RCHESTRA

Connecting European Cohorts
to Increase Common
and Effective Response to
SARS- CoV-2 Pandemic

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First report on serology in fragile populations

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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 the 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 largescale 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, 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, the Work Package 6 (WP6) aims at providing innovative laboratory capabilities combining serology, immunology, viral and human genomes, microbiota and epigenetic analysis. It aims describing markers and physiopathology of various COVID-19 outcomes including severe cases, long COVID and vaccine efficiency across various patients' 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. The goal for the serological markers part is to allow the description of the SARS-CoV-2 variants (both known variants and novel mutations) according to epidemics waves, stages of SARS-CoV-2 infection (mild vs severe), setting (outpatients vs hospitalized), and outcome (survivors vs non-survivors).

Content of the document

The present report describes the number of tested serological points and serological markers performed in the context of the prospective study in fragile populations. The objective is to allow the description of serological markers across patients included in the prospective part of the Orchestra study to allow its analysis along with viral variant, genomic, microbiota data and clinical data for all included patients with such available data.

In this report we provide the number of samples tested for all evaluated serological markers.

Dissemination level: Public

Detailed description of the conducted tests

Sample collection

All serum samples from both previously infected and uninfected solid organ transplant (SOT) patients analysed by WP6 (UAntwerpen) for this deliverable were collected in Verona (UNIVR, Italy), Vicenza (Italy), Treviso (Italy), Padova (Italy), and SAS (Spain), as described in **Table 1**.

Table 1: The numbers of included samples per cohort.

Cohort	**Number of serum samples received**	**Serology**	**Seroneutralisation**	**Harmonised data delivered on**
UNIVR SOT Cohort	523	523	523	15/10/2021
Vicenza SOT Cohort	261	261	261	15/10/2021
Treviso SOT Cohort	1354	1354	1354	05/10/2021
Padova SOT Cohort	1157	1157	1157	20/10/2021
SAS SOT Cohort	46	46	46	21/12/2021

IgG measurements in serum

IgG titres were measured in serum samples using V-PLEX SARS-CoV-2 Panel 6 Kit (IgG) from Meso Scale Discovery (MSD, MD, USA) according to the manufacturer instructions. IgG titres to the following antigens were measured: Nucleocapsid, RBD, Spike, Spike (D614G), Spike (B.1.1.7), Spike (B.1.351) and Spike (P.1).

Measurements were performed in randomized batches. Briefly, 96-well plates were blocked with MSD blocking buffer A for 30 minutes. All plates were then washed three times with PBS-Tween (0.05%). Samples were diluted 1:5,000 in Diluent 100 (MSD), loaded on the plates and incubated for two hours, after which the plates were washed three times again. Detection antibody with a sulfo-tag was added and after another one-hour incubation step 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. The detection range is described in Table 1.

Stratification of IgG responses

Antibody responses were stratified into the groups based on the quantitative IgG measurements as described in **Table 2**.

Table 2: Stratification of quantitative IgG results.

	Negative	**Inconclusive**	**Low**	**Medium**	**High**	**Units**
anti-Spike	<4.76	4.92 - <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.

ACE2 neutralisation measurements in serum

ACE2 neutralisation was measured in serum samples using V-PLEX SARS-CoV-2 Panel 6 Kit (ACE2) from Meso Scale Discovery (MSD, MD, USA) according to the manufacturer instructions. The antibodies capable of blocking the binding of ACE2 to the following antigens were measured: RBD, Spike, Spike (D614G), Spike (B.1.1.7), Spike (B.1.351) and Spike (P.1).

Measurements were performed in randomized batches. Briefly. 96-well plates were blocked with MSD blocking buffer A for 30 minutes. All plates were then washed three times with PBS-Tween (0.05%). All samples were diluted 1:50; loaded on the plates and incubated for one hour. ACE2 with a sulfo-tag was added and after another one-hour incubation step plates were washed and read with MSD Gold Read Buffer B on the QuickPlex SQ 120 (MSD).

The calibration curve was used to calculate neutralizing antibody concentrations in samples, by backfitting the measured signals for samples to the calibration curve. Neutralising antibody concentrations were measured in Units (U)/mL, which corresponds to neutralizing activity of 1 µg/mL monoclonal antibody to SARS CoV-2 Spike protein and reported as such. ULQ for the neutralizing antibody concentrations was 1,050 U/mL and LLQ was 0.26 U/mL.

Additionally, the results were reported as percent inhibition (% inhibition), calculated using the equation below:

$$\%\ Inhibition = \left(1 - \frac{Sample\ Signal}{Average\ Signal\ of\ the\ Blanc}\right) \times\ 100\%$$

These results are reported in the raw Excel database.

All corresponding results have been provided to the WP6 microbiological database.

Description of planned additional assays

Sera samples from other fragile populations, including 2699 samples from the oncology cohort, as well as M6 and M12 samples are being shared with other Orchestra WP6 partners to allow evaluation of additional innovative serological markers.

Results

Anti-Spike and anti-RBD titres consistently increased in SOT patients upon administration of COVID-19 vaccines

Despite an immunocompromised status, 22% and 20% of SOT patients developed anti-Spike and anti-RBD titres, respectively, upon the administration of the 1st dose of a COVID-19 vaccine. This number increased further upon the administration of the 2nd

dose with 55% and 57% of SOT patients developing anti-Spike and anti-RBD titres, respectively (**Figure 1, Table 3**).
A significant increase in quantitatively measured anti-Spike and anti-RBD titres as well as anti-Spike variants (D614G, B.1.1.7, B.1.351 and P.1) was observed in almost all of the of the SOT cohorts after both the 1st and the 2nd doses of a COVID-19 vaccine (**Figures 1-2, Tables 4-8**).

Titers of neutralising antibody increased against all of the studied variants upon a COVID-19 vaccine administration

Neutralising antibody titres were measured using a pseudoneutralisation assay utilising Wuhan wild type, and D614G, B.1.1.7, B.1.351 and P.1 variants. Vaccination was able to induce a significant upregulation of neutralizing titres against all studied variants in some of SOT cohorts (**Figure 3**). The absence of significant difference of neutralizing antibody titres in some of the SOT cohorts can be explained by a different cohort composition in terms of the transplant and treatment types.

Vaccination did not affect anti-Nucleocapsid titres in SOT patients

Because the vaccine administered in the studied SOT cohorts was based on the Spike protein alone, anti-Nucleocapsid titres were not affected by the vaccination in any of the studied cohorts (**Figure 2**). Overall, the number of patients negative or inconclusive for anti-Nucleocapsid IgGs remained 85-87% throughout the whole study (**Figure 1, Table 3**). Anti-Nucleocapsid titres are likely to result from an infection either prior to, or after, the administration of vaccine.

Table 3: Combined serology results from all SOT cohorts

Anti-Spike	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	938 (86%)	65 (6%)	31 (3%)	30 (3%)	27 (2%)	1091
2nd dose	600 (57%)	229 (22%)	113 (11%)	35 (3%)	82 (8%)	1059
3 months	272 (23%)	256 (21%)	230 (19%)	195 (16%)	238 (20%)	1191

Anti-RBD	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	849 (78%)	155 (14%)	28 (3%)	37 (3%)	22 (2%)	1091
2nd dose	590 (56%)	258 (24%)	94 (9%)	32 (3%)	85 (8%)	1059
3 months	278 (23%)	244 (20%)	197 (17%)	186 (16%)	286 (24%)	1191

Anti-Nucleocapsid	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	921 (84%)	30 (3%)	124 (11%)	8 (1%)	8 (1%)	1091

2nd dose	892 (84%)	36 (3%)	115 (11%)	10 (1%)	6 (1%)	1059
3 months	994 (83%)	26 (2%)	159 (13%)	10 (1%)	2 (0%)	1191

Table 4: Serology results for the UNIVR SOT cohort

Anti-Spike	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	183 (90%)	3 (1%)	6 (3%)	6 (3%)	5 (2%)	203
2nd dose	136 (68%)	38 (19%)	8 (4%)	2 (1%)	15 (8%)	199
3 months	45 (37%)	24 (20%)	23 (19%)	14 (12%)	15 (12%)	121

Anti-RBD	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	174 (86%)	13 (6%)	3 (1%)	10 (5%)	3 (1%)	203
2nd dose	139 (70%)	37 (19%)	7 (4%)	1 (1%)	15 (8%)	199
3 months	49 (40%)	23 (19%)	18 (15%)	9 (7%)	22 (18%)	121

Anti-Nucleocapsid	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	179 (88%)	6 (3%)	16 (8%)	2 (1%)	0 (0%)	203
2nd dose	173 (87%)	7 (4%)	18 (9%)	1 (1%)	0 (0%)	199
3 months	111 (92%)	4 (3%)	6 (5%)	0 (0%)	0 (0%)	121

Table 5: Serology results for the Treviso SOT cohort

Anti-Spike	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	422 (89%)	15 (3%)	16 (3%)	12 (3%)	8 (2%)	473
2nd dose	276 (60%)	92 (20%)	45 (10%)	17 (4%)	29 (6%)	459
3 months	91 (22%)	109 (26%)	91 (22%)	72 (17%)	59 (14%)	422

Anti-RBD	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	428 (90%)	13 (3%)	13 (3%)	14 (3%)	5 (1%)	473
2nd dose	302 (66%)	71 (15%)	43 (9%)	12 (3%)	31 (7%)	459
3 months	87 (21%)	101 (24%)	82 (19%)	74 (18%)	78 (18%)	422

Anti-Nucleocapsid	Negative	Inconclusive	Low	Medium	High	Total number of patients
1st dose	405 (86%)	14 (3%)	48 (10%)	2 (0%)	4 (1%)	473
2nd dose	399 (87%)	16 (3%)	39 (8%)	3 (1%)	2 (0%)	459
3 months	353 (84%)	7 (2%)	58 (14%)	4 (1%)	0 (0%)	422

Table 6: Serology results for the Vicenza SOT cohort

Anti-Spike	Negative	Inconclusive	Low	Medium	High	Total number of patients
3 months	70 (27%)	58 (22%)	52 (20%)	43 (16%)	38 (15%)	261

Anti-RBD	Negative	Inconclusive	Low	Medium	High	Total number of patients
3 months	77 (30%)	50 (19%)	46 (18%)	44 (17%)	44 (17%)	261

Anti-Nucleocapsid	Negative	Inconclusive	Low	Medium	High	Total number of patients
3 months	221 (85%)	4 (2%)	32 (12%)	2 (1%)	2 (1%)	261

Table 7: Serology results for the Padova SOT cohort

Anti-Spike	Negative	Inconclusive	Low	Medium	High	Total number of patients
1st dose	331 (80%)	47 (11%)	9 (2%)	12 (3%)	14 (3%)	413
2nd dose	176 (47%)	93 (25%)	59 (16%)	15 (4%)	35 (9%)	378
3 months	59 (16%)	63 (17%)	61 (17%)	65 (18%)	118 (32%)	366

Anti-RBD	Negative	Inconclusive	Low	Medium	High	Total number of patients
1st dose	245 (59%)	129 (31%)	12 (3%)	13 (3%)	14 (3%)	413
2nd dose	134 (35%)	147 (39%)	42 (11%)	19 (5%)	36 (10%)	378
3 months	57 (16%)	68 (19%)	49 (13%)	58 (16%)	134 (37%)	366

Anti-Nucleocapsid	Negative	Inconclusive	Low	Medium	High	Total number of patients

1st dose	335 (81%)	10 (2%)	60 (15%)	4 (1%)	4 (1%)	413
2nd dose	304 (80%)	13 (3%)	53 (14%)	4 (1%)	4 (1%)	378
3 months	295 (81%)	10 (3%)	58 (16%)	3 (1%)	0 (0%)	366

Table 8: Serology results for the SAS SOT cohort

Anti-Spike	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
2nd dose	12 (52%)	6 (26%)	1 (4%)	1 (4%)	3 (13%)	23
3 months	7 (33%)	2 (10%)	3 (14%)	1 (5%)	8 (38%)	21

Anti-RBD	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
2nd dose	15 (65%)	3 (13%)	2 (9%)	0 (0%)	3 (13%)	23
3 months	8 (38%)	2 (10%)	2 (10%)	1 (5%)	8 (38%)	21

Anti-Nucleocapsid	**Negative**	**Inconclusive**	**Low**	**Medium**	**High**	**Total number of patients**
1st dose	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2
2nd dose	16 (70%)	0 (0%)	5 (22%)	2 (9%)	0 (0%)	23
3 months	14 (67%)	1 (5%)	5 (24%)	1 (5%)	0 (0%)	21

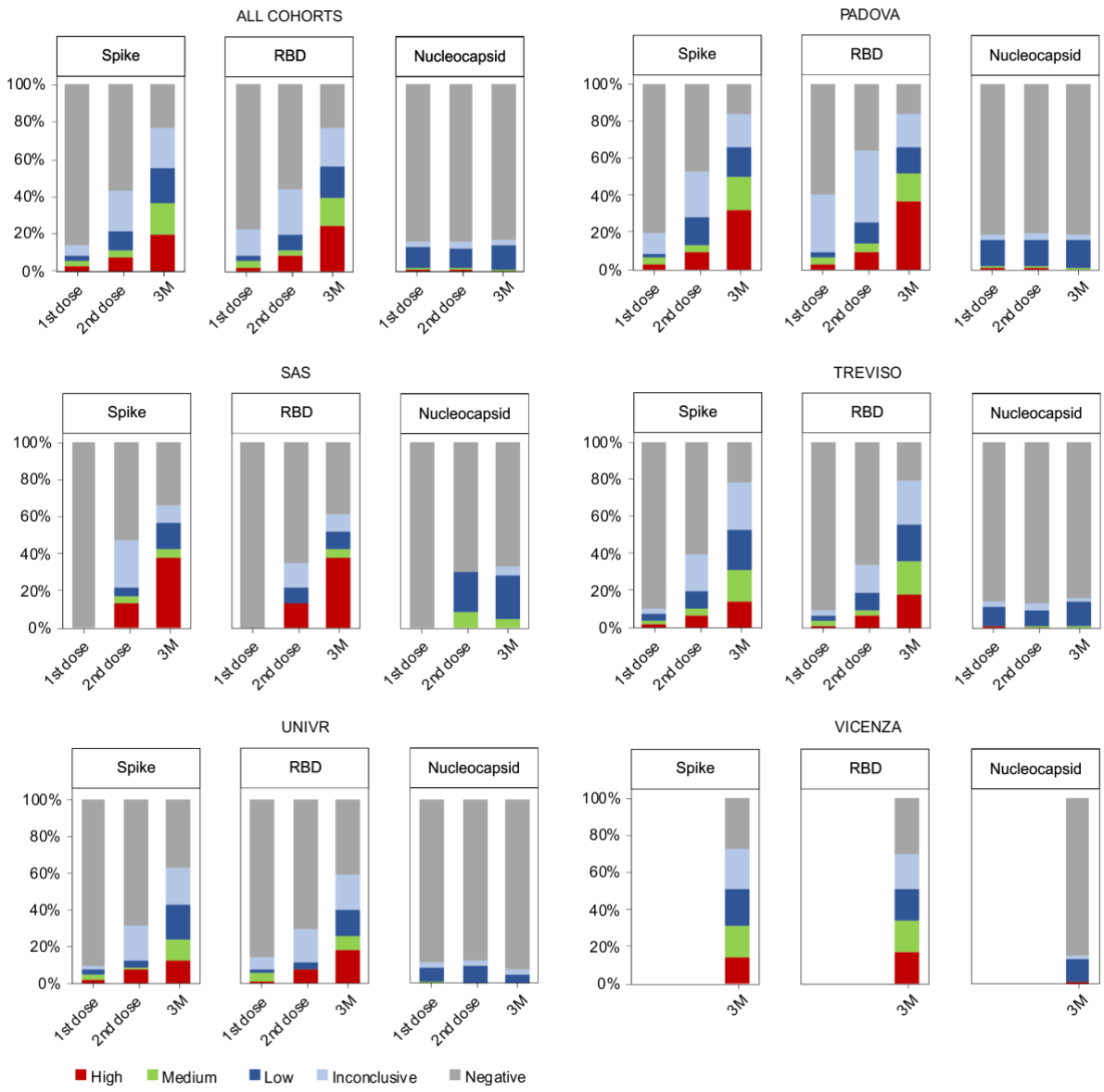

Figure 1. Qualitative serology results. Percentages of SOT patients with Negative, Inconclusive, Low, Medium and High anti-Spike, anti-RBD, anti-Nucleocapsid titres measured prior to the administration of the 1st dose, prior to the administration of the 2nd dose and 3 months after the administration of the 1st dose.

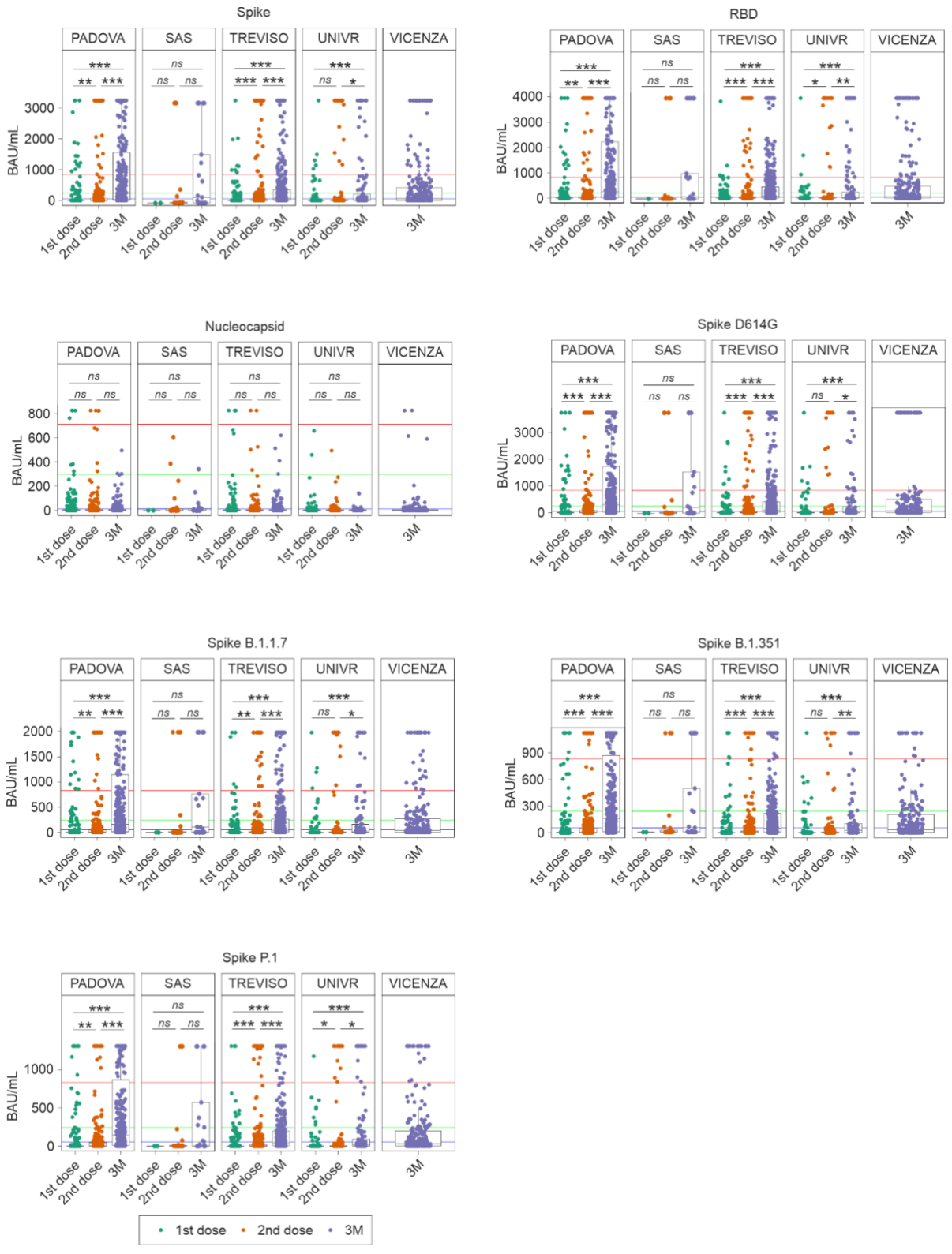

Figure 2. Serology results in different SOT cohorts. Anti-Spike, anti-RBD, anti-Nucleocapsid, anti-Spike (D614G), anti-Spike (B.1.1.7), anti-Spike (B.1.351) and anti-Spike (P.1) titres measured prior to the administration of the 1st dose, prior to the administration of the 2nd dose and 3 months after the administration of the 1st dose to the SOT patients from Padova, SAS, Treviso, UNIVR and Vicenza cohorts. Coloured lines correspond to Low (blue), Mid (green) and High (red) WHO SARS-CoV-2 reference Standards.

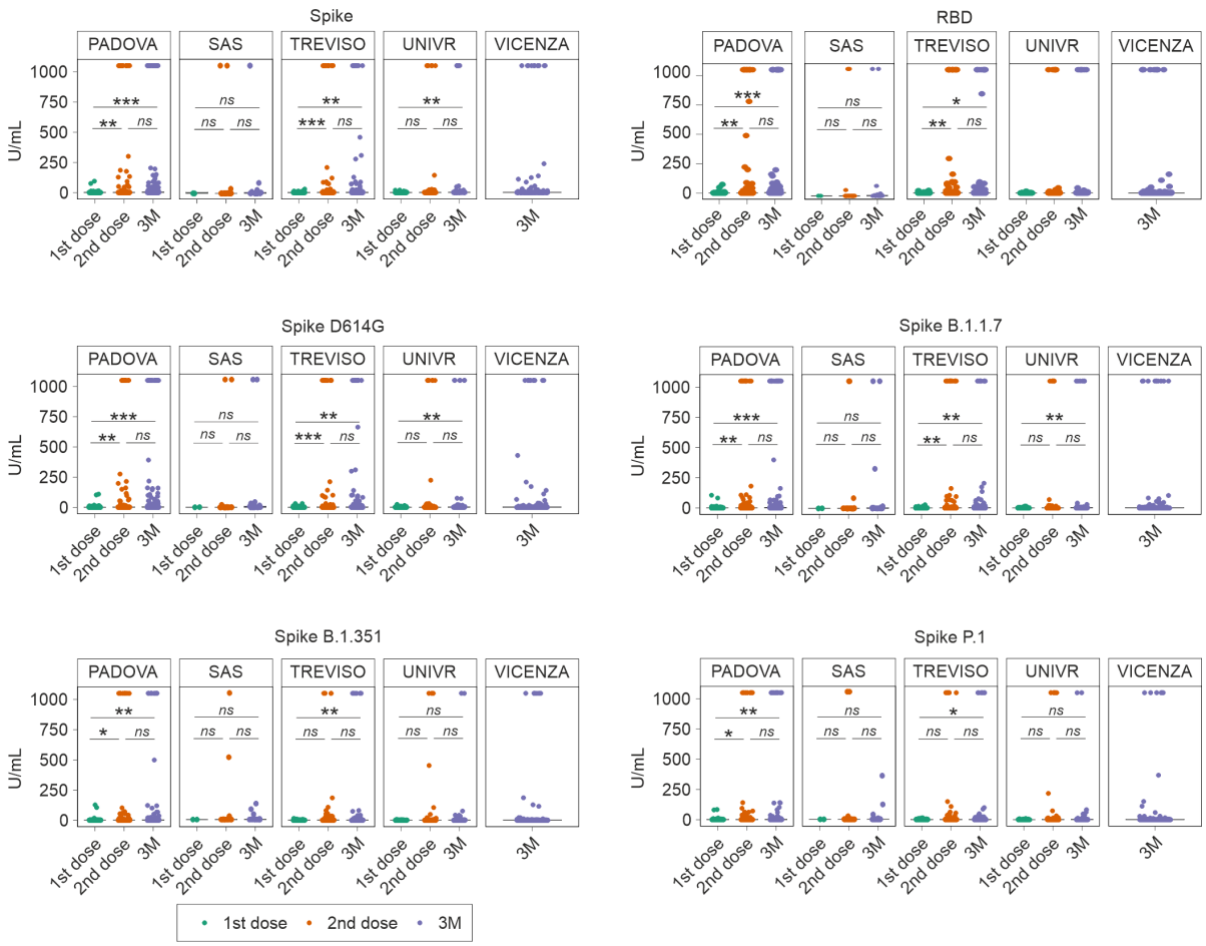

Figure 3. Seroneutralisation results in different SOT cohorts. Anti-Spike, anti-RBD, anti-Spike (D614G), anti-Spike (B.1.1.7), anti-Spike (B.1.351) and anti-Spike (P.1) titres measured prior to the administration of the 1st dose, prior to the administration of the 2nd dose and 3 months after the administration of the 1st dose to the SOT patients from Padova, SAS, Treviso, UNIVR and Vicenza cohorts.

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Vicenza SOT Cohort (WP4)
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SAS SOT Cohort (WP4)