ORCHESTRA

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

WP4_Deliverable 4.3

Quality and kinetic of early immune response to COVID-19 vaccination in fragile patients

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(UNIBO)

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Executive summary

This document is the deliverable *"D4.3 – Quality and kinetic of early immune response to COVID-19 vaccination in fragile patients"* of the European project *"ORCHESTRA – Connecting European Cohorts to Increase Common and Effective response to SARS-CoV-2 Pandemic: ORCHESTRA"*.

This document has been produced for the European Commission as report on the quality and kinetic of early immune response to COVID-19 vaccination in fragile populations. Due to the changing epidemiological scenario of the pandemic and most importantly the introduction of vaccination and booster in fragile populations, the timeline for fragile cohorts' assessment needed to be redesigned several times to be consistent with countries' legislation and access /recommendations for the booster in the fragile population. The report summarizes the first results from the biggest cohort of fragile populations included in WP4 and first to be vaccinated in all countries: transplanted recipients and subjects with HIV infection.

The results from cohorts including patients with hematological and oncological disease, cystic fibrosis, Parkinson disease, pregnant women and children are under clinical assessment and analysis. Results will be reported to the Coommission (as updated Deliverables) as soon as they complete the third dosage or at month-6 from the first two vaccination dosages in order to be consistent when comparing immunological answers among cohorts.

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Core content

Transplanted recipients cohorts

Background

To date several reports have underlined low rates of antibody response to COVID-19 vaccination in Solid Organ Transplant (SOT) recipients [1-22]. The largest report is that from Boyarsky et al. including 658 recipients of different type of SOT (322 kidney, 129 liver, 97 heart, 71 lung, 22 multiorgan and 5 pancreas) recruited across several US hospitals [2]. Antibody response after 1st and 2nd dose of mRNA COVID-19 vaccines was assessed at second dose and within 21 days after, showing a response rate of 15% and 54%, respectively [2]. Major limitations of previous studies were limited sample size, lack of control group, and limited follow-up period hampering to analyse the kinetics of antibody response. The only study assessing the kinetics of antibodies is a sub-study of 305 SOT recipients from the Boyarsky cohort where patients were sampled at 3 timepoints (before second dose, 1 month and 3 month after second dose of mRNA vaccine). The study showed that antibodies response was largely stable after 3 months of vaccination [23]. The understanding of the kinetics of antibody response over time in SOT recipients can play a pivotal role for public health officers when developing recommendations for vaccination needs and booster schedules, and for physicians to optimize monitoring studies and design tailored preventive strategies.

The aim of this study was to analyse, in a large multicentre cohort of SOT recipients, drivers of the immune response to two doses of mRNA COVID-19 vaccines and the kinetics of serological response over time compared with a cohort of healthcare workers (HCWs).

Material and Methods

The study is part of the ORCHESTRA project (https://orchestra-cohort.eu/) aiming to create a new pan-European cohort to rapidly advance the knowledge on the COVID-19 infection. The project currently includes multiple cohorts e.g. individuals at risk of infection, COVID-19 patients, HCWs, and fragile populations as SOT recipients, for a total of more than 1.300.000 subjects. Major issues encountered for the fragile population cohorts are mainly due to: high heterogeneity among countries´ policies regarding vaccination campaign; finalizing data harmonization for around 1500 clinical variables included in a unique (for all fragile populations)

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case report form hosted by a common electronic capture tool hosted by CINECA; arranging a patient/sample coding tool, to ensure a unique identification code for each enrolled patient linked with its samples

The prospective observational multicentre cohort study of SOT recipients includes all consecutive adult (≥18 years) SOT patients who received two doses of mRNA COVID-19 vaccine between January and May 2021 and is running at 6 hospitals, five from Italy (Bologna, Verona, Padova, Vicenza, and Treviso) and one from Spain (Seville). For the purpose of this analysis, patients with clinical and/or immunological evidence of prior COVID-19 were excluded. As control group, a cohort of 5045 HCWs from the Bologna ORCHESTRA cohort vaccinated with two doses of mRNA COVID-19 vaccine in the same study period and without history of SARS-CoV-2 vaccination were analysed.

Primary endpoints were the probability of positive AbR at 3±1 month of first vaccine dosage in SOT and the kinetics of AbR in SOT compared with HCWs. A positive AbR was defined as an anti-RBD titer ≥5 U/ml or ≥45 BAU/mL for Elecsys and MSD assay, respectively (see following microbiology section for details). Secondary endpoints included the clinical and epidemiological drivers of positive AbR in SOT recipients.

According to the study protocol, all SOT recipients had serological response assessed at the following timepoints: the day of first vaccine dose administration (t0); the day of second dose administration (t1, 21 or 28 days after t0 for BNT162b2 or mRNA-1273, respectively); and 3±1 month after t0 (t2).

Data were collected at t0 and included: age, sex, comorbidities other than the cause of transplant according to Charlson index criteria, type and date of transplant, current immunosuppressive regimen, receipt of induction regimen in the last 6 months, and graft function defined as good, impaired or failure according to the judgement of attending physicians. Occurrence of SARS-CoV-2 infection and clinical course was collected at each timepoint. Data collected for the HCWs at the same timepoint included age, sex, and AbR. Study variables were registered using a standardized electronic case report form (eCRF) managed by a centralised REDCap capture tool [24]. Data sources were clinical charts and hospital electronic records.

Detection of AbR was performed in two laboratories at the Bologna University, Italy (for the Bologna cohort of SOT recipients and HCWs), and at the Antwerp University, Belgium (for all the other cohorts) with Elecsys® Anti-SARS-CoV-2 ECLIA assay and V-PLEX SARS-CoV-2

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Panel 6 Kit (IgG) from Meso Scale Discovery (MSD, MD, USA). The Elecsys® Anti-SARS-CoV-2 ECLIA assay (Roche Diagnostics AG, Rotkreuz, Switzerland) was performed on the cobas e 801 analyzer (Roche Diagnostics). The cut-off value for positive reactivity anti-N was equal to 1.0 COI (cut-off index) and anti-S (RBD) is 0.8 U/mL, according to the manufacturer instructions. To establish more accurate criteria for interpretation of serological results, the assay was validated in two well-defined group of serum samples obtained from 50 HCWs before and a month after receiving second dose of vaccine. Based on the results from the 50 true-positive and the 50 true-negative SARS-CoV-2 samples, antibody responses were stratified according to Anti-N (negative: 1.0 COI; inconclusive:≥1 to <5 COI; positive: ≥5 COI) and Anti-S (negative: 0.8 U/mL; inconclusive: ≥0.8 to <5 U/ml; positive: ≥5 U/ml). The V-PLEX SARS-CoV-2 Panel was used according to the manufacturer instructions. IgG titers to the following antigens were measured: SARS-CoV-2 N, SARS-CoV-2 S1 RBD, SARS-CoV-2 Spike, SARS-CoV-2 Spike (D614G), SARS-CoV-2 Spike (B.1.1.7), SARS-CoV-2 Spike (B.1.351), SARS-CoV-2 Spike (P.1). 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 Supplemental Table 1. The overall antibody responses were stratified into non-reactive, inconclusive, positive-low, positive-mild, and positive-high according to WHO criteria (see Supplemental Table 2).

Statistical analysis

For descriptive analysis, categorical variables were presented as absolute numbers and their relative frequencies, continuous variables were presented as mean ± standard deviation (SD) if normally distributed, or as median and interquartile range (IQR) if non-normally distributed. Quantitative anti-RBD levels for positive cases were log-transformed to account for the skewness of the distribution and then normalized by dividing them by the center-specific standard error to take into account the different methods used across centers.

The comparison between SOT recipients and HCW was performed using multivariable logistic regression with AbR as dependent variable and cohort (HCWs vs. SOT recipients) as primary endpoint, after adjustment for sex, age, and time since vaccination. Sex- and age-adjusted means of anti-RBD levels were calculated among subjects with positive immune response in the two groups, based on ANOVA. Time trends in log-transformed anti-RBD levels were assessed with linear regression after application of linear splines with two knots at weeks 10 and 16.

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For the secondary endpoint, multivariable logistic regression models were fitted to estimate odds ratios (ORs) and 95% confidence intervals (CI) of AbR as dichotomous variable. The main exposure variable was time between the administration of first vaccine dose and the AbR assessment; models also included sex, age (categorical), comorbidities, type of graft, type of vaccine, time between transplant and vaccination (categorical), induction regimen in the last 6 months, immunosuppressive drugs at the time of vaccination (calcineurin inhibitors, anti-metabolites, mTOR inhibitors, steroids), graft function (good, impaired, failure) and time between first dose and assessment of AbR (categorical) as potential confounders.
Analyses were done using the STATA package, Version 16.1 (STATA/SE 16.0 for Windows. StataCorp LLC, College Station, TX, USA.), using the commands *logistic*, *glm*, *anova*, and *mksplines*.

The study, according to the Italian legislation for SARS-CoV-2 studies, was approved by the AgenziaItaliana del Farmaco (AIFA) and the Ethic Committee of Istituto Nazionale per le Malattie Infettive (INMI) Lazzaro Spallanzani (document n. 359 of Study's Registry 2020/2021). Informed consent was obtained from all the enrolled patients.

Results

By November 2021,1452 SOT recipients were included in the ORCHESTRA SOT cohort. Of them 412 were excluded from this analysis for the following reasons: results of AbR at 3±1 month were not available (n=304), SARS-CoV-2 infection before vaccination (n=76) or between dosages (n=8), and incomplete vaccination schedule (n=23).The eight breakthrough infections occurred in seven kidney and one liver transplant recipients within a mean of 16.62 days (range 8-33) from the first dose administration of BNT162b2 and mRNA-1273 vaccine in seven and one SOT recipient, respectively.
Thus, 1062 patients were analyzed. The majority of enrolled patients were males (704, 66.3%) with a mean age (±SD) of 58.28 (±13.10) years. Concomitant comorbidities (other than the cause of SOT) were present in 715 (67.3%) patients. As for type of SOT, most patients had kidney (n=677, 63.7%), followed by liver (n=182, 17.4%), heart (n=111, 16.7%), and lung (n=26, 2.5%) transplantation. In the majority of patients (n=836, 78.7%) more than 3 years were elapsed from SOT to vaccination onset. Accordingly, only one patient had received an induction regimen in the last 6 months prior to vaccination onset. The most common drugs used for maintenance immunosuppressive regimen were tacrolimus (n=763, 72%), steroids

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(n=709, 66.7%), and mycophenolate mofetil (n=626, 59%). Overall, 222 (20.9%) patients were reported as having an impaired function of the graft or graft failure. BNT162b2 andmRNA-1273 vaccines were administered to 928 (87.4%) and 134 (12.6%) patients, respectively. Cohort details are described in Table 1. Number of SOT recipients enrolled at each center and the distribution of the types of graft per center are shown in the Supplemental Table 3.
All SOT recipients tested at t_0 had negative AbR (n=622). The rate of positive AbR was 9.8% (62/631) at t_1 and 52.3% (556/1062) at t_2. The mean time from first vaccination to t_2 was 92±35 days, with the majority of patients being assessed between 70-100 (n=500, 47.08%) and 100-130 (n=298, 28.06%) days after first dose (see Table 1). The analysis of the weekly trend of odds for positive AbR showed a steadily increase in the probability of having a positive response from day 50 to day 110 after first vaccination as shown in Figure 1.

The ORCHESTRA HCWs cohort included 5,045 subjects (68.9% women, mean age 43.1 years). Serological response was detected in 99.5% of them at t_2. In the multivariable logistic regression analysis, the adjusted OR of serological response for HCW vs. SOT was 120 (95% CI 73.1-199). The adjusted mean of ln(AbR) was 6.9 (±0.01) in HCW and 5.2 (±0.05) in SOT recipients ($p<0.001$). Figure 2 shows the mean ln(AbR) in the two populations for the periods between 49 and 153 days (the overlap period of the two series of results) after vaccination in individuals with positive AbR. The ratio of ln(AbR) between HCWs and SOT recipients ranged between 1.2 and 1.7, i.e., between 3.3 and 5.5 on arithmetic scale, SOT recipients showed an increase up to 76 days, then a non-significant decrease in ln(AbR) after 118 days ($p=0.1$); conversely, HCWs experienced a strong decrease in ln(AbR) up to 76 days ($p=0.02$), and a less pronounced decrease between 76 and 118 days ($p=0.04$).

To predict drivers of AbR in SOT recipients univariable and multivariable analyses were performed. Univariable analysis found significant differences between patients with positive and negative AbR for age, presence of other comorbidities, type of graft, time from SOT, immunosuppressive drugs (tacrolimus, mycophenolate mofetil, azathioprine, everolimus and steroids), impaired graft function or graft failure, and the type of mRNA COVID-19 vaccine (see Table 1). At multivariable analysis, liver transplant (vs. other types of SOT; OR 2.71, 95%CI 1.55 4.72, $p<0.001$), ≥ 3 years from SOT to vaccination (OR 4.92, 95%CI 2.56-9.45, $p<0.001$), mRNA-1273 vaccine (3.57, 95%CI 2.25 5.67, $p<0.001$), use of azathioprine (OR 3.43, 95%CI 1.20-9.82, $p=0.02$), and longer time from vaccination to serological assessment (OR 1.30, 95%CI 1.10-1.53, $p<0.001$) were associated with positive AbR. While, older age (OR 0.68, 95%CI 0.60-0.77, <0.001), presence of other comorbidities (OR 0.60, 95%CI 0.43

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0.83,p=0.002), use of mycophenolate mofetil (OR 0.29, 95%CI 0.20 0.43, p<0.001), steroids (OR 0.44, 95%CI 0.30-0.65, p<0.001), and impaired graft function or graft failure at vaccination (OR 0.38, 95%CI 0.26-0.55, p<0.001) were associated with lower probability of positive antibody response at t_2 (see Table 2).

Discussion

The ORCHESTRA SOT cohort is the largest cohort of SOT recipients assessed for serological response to SARS-CoV-2 vaccines reported to date. Assessment of immune response at month 3±1 after two doses of mRNA COVID-19 vaccine found that 52.3% among 1062 naïve SOT recipients had positive antibodies response versus 99.5% detected in HCWs. A steadily increase in the probability of having a positive response from day 50 to day 110 after first dose administration was observed. Furthermore, as expected among responders, the mean levels of antibodies were significantly higher in HCWs than in SOT recipients, but the kinetics was different. In HCWs there was a significant reduction in antibody levels during the first two months followed by a less pronounced decrease thereafter; while in SOT recipients after increasing during the first two months the mean levels of antibodies were stable during the following two months starting to slowly decrease from month 4. These results suggest that an early assessment of AbR in SOT could have missed the subsequent increase in AbR, and that SOT recipients maintained good antibody levels during a limited period of time after a standard vaccination schedule. The rate of seroconversion was heterogeneous by type of SOT as already observed [25] and varied from 46% in kidney to 79% in liver transplant recipients. Note of worthy age, comorbidities, type of graft, time from SOT, graft function, and type of immunosuppressive regimens were associated with the AbR within 3 months, suggesting that prevention strategies, other than vaccination, could be considered in this setting based on patient specific characteristics.

Higher intensity of immunosuppressive regimen, in particular the use of anti-metabolites drugs, has been associated with lower antibody response [25]. For this reason, some authors have proposed temporary suspension of mycophenolate during vaccine administration, although this practice has been discouraging from international transplant Societies due to safety concerns and lack of data about its efficacy (https://ishlt.org/ishlt/media/documents/ISHLT-AST_SARS-CoV-2-Vaccination_5-11-21.pdf). A clinical trial is currently ongoing assessing the seroconversion rate after third dose among patients receiving mRNA vaccination with or without temporary suspension of mycophenolate [26]. We confirmed the negative role of mycophenolate, along with steroids, on the probability to achieve a seroconversion rate at 3±1

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month from vaccination, while patients on azathioprine were more likely to show a positive AbR. We deem that this result could be relevant for future strategies to improve efficacy of vaccination in SOT recipients, considering azathioprine as a temporary alternative to mycophenolate, in order to improve immunological response and minimize rejection risk at the same time.

Data on the kinetics of antibody response to SARS-CoV2 infection or vaccine, as well as to other infectious agents such as influenza, are limited [27]. Most studies focused on duration of antibody response, being the waning of antibodies the main concern of physicians and public health officers during the pandemic of SARS-CoV-2. However, the knowledge of the time needed to mount a protective AbR in SOT could be very important to provide correct advices to patients and plan adequate monitoring activities. Our study shows a steady increase in the probability of having a positive antibody response between day 50 to 110 after first vaccination suggesting that this could be the best time interval to assess antibody response to vaccination in SOT recipients. Similarly Bovarsky et al. observed an increase from 13.5% to 67% at month 3 after the second dose [23]. Very interestingly our data also showed a different kinetics of the antibody response in SOT recipients compared to HCWs that merits further investigations. Our data confirmed a lower level of antibodies among SOT than HCW responders, that was maintained during a limited period of time, supporting the current strategy of booster dosage in this setting. On this regard, preliminary data were controversial showing a moderate rate of seroconversion [28,29]. According to our and prior data, low-level responders could be those who most benefit from booster dosages.

A limitation of our study is the lack of cellular immune response analysis. Indeed, several authors have shown that the analysis of cellular immune response increase the rate of immunological response among vaccinated SOT recipients, mainly among those receiving hybrid (vector/mRNA) COVID-19 vaccination [5,11,30]. We did not collect dosage of steroids at vaccination which could impact on AbR to vaccines in SOT recipients. Strengths of our study, in addition to the large study population, include the extensive amount of clinical data available for analysis, enabling a detailed investigation of characteristics of SOT recipients associated with immunology response to mRNA vaccines, the fact that a 3-month follow-up was available for essentially all subjects, and the use of a comparison population of HCWs.

In conclusion, we showed that the timing of sampling was significantly associated with the probability of finding a positive antibody response to COVID-19 vaccine in SOT recipients with a progressive increase in the seroconversion odds between day 54 to day 110. In addition, the

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magnitude and kinetics of antibody response was different between SOT recipients and HCWs, being lower and limited in time among SOT. These results could be helpful to optimize strategies of immune monitoring after COVID-19 vaccination and to give indications about timing for booster dosages in the setting of SOT recipients.

These data have been submitted for publication.

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Tables

Table 1. General characteristics of study population

	Total N=1062 (%)	**Positive antibody response at 3±1 month N=556 (%)**	**Negative antibody response at 3±1 month N=506 (%)**	***p***
Demographic data				
Age (mean±SD) (years)	58.28 ± 13.10	56.47 ± 13.61	60.26 ± 12.23	<0.001
Age group				<0.001
< 39 y	93 (8.76%)	63 (67.74%)	30 (32.26%)	
40-49 y	156 (14.69%)	93 (59.62%)	63 (40.38%)	
50-59 y	268 (25.24%)	146 (54.48%)	122 (45.52%)	
60-69 y	309 (29.10%)	148 (47.90%)	161 (52.10%)	
>= 70 y	236 (22.22%)	106 (44.92%)	130 (55.08%)	
Sex				0.967
Male	704 (66.29%)	368 (52.27%)	336 (47.73%)	
Female	353 (33.40%)	185 (52.41%)	168 (47.59%)	
Comorbidities				0.005
No	347 (32.67%)	203 (58.50%)	144 (41.5%)	
Yes	715 (67.33%)	353 (49.37%)	362 (50.63%)	
Type of graft				<0.001

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Kidney	677 (63.75%)	312 (46.09%)	365 (53.91%)	
Heart	177 (16.67%)	86 (48.59%)	91 (51.41%)	
Liver	182 (17.14%)	144 (79.12%)	38 (20.88%)	
Lung	26 (2.45%)	14 (53.85%)	12 (46.15%)	
Type of vaccine				<0.001
BNT162b2	928 (87.38%)	463 (49.89%)	465 (50.11%)	
mRNA-1273	134 (12.62%)	93 (69.40%)	41 (30.60%)	
Time from transplant to vaccination				<0.001
Less than 1 year	58 (5.46%)	21 (36.21%)	37 (63.79%)	
1 to 3 years	166 (15.63%)	68 (40.96%)	98 (59.04%)	
More than 3 years	836 (78.72%)	465 (55.62%)	371 (44.38%)	
Induction regimen in the last 6 months				0.340
No	1061 (99.90%)	555 (52.31%)	506 (47.69%)	
Any	1 (0.09%)	1 (100%)	0 (0.00%)	
Immunosuppressive drugs at the time of vaccination				
Calcineurin inhibitors	1007 (94.82%)	520 (51.64%)	487 (48.36%)	0.046
Tacrolimus	763 (71.85%)	384 (50.33%)	379 (49.67%)	0.035
Cyclosporine	246 (23.16%)	136 (55.28%)	110 (44.72%)	0.294
Anti-metabolites	663 (62.43%)	284 (42.84%)	379 (57.16%)	<0.001

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Mycophenolate mofetil	626 (58.95%)	252 (40.26%)	374 (59.74%)	<0.001
Azathioprine	37 (3.48%)	32 (86.49%)	5 (13.51%)	<0.001
mTOR	144 (13.56%)	90 (62.50%)	54 (37.50%)	0.009
Everolimus	128 (12.05%)	78 (60.94%)	50 (39.06%)	0.038
Sirolimus	16 (1.51%)	12 (75.00%)	4 (25.00%)	0.068
Steroids	709 (66.76%)	312 (44.01%)	397 (55.99%)	<0.001
Impaired graft function				<0.001
Good	830 (78.15%)	475 (57.23%)	355 (42.77%)	
Impaired or Failure	222 (20.90%)	75 (33.78%)	147 (66.22%)	
Time between first dose and assessment of antibody response				0.038
40-70d	170 (16.01%)	74 (43.53%)	96 (56.47%)	
70-100d	500 (47.08%)	262 (52.40%)	238 (47.60%)	
100-130d	298 (28.06%)	170 (57.05%)	128 (42.95%)	
130-160d	75 (7.06%)	37 (49.33%)	38 (50.67%)	
>160d	19 (1.79%)	13 (68.42%)	6 (31.58%)	

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Table 2. Multivariable analysis of predictors of antibody response at 3±1 months after first dose administration of mRNA COVID-19 vaccine in SOT recipients

VARIABLE	OR (95% CI)	P-VALUE (α=0.05)
Sex	-	-
Male	1 (ref)	-
Female	0.91 (0.67 1.24)	0.568
Age	-	-
Categorical increase (< 39 y; 40-49 y; 50-59y; 60-69y; >= 70y)	0.67 (0.60 0.76)	<0.001
< 39 y	1 (ref)	-
40-49 y	0.58 (0.31 1.09)	0.091
50-59 y	0.38 (0.21 0.68)	0.001
60-69 y	0.26 (0.14 0.48)	0.000
>= 70 y	0.20 (0.11 0.38)	0.000
Type of graft	-	-
Kidney	1 (ref)	
Heart	0.64 (0.39 1.07)	0.090
Liver	2.71 (1.55 4.72)	<0.001
Lung	1.16 (0.46 2.95)	0.750
Time from transplant to vaccination	-	-
Less than 1 year	1 (ref)	
1 to 3 years	1.79 (0.87 3.67)	0.111
More than 3 years	4.92 (2.56 9.45)	0.000
Time from vaccination onset to serological assessment		
Categorical increase (40-70d; 70-100d; 100-130d; 130-160d; >160d)	1.30 (1.10 1.53)	<0.001
40-70d	1 (ref)	
70-100d	1.77 (1.12 2.80)	0.015
100-130d	2.49 (1.46 4.25)	0.001
130-160d	4.25 (1.82 9.93)	0.001
>160d	8.72 (2.38 31.89)	0.001
Comorbidities		
No	1 (ref)	

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Yes	0.60 (0.43 0.83)	0.002
Type of vaccine		
BNT162b2	1 (ref)	
mRNA-1273	3.57 (2.25 5.67)	<0.001
Immunosuppressive drugs at the time of vaccination		
Cyclosporine	0.71 (0.30 1.67)	0.429
Tacrolimus	0.52 (0.23 1.16)	0.111
Azathioprine	3.43 (1.20 9.82)	0.022
Mycophenolates	0.29 (0.20 0.43)	<0.001
Sirolimus	0.70 (0.18 2.66)	0.598
Everolimus	0.72 (0.43 1.20)	0.212
Steroids	0.44 (0.30 0.65)	0.000
Impaired graft function		
Good	1 (ref)	
Impaired, Failure, and others	0.38 (0.26 0.55)	<0.001

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Figures

Figure 1. Weekly trend of odds (number of positive cases in the specific time interval/number of negative cases in the same interval) for positive AbR, and of rates (positive/total) of positive AbR

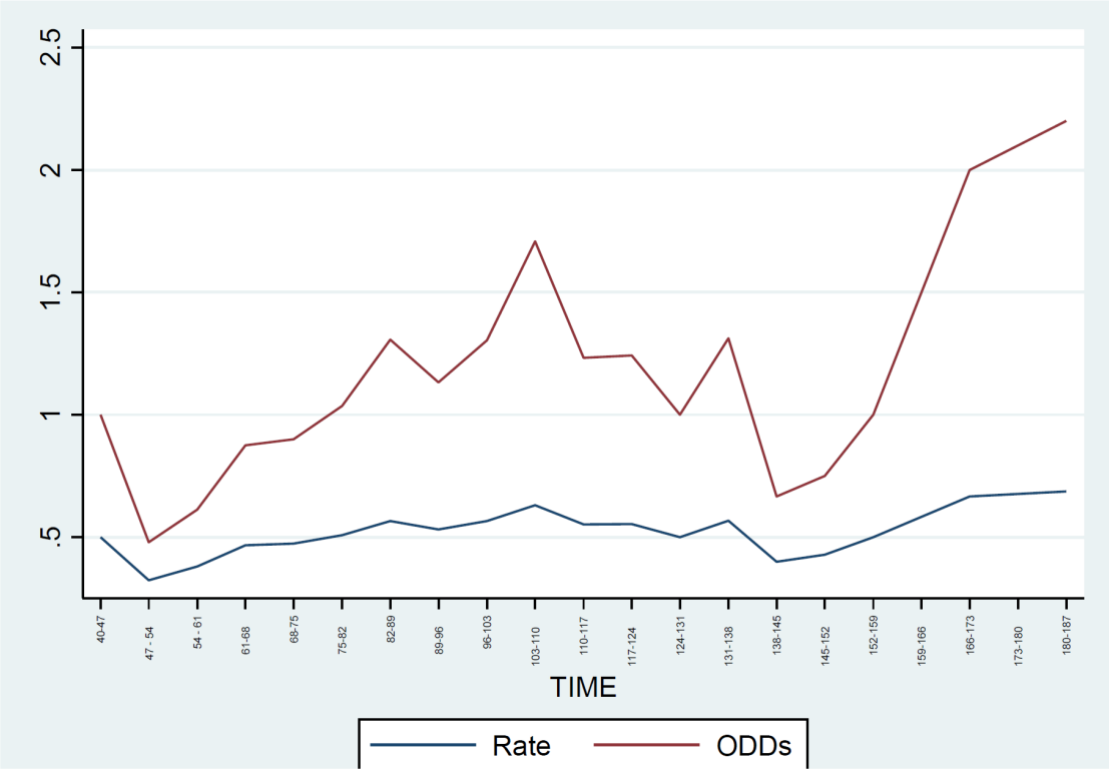

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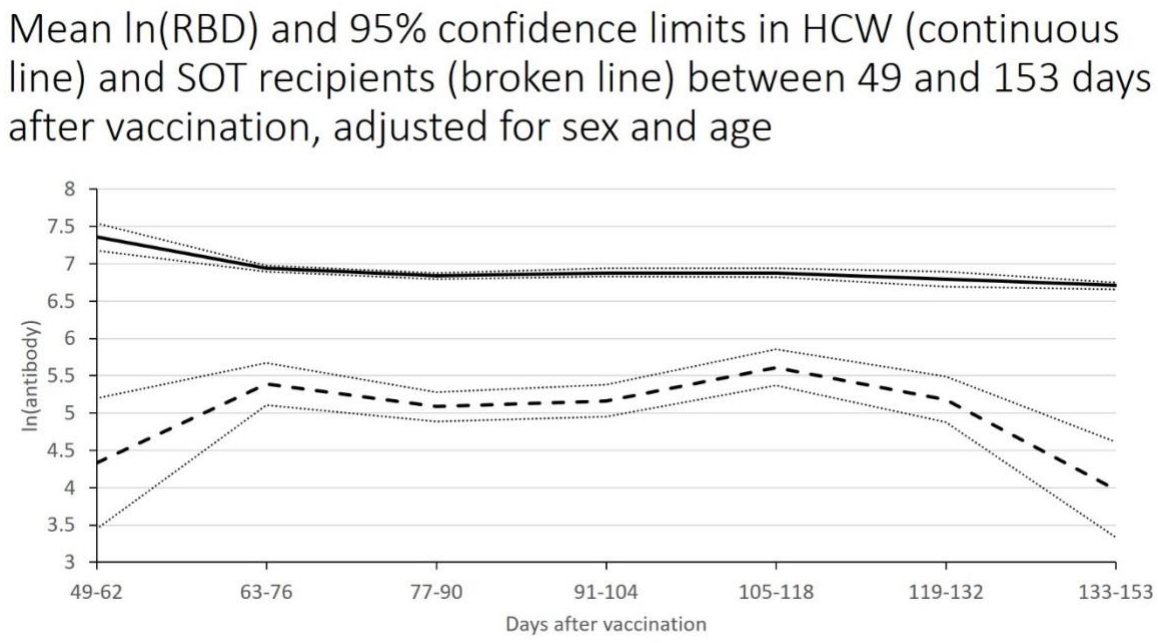
Figure 2. The mean ln(AbR) in the two populations for the periods between 49 and 153 days after vaccination in individuals with positive AbR. The ratio of ln(AbR) between HCWs and SOT recipients ranged between 1.2 and 1.7, i.e., between 3.3 and 5.5 on arithmetic scale, SOT recipients showed a significant increase up to 76 days ($p<0.001$), then a non-significant decrease in ln(AbR) after 118 days ($p=0.1$); conversely, HCWs experienced a strong decrease in ln(AbR) up to 76 days ($p=0.02$), and a less pronounced decrease between 76 and 118 days ($p=0.04$).



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Supplemental tables

Supplemental Table 1. Lower Limits of Quantification (LLQ) and Upper Limits of Quantification (ULQ) for IgG measurements.

	Nucleocapsid	RBD	Spike	Spike (D614G)	Spike (B.1.1.7)	Spike (B.1.351)	Spike (P.1)
LLQ	0.20	0.96	0.79	0.91	0.48	0.27	0.32
ULQ	826	3 944	3 244	3 739	1 982	1 126	1 306

Supplemental Table 2. Stratification of antibody response according to WHO criteria

	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

Supplemental Table 3. Number of SOT recipients enrolled at each centre and the distribution of the types of graft per center

	Overall SOT enrolled	Kidney N (% per organ) [% per centre]	Heart N (% per organ) [% per centre]	Liver N (% per organ) [% per centre]	Lung N (% per organ) [% per centre]
Padova	148 (13.94%)	52 (7.68%) [35.14%]	77 (43.5%) [52.03%]	19 (10.44%) [12.84%]	0
Bologna	487 (45.86%)	230 (33.97%) [47.23%]	100 (56.50%) [20.53%]	132 (72.53%) [27.10%]	25 (96.15%) [5.13%]
Verona	97 (9.13%)	69 (10.19%) [71.13%]	0	28 (15.38%) [28.87%]	0

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Vicenza	211 (19.87%)	209 (30.87%) [99.05%]	0	1 (0.55%) [0.47%]	1 (3.85%) [0.47%]
Treviso	119 (11.21%)	117 (17.28%) [98.32%]	0	2 (1.10%) [1.68%]	0
Seville	23 (2.12%)	22 (3.15 %) [95.65%]	0	0	1 (3.70 %) [4.35%]

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HIV infected patients cohort

Background

People living with HIV (PLWH) may experience a chronic immune dysregulation, despite the effect of antiretroviral therapy, causing a not fully restored immune health [1]. Some studies suggested a higher risk of severe COVID-19 and/or death in PLWH compared to general HIV negative population [2-3]. This higher risk passes through complex mechanisms, intersecting biological and structural determinants: the excess burden of age-associated comorbidities related to HIV [4], unsuppressed HIV viremia and severe immune-depression [5] as well as socio-economic determinants in common both for the acquisitions and clinical impact of SARS-CoV-2 and HIV [6].

The excess of risk could be also explained by a lower neutralizing antibody titers and a diminished serological response to SARS-CoV-2 natural infection in PLWH [7]. Consistently, data on serological response to viral agents vaccines like influenza and HBV [8-9] showed a poorer immunogenicity in PLWH.

PLWH have been therefore considered by WHO and several countries a priority group for COVID-19 vaccination, but in the registrative clinical trials of COVID-19 vaccine only less than 3% are PLWH [10-13], and complete data on immunogenicity (humoral, neutralizing and cell-mediated response) of COVID-19 vaccine as well as data on durability of protection for this vulnerable population are still insufficient.

Few studies have been published on the immunogenicity of SARS-CoV-2 vaccination with adenovirus-vectored vaccine in PLWH. Preliminary data on PLWH are published from a single-arm open-label vaccination sub-study within the setting of large phase 2/3 trial in UK (COVV002), and showed that ChAdOx1-nCoV-19 vaccine, given as prime-boost dosing 4–6 weeks apart, was safe and induce a consistent anti-spike (anti-s) IgG response in PLWH aged 18-55 years, on ART with HIV-RNA<50 copies and with CD4 counts above 350 cells/mm3. Magnitude and persistence of response was similar compared to HIV negative [14]. Frater et al. did not found any correlation between the anti-S IgG response at day 56 and CD4 cell count or age, but the characteristics of study population included in the trial in terms of CD4 strata and age did not fully fit these association analyses [14]. Similarly, data from the randomized, double-blind phase 1b/2 trial (COV005) on the safety and immunogenicity of the same ChAdOx1-nCoV-19 vaccine in South Africa, showed comparable safety and immunogenicity between PLWH, with a high median CD4 cell count of (695 cell/mm^3) and the HIV-negative group [15].

Several observational studies, instead, has been published on mRNA vaccines BNT162b2 and mRNA-1273 in PLWH. Levy I et al. compared the RBD-binding IgG response and neutralizing antibody levels after the second dose of BNT162b2 in 143 PLWH (with a mean CD4 count of 700 cell/ mm3 with a mean CD4 count of 700 cell/ mm3) and 261 HIV negative controls, showing comparable proportion of

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detectable RBD IgG response (98%) in HIV positive and negative but a lower titer – both for the RBD IgG and the neutralizing antibody levels – in PLWH [16]. A small study, by Woldemeskel and colleagues, on 12 PLWHs with a median of 913 CD4 T-cells/mm3 receiving BNT162b2, observed a robust humoral and cellular immune response, comparable to that observed in healthy donors [17]. Other confirms of an uniformly high anti-RBD response after two doses has been found by Ruddy et al. [18], on 14 PLWH receiving BNT162b2 or mRNA-1273, with excellent virological and immunological control (100% viral suppression, 86% with a CD4 count >200 cell/mm^3).

All of these studies showed substantially a good immunogenicity of SARS-CoV-2 vaccination in PLWH but data on subjects with low CD4 cell count is lacking. First data on this vulnerable subgroup has been showed by Antinori A at 18th European AIDS Conference, in a observational study of 127 PLWH and 169 non-matched HIV negative healthcare workers, the humoral and cell-mediated immune response against SARS-CoV-2 has been significantly poorer in those with CD4 cell <200/mm^3 compared to those with >500 cell/mm^3 and HIV-negative controls [19]. Moreover the role of CD4/CD8 ratio, a well studied marker of overall immune dysfunction and may solid biomarker for disease progression, response to treatment, morbidity, and mortality for the virally suppressed HIV [20], has never been investigated as predictors of COVID-19 vaccine response but data suggests that patients with a low CD4/CD8 ratio had a decreased ability to respond to SARS-CoV-2 infection [21]. Understanding the effect of the CD4 cell count and CD4/CD8 ratio markers on predicting vaccine response in PLWH using the larger sample size of the Orchestra study is important for decide additional dose schedule and design appropriate preventive strategies, for this reason first two studies within the orchestra WP3 HIV cohort have been focused on these two markers

Study 1: *Poor humoral immunogenicity to SARS-CoV-2 vaccination in people living with HIV (PLWH) with low CD4 count*

Aims

Aim of this analysis was to investigate the association between CD4 count and anti-S response after primary vaccination in a real-world setting.

Methods

We included PLWH of the VAXICONA-ORCHESTRA cohort who received SARS CoV2 vaccine and for whom anti-S serology was available. Serologic titres were standardized in BAU/mL. Participants were stratified by CD4 count pre-vaccination (T0) (LCD4=CD4 count <200 cell/mm3; ICD4=CD4 count 201-500 cell/mm3; HCD4=CD4 count >500 cell/mm3). Immune response was defined as having anti-S ≥7.1

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BAU/mL for Abbott, ≥0.82 BAU/mL for Roche and ≥4.8 BAU/mL for DiaSorin, while low response was defined as ≤46 BAU/mL regardless of assay. ANOVA was used to compare titres (log2 scale); association between CD4 groups and risk of undetectable/low level anti-S was evaluated by means of logistic regression.

Results

1,229 PLWH were included (LCD4=111; ICD4=372; HCD4=746); median age 53 yrs (IQR 45-59), median time from HIV diagnosis 12 years (6-24), median CD4 nadir 179 cell/mm3 (55-343), 89% HIV-RNA <50 copies/mL, 24% with a previous AIDS diagnosis, 27% with ≥1 comorbidity. The proportion with undetectable/low immune response after 1st dose was 37.4/79.1% for LCD4, 9.3/58.7% for ICD4 and 4.3/46.2% for HCD4(P<0.0001/P<0.0001). Odds ratios from fitting a logistic regression are reported in Table 1. After a median of 35 days (30-63) from 2nd dose, the proportion with undetectable/low response were 11.7/32.4% for LCD4, 1.6/8.6% for ICD4 and 0.7/5.8% for HCD4 (P<0.0001/P<0.0001). The adjusted mean (SD) levels of anti-S were 6.6 (4.0) log2 BAU/mL for LCD4, 8.7 (2.6) for ICD4 and 9.2 (2.3) for HCD4 (Fisher test P<0.0001, Figure 1).

Table 1. Odds ratios of having undetectable/low level of anti-S response after priming dose and after full cycle of vaccination from fitting a logistic regression analysis. OR=odds ratio; AOR=adjusted odds ratio. All models were controlled for age, CD4 nadir, HIV-RNA at T0 and number of concomitant co-morbidities.

	Logistic regression of the probability of anti-S response post vaccination				
	Unadjusted		Adjusted*		
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	&Type III p-value
CD4 count					
	Undetectable - first dose				
500+	1		1		<.001
201-500	2.27 (1.32, 3.91)	0.003	2.61 (1.32, 5.15)	0.006	
0-200	13.17 (7.38, 23.49)	<.001	9.19 (4.23, 19.97)	<.001	
	Low level (below 46 BAU/mL) - first dose				
500+	1		1		<.001
201-500	1.66 (1.26, 2.18)	<.001	1.90 (1.35, 2.69)	<.001	
0-200	4.42 (2.60, 7.51)	<.001	4.35 (2.21, 8.58)	<.001	
	Undetectable - full cycle				
500+	1		1		<.001
201-500	2.43 (0.74, 8.01)	0.145	2.37 (0.71, 7.89)	0.161	
0-200	19.66 (6.86, 56.33)	<.001	11.64 (3.55, 38.15)	<.001	
	Low level (below 46 BAU/mL) - full cycle				
500+	1		1		<.001
201-500	1.54 (0.96, 2.48)	0.076	1.47 (0.88, 2.45)	0.143	
0-200	7.85 (4.75, 12.97)	<.001	5.24 (2.88, 9.55)	<.001	

**adjusted for age, CD4 nadir, VL<=50 copies/mL at T0 and no. of comorbidities*
&from the adjusted model

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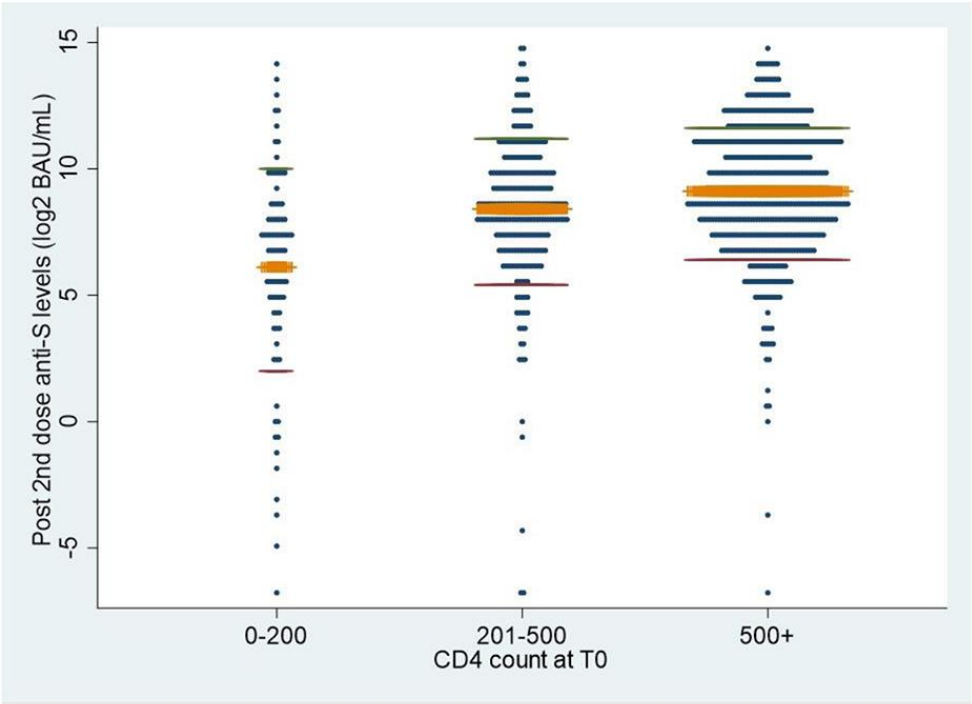
Figure 1. Magnitude of anti-S response (log_2 BAU/mL) after second dose of SARS-CoV-2 vaccine according to CD4 count at the time of priming dose. Data are expressed as geometric mean titres (line orange) and SD (line green and red). P value at Fisher test <0.0001; P value after Dunn-Bonferroni correction for multiple comparisons: CD4 ≤200 vs CD4 201-500 P<0.0001; CD4 ≤200 vs CD4 >500 P<0.0001; CD4 201-500 vs CD4 >500 P=0.0028.



Conclusions

Humoral immunogenicity after primary SARS CoV2 vaccination was lacking or poorly elicited in a substantial proportion of PLWH with CD4count <200/mm3. Having >500 as the comparator, a significantly higher risk of lack of response after 1st dose and lower average levels after 2nddose was also observed in PLWH with CD4 count of 201-500/mm3. A third additional dose is mandatory in PLWH with CD4 <200/mm3 and should be considered in those with CD4 between 200-500 cell/mm3.

Data have been submitted to the ECCMID *"Poor humoral immunogenicity to SARS-CoV-2 vaccination in people living with HIV (PLWH) with low CD4 count"* (#04099) was submitted on date 27th November 2021 for the 32nd European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) Congress 2022).

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Study 2: *CD4/CD8 to predict COVID-19 vaccine response in not severely immunosuppressed PLWH*

Aims

The aim of the analysis was to evaluate anti-S response in not severely immunosuppressed PLWH and the role of CD4/CD8 in predicting this response.

Methods

We included PLWH of the VaxIcona-Orchestra cohort vaccinated for SARS Cov2 and for whom anti-S serology is available. Other inclusion criteria: CD4>200 cells/mm3 at first dose (T0) and no previous AIDS. Serology values were standardized in BAU/mL. Participants were stratified by CD4/CD8 ratio according to the tertiles at T0 (low ratio LR:0-0.68; intermediate ratio IR: 0.69-1.08; and high ratio HR:1.09+). Response were defined as having anti-S ≥7.1 BAU/mL for Abbott, ≥0.82 BAU/mL for Roche and ≥4.8 BAU/mL for DiaSorin while low response was defined as ≤46 BAU/mL regardless of the assay. ANOVA was used to compare titers (log2 scale); association between CD4/CD8 and the risk of undetectable and/or low level anti-S was evaluated by means of a logistic regression model controlled for age, CD4 nadir, HIV-RNA at T0 and number of concurrent co-morbidities. We ran a sensitivity analysis after excluding participants with ≥1 comorbidity.

Results

We included 916 PLWH (n=305 LR, n=306 IR, n=305 HR): median age 51 years (IQR 41-58), median CD4 nadir 256 cell/mm3 (120-400), 94% HIV-RNA <50 copies/mL, 36% with ≥1 comorbidity. The proportion with undetectable/low levels to 1st dose were 9.2/48.9% LR, 4.3/40.9% IR and 1.6/37.6% HR (p=0.0003/p=0.03). Figure 1 show the corresponding odds ratios. After the 2nd dose (a median [IQR] of 59 [57-64] days after T0) adjusted mean levels of anti-S were 8.4 (2.7) in LR, 8.8 (2.7) in IR and 8.7 (2.8) log2 BAU/ml in HR (Fisher test p=0.14, Figure 2). Results were similar in the subset of participants without comorbidities.

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Figure1. Forest Plot of odds ratio (OR)from fitting a logistic regression model

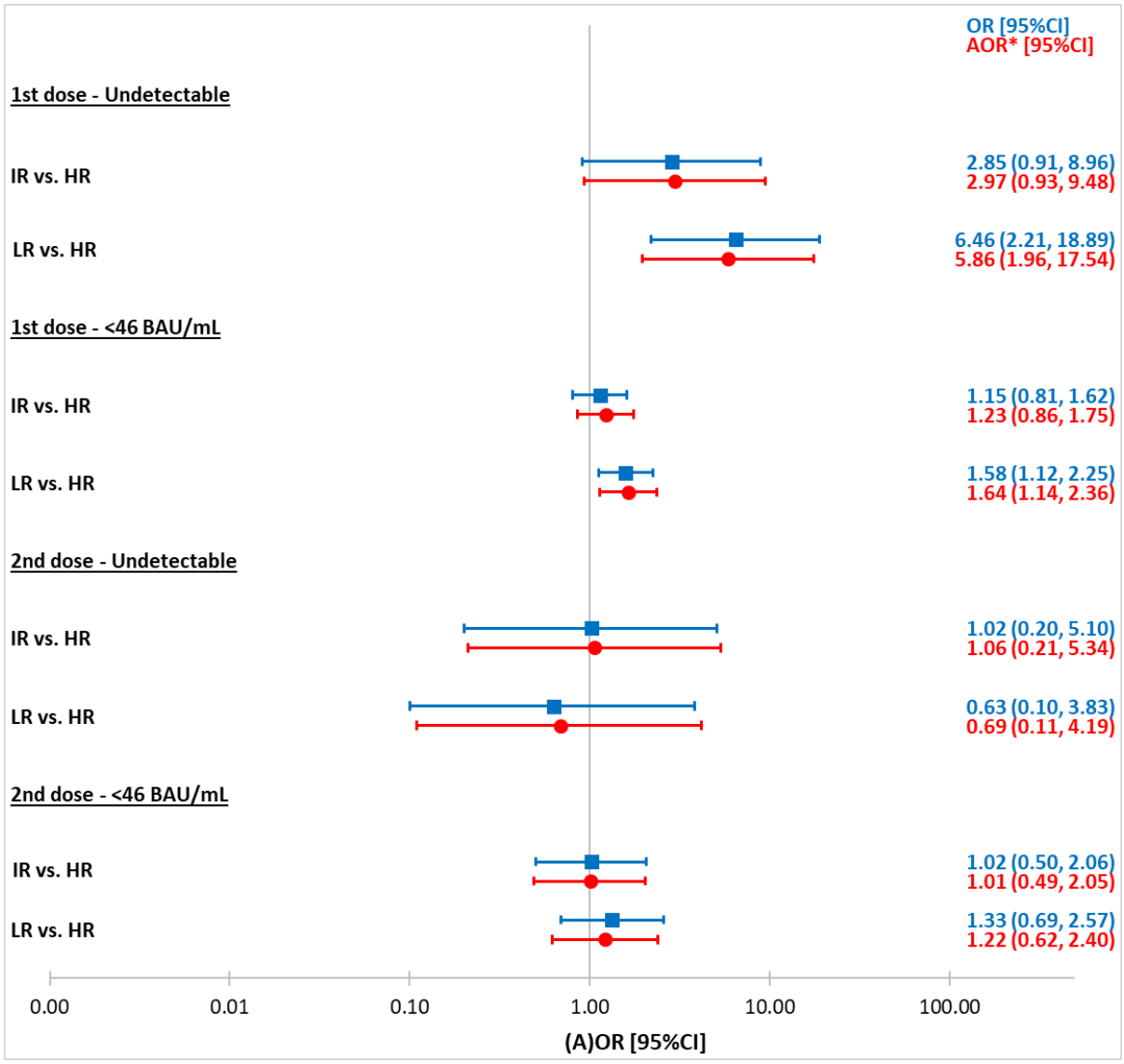

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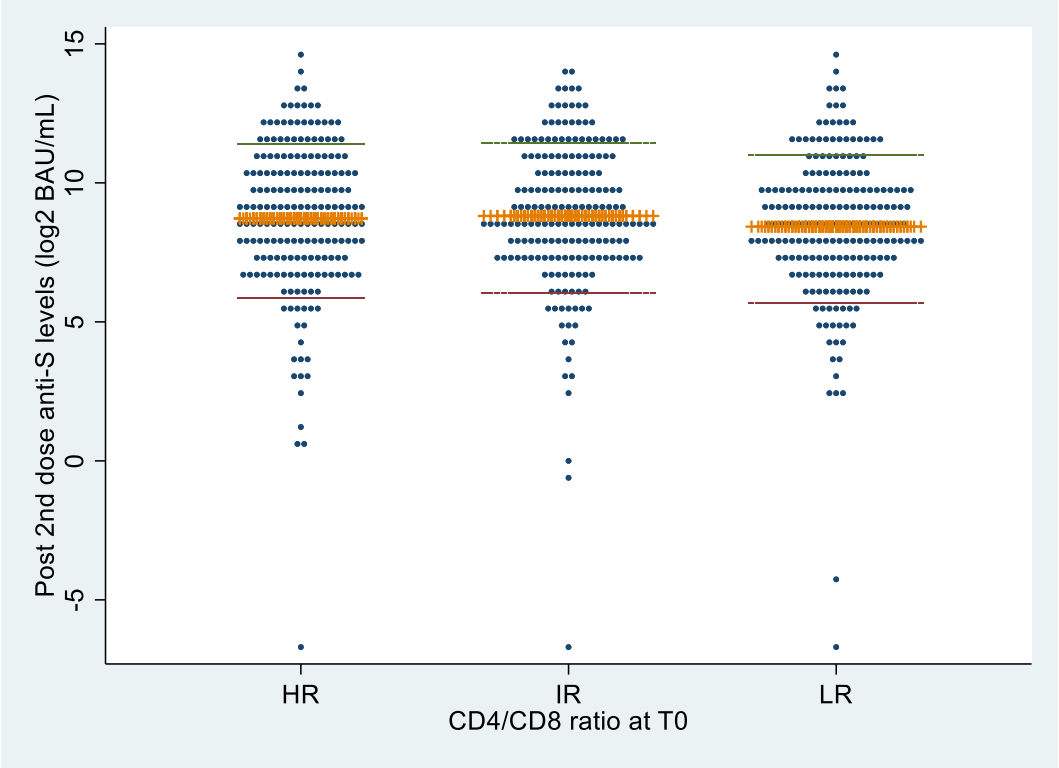
Figure 2. Dot plot with mean and SD of anti-S levels post 2nd dose of vaccine by CD4/CD8 ratio groups



Conclusions

In PLWH with >200 cells/mmc, those with a ratio below 0.68 showed a higher risk of having low anti-S responses vs. those with a ratio >1.08 only after one dose; there was no evidence for a difference in mean titers or proportion with a response after the full cycle. Although power of the analysis is limited, CD4/CD8 ratio prior to vaccination does not seem to be helpful to decide prioritization for BS in not severely immunosuppressed PLWH.

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Enrolment status for other the fragile cohorts

Number of patients tested for antibody response

		time-points				
		1st dose	2nd dose	3 months	6 months (NO 3rd dose)	1 month after 3rd dose
ORCHESTRA cohort	Cancer patients (UNIBO, UNIVR, SAS)	1378	1240	811	276	236
	Hematological patients (UNIBO, UNIVR, SAS)	405	416	50	222	955
	Parkinson's Disease patients (LIH)	-	-	-	-	45
	Pregnant women (ZikaAlliance, PENTA)	-	-	-	-	-
	Children (PENTA)	starting now				

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