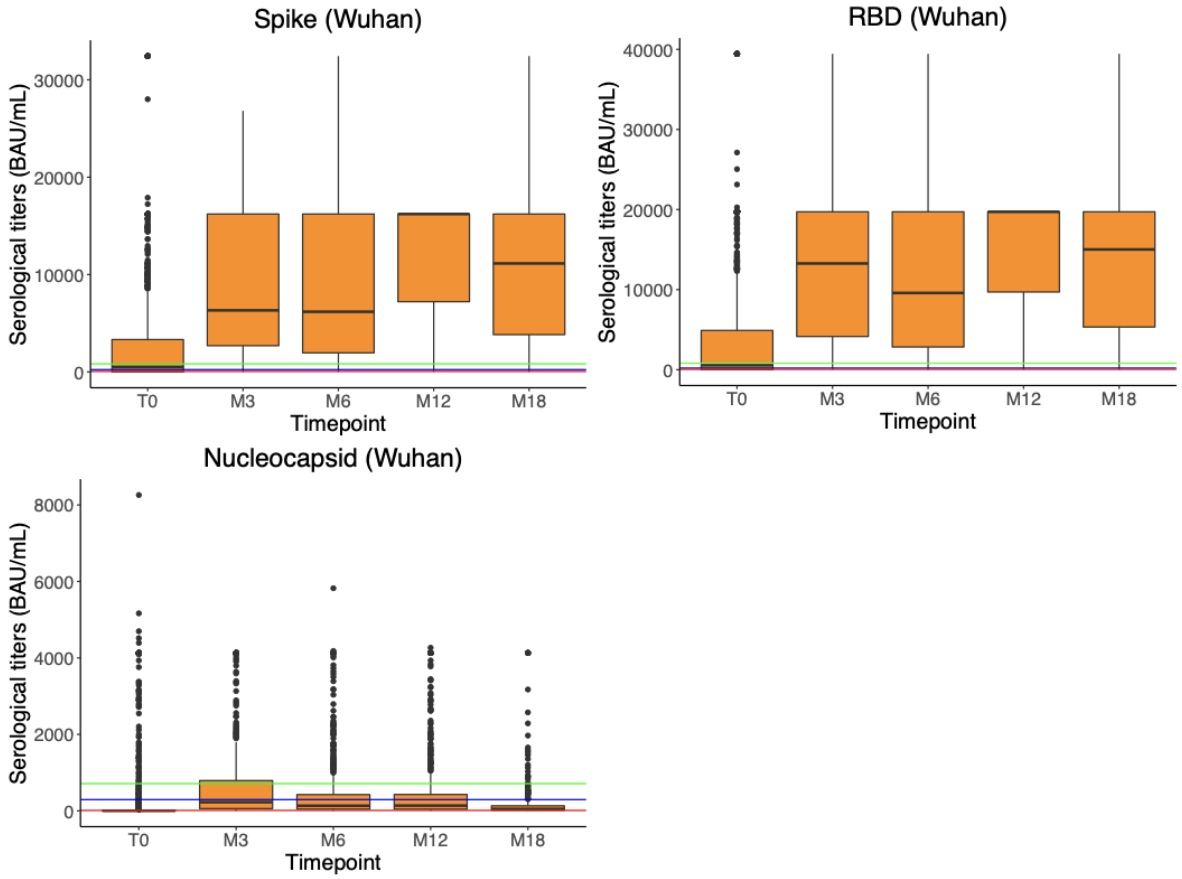

Figure 2. Anti-S, anti-RBD, and anti-N serology titres for the Wuhan variant of COVID-19 patients immediately after infection, at M3, M6, M12, and M18. Red, green, and blue lines indicate SARS-CoV-2 WHO reference standard values for low, medium, and high antibody titres, respectively. Box plots indicate median (middle line), 25th, 75th percentile (box), and 5th and 95th percentile (whiskers). BAU: Binding antibody units

Serology results for the Omicron sub-variants

In order to evaluate post-COVID-19 protection against currently circulating variants, we looked in anti-spike serology titres against Omicron sub-variants, BA.1, BA.2, BA.3, BA.4, and BA.5. Similar to the anti-spike responses for the Wuhan variant, we have observed a gradual increase in titres until 12 months after COVID-19 (**Figure 3**). However, anti-spike titres against Omicron sub-variants were significantly lower than anti-spike titres against the Wuhan variant at all studied timepoints.

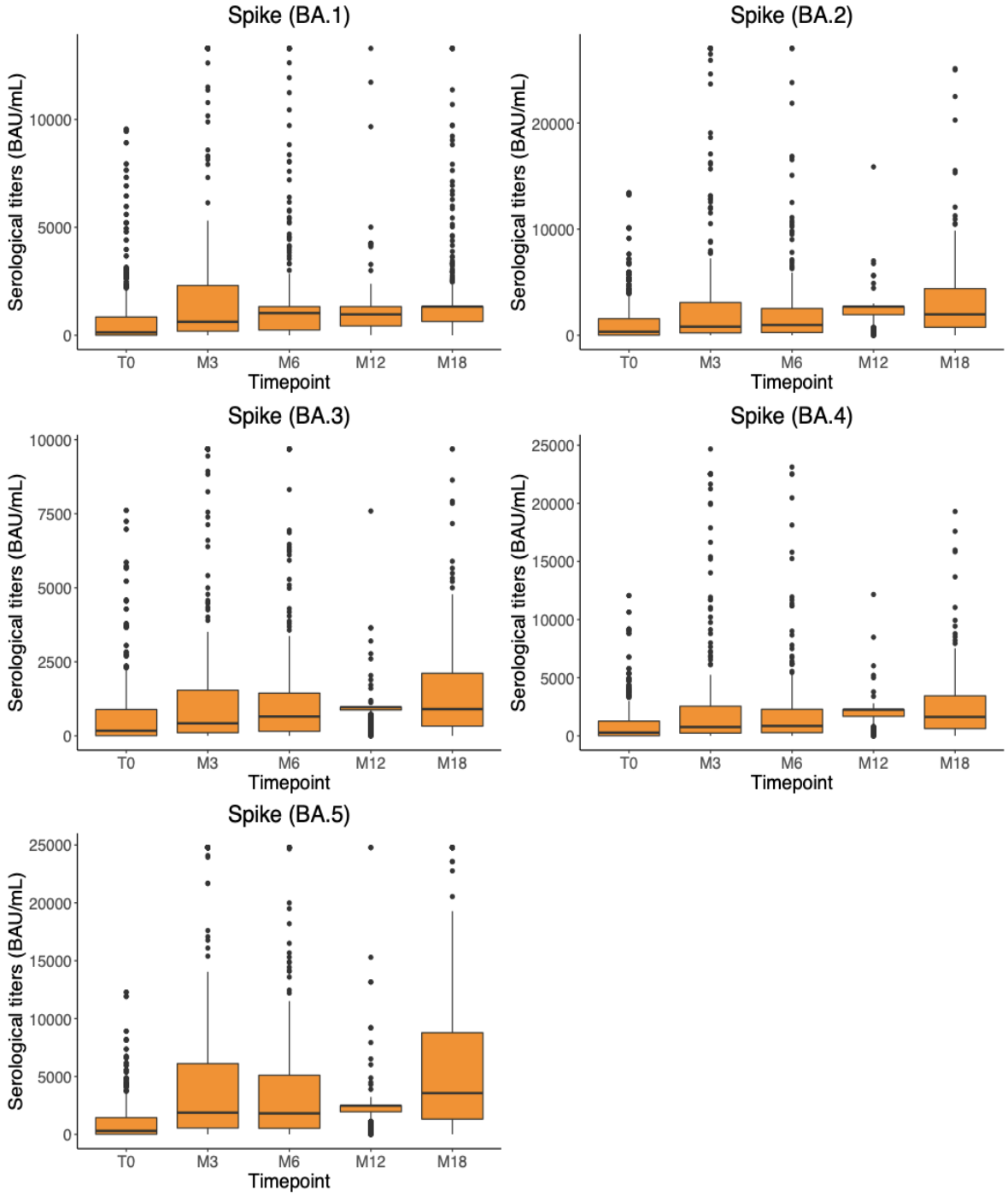

Figure 3. Anti-S serology titres for Omicron sub-variants (BA.1, BA.2, BA.3, BA.4, and BA.5) of COVID-19 patients immediately after infection, at M3, M6, M12, and M18. Red, green, and blue lines indicate SARS-CoV-2 WHO reference standard values for low, medium, and high antibody titres, respectively. Box plots indicate median (middle line), 25th, 75th percentile (box), and 5th and 95th percentile (whiskers). BAU: Binding antibody units

Conclusions

Most patients developed high anti-spike and anti-RBD titres after COVID-19 infections. The titres progressively increased throughout the study, reaching peak levels at 12 months after COVID-19. A similar trend was observed for anti-Omicron antibodies, although the extent of the increase of the titres was lower compared to responses against infecting non-Omicron variants. These data suggest that the protection against Omicron sub-variants might be compromised.

Acknowledgments

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