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Immunogenicity of two COVID-19 vaccines currently used in India: Experience in health care workers from a tertiary care hospital

Brief Title-

Immunogenicity evaluation of COVISHIELD and COVAXIN, India

Authors-

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Abstract:
Background: Being a long-term preventive measure, COVID-19 vaccines are used in global populations. In India, country-wide immunization drive was initiated in January 2021.

Methods: To assess immune response of health-care-workers to COVISHIELD(n=187) and COVAXIN(n=21), blood samples collected pre-vaccination and 1month-post-1/post-2 dose, administered 28days apart, were tested for IgG-anti-SARS-CoV-2 (ELISA) and neutralizing (Nab, $PRNT_{50}$) antibodies. Spike protein-specific T cells were quantitated by IFN-γ ELISPOT and Flow-cytometry-based Intra-cellular-secretion (ICS) for IFN-γ/IL2.

Findings: Among pre-vaccination-antibody negative (pre-negatives, n=120) and positive (pre-positives, n=67) COVISHIELD recipients, %Nab seroconversion and median (IQR) Nab titers were 55.1%/95.6% and 16(IQR 2.5-36.3)/(64.5, IQR 34.5-154.2, p<0.000) and independent of age/gender. In pre-positives, Nab titers increased from 75 (IQR 29-129) before vaccination to 3050 (1282-3998, p< 0.001, n=42) post-1st dose, but declined to 1740(911-3116, p<0.05) post-2nd dose. Though the number of COVAXIN recipients was small, post-2nd dose humoral response was lower than COVISHIELD (50% seroconversion and median titer 6.75, IQR 2.5-24.75, p<0.0001). Despite higher age, COVAXIN recipients elicited superior IFN-γ-T cell response than COVISHIELD, as measured by ELISPOT (100%; 1226, 522-3628 spot forming units, SFU/million PBMCs v/s 57.8%; 21.7, 0-2149; p<0.001) and ICS (56.9%, 24.8-78.6 v/s 30%, 8.1-90.4, p<0.05). During immunization, COVID-19 cases were detected among COVISHIELD (n=4) and COVAXIN (n=2) recipients.

Interpretation: This first-time, systematic, real-world assessment revealed stronger humoral (COVISHIELD) and cellular (COVAXIN) immune responses respectively. Relation of dose interval and post-2nd decline in Nab titers in pre-positives (COVISHIELD) needs evaluation. Immunogenicity/efficacy of vaccines will change with the progression of the pandemic and needs to be assessed in field-setting.

Key words- SARS CoV-2, COVISHIELD, COVAXIN, Immunogenicity, T cell response

Research in Context

Evidence before this study:

We used the terms "COVID-19 vaccine COVISHIELD", "COVISHIELD vaccine, clinical trials" and "COVISHIELD vaccine, immune response" to search available data for COVISHIELD vaccine earlier and on 16th September 2021. Similar terms were used for Covaxin as well. Since the source of both AZD1222 and COVISHIELD vaccines is the candidate developed by Oxford, references related to AZD1222 appeared as well. AZD1222 vaccine has been extensively studied in relation to age, dose of the vaccine and duration between two doses, HIV positives, infections with different variants, efficacy and more recently the third dose. We identified studies on COVISHIELD related to adverse effects following immunization, neutralization with delta variant and one study analysing antibody response by chemiluminescence and enumeration of immune cell subsets after dose1. A study employing pan-India sampling of doctors did compare anti-spike antibody titers post-1st and 2nd dose[1]. However, in the absence of pre-immunization samples, asymptomatic prior infections were clubbed with pre-antibody negatives and therefore the data may not reflect true picture. Thus, a systematic study analysing neutralizing antibodies and T cell response to COVISHIELD vaccine was not found. For COVAXIN, reports of phase1, 2, 3 clinical trials are available. The vaccine was shown to be highly immunogenic inducing both neutralizing antibodies and T cell response. Overall, both vaccines were shown to elicit desired immune response, mostly in clinical trials.

Added value of this study

This study reports immunogenicity of two COVID vaccines in terms of live virus neutralization titers when used in the national program. As immunization in India was initiated at the end of the first wave and continued through the second wave, a large proportion of the adult population (~40%) was already exposed to SARS-CoV-2. Our data presents immune response among individuals positive for antibodies before immunization (pre-positives, ratio of clinical: subclinical infections 1:1.86) and the negatives (pre-negatives), relevant in the current scenario with second wave affecting still larger adult population (~60%). At the time of this study, the policy makers recommended interval of 4weeks between two doses, that was increased subsequently. Neutralizing antibody (Nab) titers ($PRNT_{50}$) were independent of age and gender. Irrespective of symptomatic or asymptomatic prior infection, (Nab) titers, increased exponentially after first dose. Of concern, the second dose did not boost the Nab titers, but, 1.75fold reduction was noted. In pre-negatives, a clear boosting was seen post-2nd dose. Titers among pre-positives were 190.6fold (1st dose) and 27.2fold (2nd dose) higher than in pre-negatives. T cell response was independent of prior exposure.

During the stipulated period, samples from only 21 Covaxin recipients could be collected post-2nd dose. Though the numbers are small, as compared to COVISHIELD the humoral response was lower while the T cell response was universal and 56.5fold higher. This could probably be attributed to the use of the adjuvant enhancing cell mediated immunity. Further assessment with larger numbers is warranted. Our study emphasizes necessity to monitor immune response against COVID vaccines when used in immunization of populations, the major weapon used in the global fight against this pandemic.

Implications of all the available evidence

Our results along with earlier reports suggest that in populations with high exposure rates, immunization with COVID-19 vaccines will lead to high antibody titers that should protect from emerging variants. Though a single dose seems ideal but not practical. In SARS-CoV-2 naïve people, a booster should be recommended for the persistence of antibodies for protection against divergent variants. In Indian health care workers immunized with two doses at 4weeks, COVISHIELD elicited higher humoral response while COVAXIN generated stronger T cell response. Results of clinical trials that are more regulated should be confirmed when the vaccines are used in public health systems.

Introduction:

The current pandemic of COVID-19 caused by SARS-CoV-2 continues to affect global population. India witnessed the first COVID-19 case in a traveler student returning from Wuhan [2]. After initial detection of cases among travelers from endemic countries, local transmission was established in different states at different times, State of Maharashtra and Pune city being the hotspots. Highest number of cases during the first and second waves in India (Pune) being 97,859 (2,120) in Sept 2020 and 414,433 (7,010) in May 2021 respectively [3],[4],[5]. In addition to the development and use of vaccines in record time, the pandemic is also characterized by the emergence of viral variants that can escape immune response generated by the earlier viral strain that was also used for vaccine development[6]. In addition to the conventional inactivated whole virus-based vaccines (n=9), adenovirus vectored (n=4), subunit protein-based (n=5), novel RNA-based (n=2) and DNA (n=1) vaccines have been approved for population immunization in different countries[7].

Following grant of "Emergency Use Authorization" to COVISHIELD and COVAXIN, the government of India initiated nationwide immunization program on Jan 16th January, 2021. Of these, COVISHIELD is developed by Oxford University (UK, chAdOx1 nCoV-19 vaccine) and manufactured in India by the Serum Institute of India Pvt Ltd (SIIPL) while COVAXIN is a whole virus-inactivated and adjuvanted vaccine developed and produced by Bharat Biotech International Ltd (BBIL), India. As on 11th September 2021, over 738 million vaccine doses have been administered in India including 641.65 million doses of COVISHIELD (86.9%) and 85.32 million doses of COVAXIN (11.6%). The results of phase1[8], phase2[9] and, phase 3[10] clinical trials of COVAXIN have been reported. However, though AZD1222, the vaccine developed by Oxford and manufactured at AstraZeneca has been studied extensively[11],[12],[13],[14], so far, similar data for COVISHIELD and Indian population are not available in the public domain. In the field settings, adverse effects following immunization with COVISHIELD[15] and Covaxin[16] were mild and short-lived. Short term efficacy of these vaccines in reducing infections [17] and mortality in COVID-19 patients post-two doses has been reported [18],[19].

So far, correlates of protection in SARS-CoV-2 infections in humans are not well defined. However, neutralizing antibodies and Th1-driven T cell response have been associated with recovery[20],[21],[22] and are evaluated during the clinical trials. In view of the scale of the immunization drive in the shortest possible time, it would be essential to assess immune response of recipients of both the vaccines (and the newer vaccines when introduced) in the field setting. We report immunogenicity of two vaccines used during the early phase of the national program.

Materials and Methods:

Recruitment, vaccination and sampling were done at Bharati Vidyapeeth (deemed to be) University Medical college and hospital (BVDTUMCH), a tertiary care hospital and designated immunization centre for COVID vaccines, at Pune, India. The study was approved by the "Human Ethics Committee" of BVDUMCH. Written informed consent was obtained from all the participants. Immunization was dependent on the availability of a particular vaccine on a given day and not as per choice.

Vaccines and vaccination schedules:

Vaccines:

1. COVISHIELD: University of Oxford (Oxford, UK) developed the chimpanzee adenoviral vectored vaccine with full length SARS-COV-2 spike insert (chAdOx1 nCoV-19 vaccine). This vaccine is manufactured at AstraZeneca, UK (AZD1222) and Serum Institute of India Pvt Ltd, India (COVISHIELD). For both, one vaccine dose contains $5X10^5$ viral particles.
2. COVAXIN: This is a whole-virion inactivated SARS-CoV-2 vaccine (BBV152) manufactured at Bharat Biotech International Ltd, India. It is adjuvanted with Algel- imidazoquinoline molecule (IMDG). IMDG is a TLR7/8 agonist used to augment cell-mediated responses. One dose contains 6ug of whole-virion inactivated SARS CoV-2 antigen.

Vaccination schedules:

Vaccine supply was made by the government through local public health administration. Vaccines were administered irrespective of COVID-19 in past. Depending on the type of the vaccine supplied on a day, inoculations were performed by the trained staff. Eligibility for vaccination was strictly followed as per the recommendations of the manufacturers. At the time of conducting this study, the national policy was to immunize HCW with two doses of the vaccines at 4weeks interval.

Study population and sample collection:

The number of study participants was primarily dependent on the feasibility of immunological analyses and enrolment at a single centre. In December 2020, IgG-anti-SARS-CoV-2 positivity among blood donors from BVDTUMCH was found to be 39.3% (our unpublished observations) indicative of exposure of a large proportion of the population to SARS-CoV-2. With 40% positivity and 50% dropouts at the time of sampling at one-month post-2nd dose, a sample size of 400/vaccine was estimated. This would allow us to analyse 120 and 80 recipients of each of the two vaccines. History Of (H/O) COVID was obtained before each sampling. Blood samples were collected before vaccination, before 2nd dose and one-month post-dose-2, in EDTA tubes. PBMCs

were separated within 4hours of blood collection by ficoll-histopaque based density gradient method. PBMCs and plasma samples were stored at -80^{0}C in aliquots. This would allow us to analyse 120 and 80 recipients of each of the two vaccines.

Serology

All the samples were tested for the (1) presence of IgG-anti-SARS-CoV-2 antibodies by ELISA (SCoV-2 Detect IgG ELISA, Inbios International, Inc., USA) and (2) presence/ titers of neutralizing antibodies by 50% plaque reduction neutralization test ($PRNT_{50}$) using live virus, as per the protocol described earlier[23]. Samples with PRNT titer ≥ 10 were considered positive for neutralizing antibodies (Nabs). For statistical analysis, negatives were assigned a titer value of 2.5.

Assays for cellular responses:

IFN-γ ELISPOT

Human IFN-γ ELISPOT^{Pro} kit (Mabtech) was employed to assess T cell responses against SARS CoV-2 spike protein following the manufacturer's instructions. Briefly, cryopreserved PBMCs were revived and rested overnight at 37°C in humidified CO2 incubator before assay. After trypan blue based live cell count, approximately, 4×10^5 PBMCs per well were stimulated in duplicate with peptide pool comprising 15mer overlapping peptides spanning the whole Spike protein (Source-BEI NIH, USA) at a concentration of 2 μg ml−1 of individual peptide for 24 hrs. Negative controls comprising 0.1% DMSO, complete culture media and positive controls (anti-CD3 & CD28) were included for each sample. Spots were counted using CTL S6 Macroanalyser (CTL Biospot) and presented as spot forming units (SFU)/million PBMCs. Based on the testing of 22 SARS CoV-2 IgG negative subjects, the cut off value (mean + 3 SD) was ≥14 SFU/million PBMCs.

Flow cytometry based intracellular cytokine secretion assay

To quantitate IFN-γ and IL-2 secreting T cells, 0.7- 1 X 10^6 PBMCs were exposed to Spike peptide pool (spanning whole Spike protein) in the presence of Golgi inhibitors. Post-incubation, the cells were stained with anti-CD3 APC A750, fixed and permeabilized to stain intracellular cytokines using anti-Human-IFN-γ (PECY7) and anti-Human-IL-2 (BV605) antibodies. The cells were then acquired using CytoFLEX LX (Beckman Coulter) and analysed with Cytexpert 2.4 software. The T cells secreting IFN-γ (CD3+IFN-γ+) and IL-2 (CD3+ IL-2+) were quantified. The cut off values for IFN-γ+ T cells and IL-2 +T (mean + 2SD) were 16% and 9% respectively.

Statistical Analysis

For statistical analysis Graph pad Prism (5.01 version) and "R" software (version 4.05) were used. For comparison between the groups, Mann Whitney U test was performed whereas for the comparisons of $PRNT_{50}$ titers and T cell responses, Wilcoxon Signed Rank sum test was used.

Results:

COVISHIELD:

Enrolment, vaccination and demographics:

Table 1 describes the details of the COVISHIELD recipients and numbers available at different time points. Majority (89.9%) of the recruits were ≤ 55 years while 10.1% were >55 years age. Of these, 57 individuals (13.4%) gave H/O COVID-19 as confirmed by viral RNA positivity. Disease severity was mild (n=52), moderate (n=3) and severe (n=2). The mean ages for pre-antibody negatives (pre-antibody positives) were 35.5 ± 9.8 (33.2± 10.3) in ≤55years group and 62.8 ± 6.9 (57.5± 1.5) in > 55years group and, overall, 41.2±14.4 years (33.2±10.3). Post-second dose, 120 samples from antibody-negatives (pre-negatives) and 67samples from antibody positive (Pre-positives) vaccine recipients were collected.

Evidence of previous exposure to SARS-CoV-2 as indicated by IgG-anti-SARS-CoV-2 positivity was seen in 163 (38.3%) individuals. Thus, 57 and 106 respectively were symptomatic and asymptomatic infections, the ratio of clinical: subclinical infections being 1:1.86. Nab titers did not differ in these categories (median 57, IQR=22-97 and 84, IQR=35-147 respectively, p>0.1.)

Clinical COVID-19 during vaccination:

Before the post-2nd dose sampling, 4 mild COVID-19 cases were recorded, none in the elderly. All were pre-negatives. The disease onset was post-1st dose in one and post-2nd dose in three. These were removed from further analysis.

Post-immunization antibody responses:

When the vaccination drive was initiated, the first wave of COVID-19 had declined and the second wave was not yet started. As vaccines were administered irrespective of prior antibody positivity, both antibody negatives and positives received the vaccine. These groups were analysed separately.

Antibody response among IgG-anti-SARS-CoV-2 negatives:

Post-first dose, 130/136 (95.6%) pre-negatives seroconverted to IgG-anti-SARS-CoV-2 antibodies (ELISA) while Neutralizing antibodies were detected in 55.1% (75/136, PRNT). Post 2nd dose, seroconversion was 100% (ELISA) and 95% (PRNT); 6 vaccinees (22F, 26F, 36F, 63F, 41M and 60M) were negative for neutralizing

antibodies (5%). The PRNT titers were <100 in 65/120 (54.2%; <20=6, 20-<50= 36 and 50-<100=29) and ≥ 100 in 49/120 (40.8%) vaccinees. Figure-1 depicts antibody titers among pre-negatives. Overall, the median Nab titers post-first dose was 16 (IQR 2.5-36.3) increasing 4fold (64.5; IQR 34.5-154.2) after second dose (p<0.000). Similar rise was seen when Nab titers were compared in relation to age groups (p<0.000 for both) and gender (p<0.000 for males and females). Nab titers after both the doses were independent of age (p>0.1 for both) and gender (p >0.1 for both).

Antibody response among individuals with prior exposure to SARS-CoV-2:

Of the 67 antibody positives, only 3 were >55 years age (Figure-2). The median Nab titers increased 40.7fold from 75 (IQR 29-129) before vaccination to 3050 (1282-3998, p< 0.001) post-1st dose (n=42). However, post-2nd dose (n=67), no boosting effect was seen, the median titers declining 1.75fold to 1740 (911-3116, p<0.05). Nab titers post-both doses were independent of gender (p>0.1); the decline in males (p>0.1) and females (p>0.05) were comparable. Vaccinees with prior exposure through asymptomatic (n=38) or symptomatic (n=29) infections did not differ in Nab titers before vaccination or post-both doses (p>0.1). Though post-2nd dose decline was apparent, the difference was not significant (p>0.1, figure2)

T cell responses (γ-interferon ELISPOT and Intracellular cytokine secretion):

Antigen-specific T cell responses were measured in 63 COVISHIELD recipients by IFN-γ ELISpot and intracellular cytokine staining of IFN-γ and IL-2 positive T cells in the PBMCs stimulated with spike peptide pool (table-2 and figures 3A and 3B). The selection of the subjects was based on the post-dose-2-PRNT titers. Among the pre-negative vaccinees (n=45), 57.8% were responsive to IFN-γ ELISPOT while IFN-γ+ and IL2+ T cells were detected in 68.9% and 8.9% recipients; the respective positivity rates among pre-positive vaccine recipients (n=18) were 61.1%, 61.1% and 27.8%. Of the 6 non-responders with respect to PRNT, 4 displayed T cell response. Testing of 16 paired PBMC samples from pre-negatives revealed absence of any reactivity prior to vaccination and robust, variable IFN-γ ELISpot response post-vaccination (figure 3C). When all the three parameters of T cell response were considered, an excellent correlation between IFN-γ based ELISpot and ICS (r=0.848, p=0.0001) was observed. These responses did not correlate with PRNT titers (r=0.020 and 0.063 respectively).

Comparison among vaccinees without or with prior exposure to SARS-CoV-2:

Post-dose1, titers among pre-positives were 190.6fold higher than pre-negatives. However, due to the lack of boosting effect post-dose-2 in the pre-positives and 4fold rise in pre-negatives, the difference was reduced to 27.2fold. At both time points, Nab titers among prepositives were higher than pre-negatives at both points (p <0.0001).

IFN-γ-specific T cell responses were not different among pre-negatives and pre-positives (figures 3A and 3B), the median SFU/million PBMCs being 21.7 (range: 0-2149,) and 19.8 (range: 0-633, p>0.1) while the proportions of IFN-γ+ T cells were 30% (range: 8.1-90.4) and 28% (range: 4.3-84, p>0.1) respectively. Higher proportion of prepositives (5/18, 27.8%) elicited IL2+ T cells than those without the exposure (4/45, 8.9%; P<0.001).

Immune response to COVAXIN:

During the stipulated period, we could recruit only 65 individuals. However, majority of these (n=44) received second dose at different centres and hence no blood samples could be collected. The data is limited to only 21 COVAXIN recipients. Of these, 8 were ≤ 55 years (23-50years; 4males and 4females) and 13 were > 55 years age (57-81years; 10males and 3females); 2/8 and 1/13 respectively were IgG-SARS-CoV-2 positive before vaccination. Two of the pre-negatives (65M and 81M) developed clinical COVID19 post-2nd dose; The 65M diagnosed on 12th day post-2nd dose had mild disease with Nab titer of 3123 three weeks later. The 81M was ELISA/PRNT negative after 1st dose, developed COVID19 4weeks post-2nd dose, was hospitalized and recovered without oxygen support. No sample was collected. Of the remaining 16 pre-negatives, % seroconversion post-second dose was 62.5% (ELISA) and 50% (PRNT, 4/8 each in ≤ and > 55years; median titer 6.75, IQR 2.5-24.75). Nab titers among three pre-antibody positives increased from median 119 (IQR 112.5-318.5) to 469 (median 680, IQR 574.5-1049).

Figure 4 depicts T cell response elicited by COVAXIN recipients (n=13). IFN-γ response detected by ELISPOT and ICS was elicited by all the participants (100%). Among 11 pre-negatives, the median SFU and % IFN-γ+ T cells were 1226 (522-3628) and 56.9 (24.8-78.6) respectively. Importantly, IFN-γ responses among vaccinees with no/low neutralizing antibodies were similar to the pre-positive vaccinee (31M, PRNT titer469) and the one developing mild disease post-second dose (65M, PRNT titer 3123). Both proportion and magnitude of IL2+ T cells were lower.

Comparison of immune response to COVISHIELD and COVAXIN vaccines:

Table 3 displays comparative immune response of the recipients of COVISHIELD and COVAXIN at 1month post-dose2. Though the numbers are small, Nab titers of COVAXIN recipients were lower than the COVISHIELD recipients (p<0.0001). Of note, the proportion of vaccinees > 55years was higher with COVAXIN (13/21, 61.9%) than COVISHIELD (10.9%). Despite higher age, COVAXIN induced superior T cell response as measured by ELISPOT (56.5fold, p<0.001) and ICS (1.8fold, p<0.05) for IFN-γ. Detection of

IL2+Tcells was lower with both the vaccines (p>0.1). Among prepositives, the titers after second dose were higher with COVISHIELD (n=67, median titer 1740) than with COVAXIN (n=3, median titer 680).

Discussion:

This study was designed to assess immune response of Indian subjects to COVISHIELD and COVAXIN used during the nationwide immunization program. Though our plan was to assess immunogenicity of both the vaccines, we were not able to recruit and follow up enough numbers of COVAXIN recipients. As a result, the study mainly focuses on COVISHIELD with a small data for COVAXIN. An efficacious COVID vaccine is expected to generate adequate neutralizing antibodies and a Th1-driven cell-mediated immunity. We therefore determined neutralizing ab titers and spike protein-specific T cell responses by IFN-γ -ELISPOT and enumeration of IFN-γ /IL2 positive T cells by flow cytometry.

At the time of initiation of vaccination in February, the number of COVID-19 cases during the first wave of the disease at Pune were reduced to the minimum[5]. Before vaccination, 38.3% vaccinees were exposed to SARS-CoV-2. With such high positivity, immune response was compared in relation to prior exposure. Importantly, the second wave caused by Kappa and Delta variants started in March 2021 in Pune. Thus, the vaccine efficacy was naturally tested against the divergent variants.

The origin of COVISHIELD and AstraZeneca vaccines is same. For the AstraZeneca vaccine manufactured earlier, results of clinical trials evaluating humoral and cellular responses in relation to the crucial parameters have been extensively reported [13],[17],[24]. However, similar data for COVISHIELD is not available in public domain. When the same two doses (5×10^{10} viral particles/dose) at 28days interval and $PRNT_{50}$ as the assay were considered[13] , post-dose-1 seroconversion rates and Nab titers during our study were lower with COVISHIELD (62.8% in ≤55 years age group) than AstraZeneca (35/35, 100%); the median titers were 218 (IQR 122–395) and 16 (2.5-39) respectively. Post second dose titers were not determined by PRNT in the AstraZeneca study. It is pertinent to note that here that the median titers of pre-immunization samples from the AstraZeneca study were 23 (10, 34), i.e., the analysis included prepositives (number not known) while a separate analysis for pre-positives and negatives was done in our study. When prepositives were considered, the median Nab titers increased from 75 to 3050 (Post 1st dose).

COVISHIELD vaccine was immunogenic and elicited neutralizing antibodies in 95% of the Indian HCW. However, for the generation of higher antibody titers and possible longer persistence at raised levels, a booster dose will be required among ab negatives. With AstraZeneca vaccine, antibody response was shown to be better when the duration between two doses was extended to 8-12weeks[13]. Whether longer duration between the doses (≥ 45 days) improved the immune response among Indian recipients vaccinated later needs to be evaluated.

Though generation of high tittered neutralizing antibodies among previously exposed individuals after one dose is satisfying, significant reduction following the second dose remains a matter of concern. Post-1st dose rise in antibody titers of mRNA vaccines recipients with clinical disease (clinical disease+) was higher than those with asymptomatic infections[25]. Notably, antibody response in clinical disease+ group was comparable after 1st and 2nd dose. In the absence of pre-vaccination screening, a cumbersome and expensive process for a national immunization program of a populous country like India, the apparent wastage of one dose per pre-exposed individual seems to be unavoidable, especially in view of the rampant subclinical infections. In any case, the titers among pre-positives were higher post-both doses (191fold and 27fold). In the blood donors examined during April 2021 in Pune when the second wave was ongoing, prevalence of IgG-anti-SARS-CoV-2 increased to 60% (our unpublished observations). Clearly, in future, a large population would have antibodies through prior exposure and likely to produce neutralizing antibodies at high titers. For the generation of higher Nab titers in pre-negatives that can possibly protect against the emerging variants, a booster dose seems necessary. However, only after the majority of the population receives two doses, administration of a booster dose will have to be considered. Very recently, lower reactogenicity and higher humoral as well as T cell responses were shown post-3rd dose of AZT1222 vaccine.

We could demonstrate T cell responses in the majority (77.8%) of COVISHIELD recipients (figure 3A and B). Of note, 4/6 non-responders elicited spike-specific T cell response. Thus, the vaccine induced desired Th1 driven T cell response that is shown to be associated with recovery from SARS-CoV-2 infection [22].

Though the number of COVAXIN recipients was small, comparisons of humoral and cellular responses led to significant findings. COVISHIELD led to higher neutralizing antibody response while COVAXIN elicited strong cellular response. During COVAXIN trials, formulation with the same composition led to seroconversions in 91.9% (phase-1 MNT) [8]and 98.3% (phase-2, PRNT) recipients [9]. Further the GMTs (MNT50) varied between 121.2 -130·3 when three lots of the vaccine were evaluated[10]. In view of such good response during clinical trials, it is indeed intriguing to note the lower humoral response observed during the current study, albeit with small numbers. Comparison of anti-spike antibody response among COVISHIELD (n=370) and Covaxin (n=87) recipient pan-India doctors without H/O COVID revealed lower seropositivity

(97.8% and 79.3%) and titers (115.5 v/s 5IAU) in Covaxin recipients[1]. In the absence of prevaccination samples, subjects with asymptomatic infections were clubbed with pre-antibody negatives making it difficult to compare true pre-negatives and positives. Antibody titers in the previously infected group receiving CoronoVac (inactivated vaccine, Sinovac)[26] or BNT162b2 Vaccines[27] increased significantly after 2nd dose.

We observed higher IFN-γ ELISpot response 1226 (522-3628 SFU/million PBMCs) than reported during the phase-1 clinical trial (peak of 100–120 SFU/million PBMCs) on day 28. Further, the rise in Nab titers in pre-positives was 1.65fold higher (n=48) [8] while we observed 5.7fold rise in the three vaccinees with prior exposure to SARS-CoV-2. Taken together, we observed stronger T cellular, lower humoral and higher Nab boosting effect among pre-positives. The results emphasize need for extending the study to a larger series. Further, the data underscores the importance of periodic monitoring of immune response to COVID vaccines when used for mass immunization that will ensure desired benefit of the immunization drives being undertaken globally.

In conclusion, we demonstrate excellent immunogenicity of COVISHIELD in Indian population. Though the number with COVAXIN was small, stronger T cell response with Covaxin and higher humoral response with COVISHIED were apparent. There is a definite need to assess immunogenicity of COVID vaccines during mass immunization programs.

Author Contribution:
VA conceived and designed the study. AK & RK were responsible for ELISPOT, ICS and PRNT work. SP, JO & SL were responsible for the recruitment of vaccine recipients and collection of samples/relevant information. ACM reviewed the results and commented on manuscript. All authors contributed to the article and approved the submitted version.

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Declaration of interests:
Authors declare no any commercial or financial conflict of interest.

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The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation upon reasonable request.

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Table 1: Demographic characteristics of the COVISHIELD recipients studied at different time points

Parameters	IgG Negatives	IgG positives	Total
Number consented for follow up (pre-vaccination)	155	67	222
Males: Females	72:83	35:32	107: 115
Age ±55: >55 years	127:28	64:3	191:31
Number tested Pre-2nd dose	136	42	178
Males: Females	55:81	22:20	77:101
Age ≤ 55: >55 years	121:15	41:1	162:16
Number tested post-2nd dose	120	67	187
Males: Females	53:67	35:32	88:99
Age ≤ 55: >55 years	95:25	64:3	159:28

Table 2: COVISHIELD: ELISPOT reactivity in relation to PRNT titers and prior exposure to SARS-CoV-2

PRNT50 titer	Positive in ELISPOT/ No tested (SFU/million PBMCs for positives)	
	Vaccinees without prior exposure	Vaccinees with prior exposure
Negative	4/6 (303, 675, 480, 192)	None*
<20	2/4 (21, 61)	None*
21-50	5/10 (41,33, 220,22, 25)	None*
51-100	4/9 (20, 41, 33, 141)	1/2 (69)
101-500	5/8 (429,20,1488,673,2149)	2/2 (14,23)
501-4000	6/8 (146,450, 88, 1784,52,99)	3/9 (633,165,391)
>4000	None*	1/5 (33)
Total	26/45,57.7% (median:19.8; range: 0-633)	7/18, 38.9% (median:21.7; range:1.7-2149)

*None exhibited these titers.

Table 3: Comparison of immune response of pre-antibody negative vaccinees receiving two doses of COVISHIELD or COVAXIN at 1month post-second dose

Parameters	COVISHIELD	COVAXIN	P value
IgG-anti-SARS-CoV-2 ELISA	120/120 (100)	10/16 (62.5)	<0.001
Nab titers: No Pos/No tested (median±SE)	114/120 (95) 64.5±55.7	8/16 (50) (2.5-24.8)	<0.001 <0.0001
Number of ELISPOT Positives (%) Median SFU/million PBMC (range)	26/45 (57.8) 21.7 (0-2149)	11/11 (100) 1226 (522-3628)	<0.05 <0.0001
Number eliciting IFN-γ+ T (%) %Median (range)	31/45 (68.9) 32.1 (9.2-90.4)	11 (100) 56.9 (24.8-78.6)	>0.05 <0.05
Number eliciting IL-2+ T cells % Median (range)	4/45 (8.9) 0-13.7	2 (18.8) 0.61-9.7	>0.1 >0.5

Legends to the figures:

Figure-1: **SARS CoV-2 $PRNT_{50}$ titers in Prenegatives**

$PRNT_{50}$ titers (median±SE) among COVISHIELD vaccine recipients negative for IgG-anti-SARS-CoV-2 antibodies prior to vaccination. The numbers above the bars represent the number of samples available in the respective categories.

Figure-2: **SARS CoV-2 $PRNT_{50}$ titers in Prepositives**

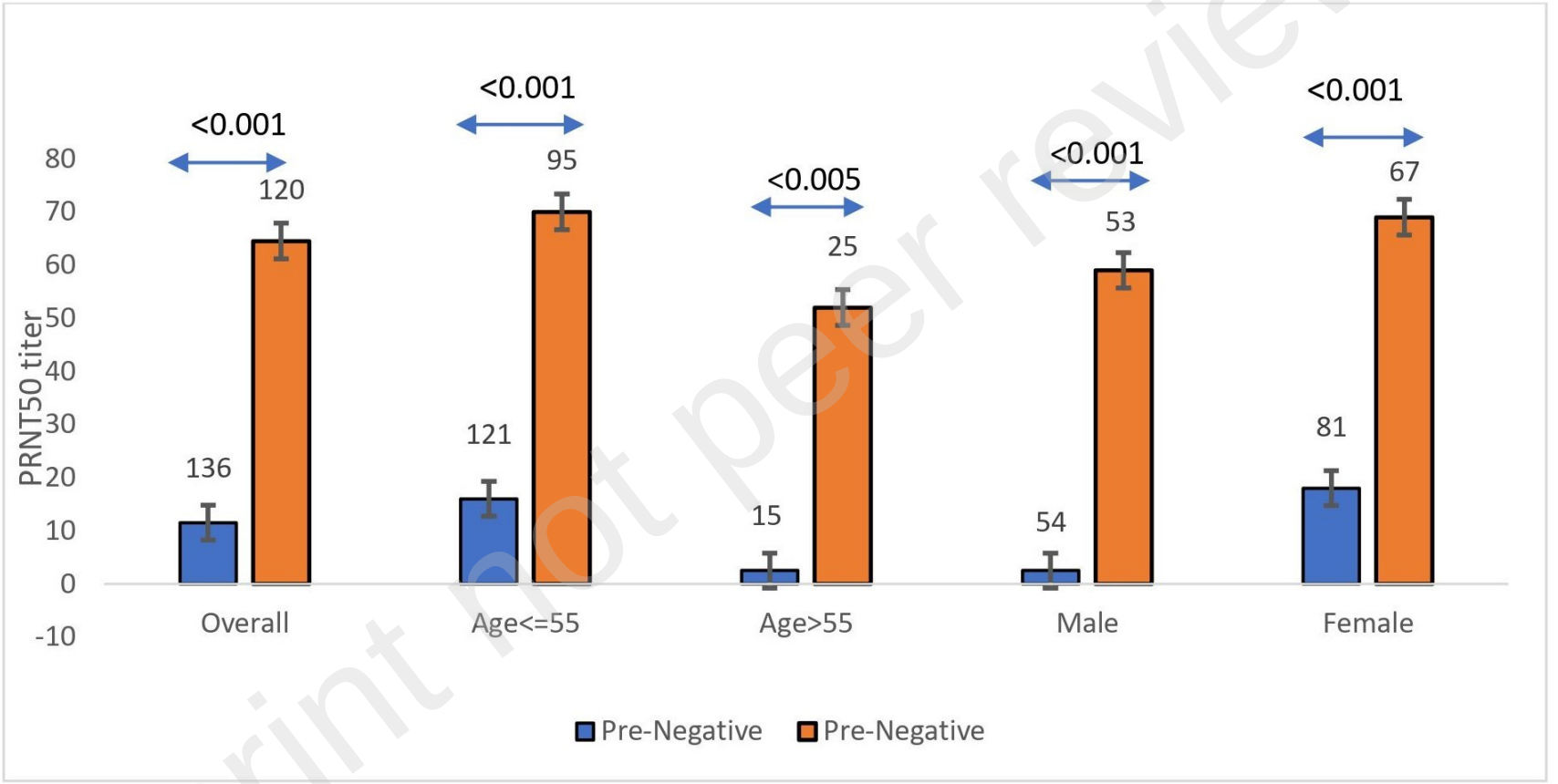
$PRNT_{50}$ titers (median±SE) among COVISHIELD vaccine recipients positive for IgG-anti-SARS-CoV-2 antibodies prior to vaccination (n=67). The numbers above the bars represent the number of samples available at different time points in the respective categories. * Indicates p value < 0.001.

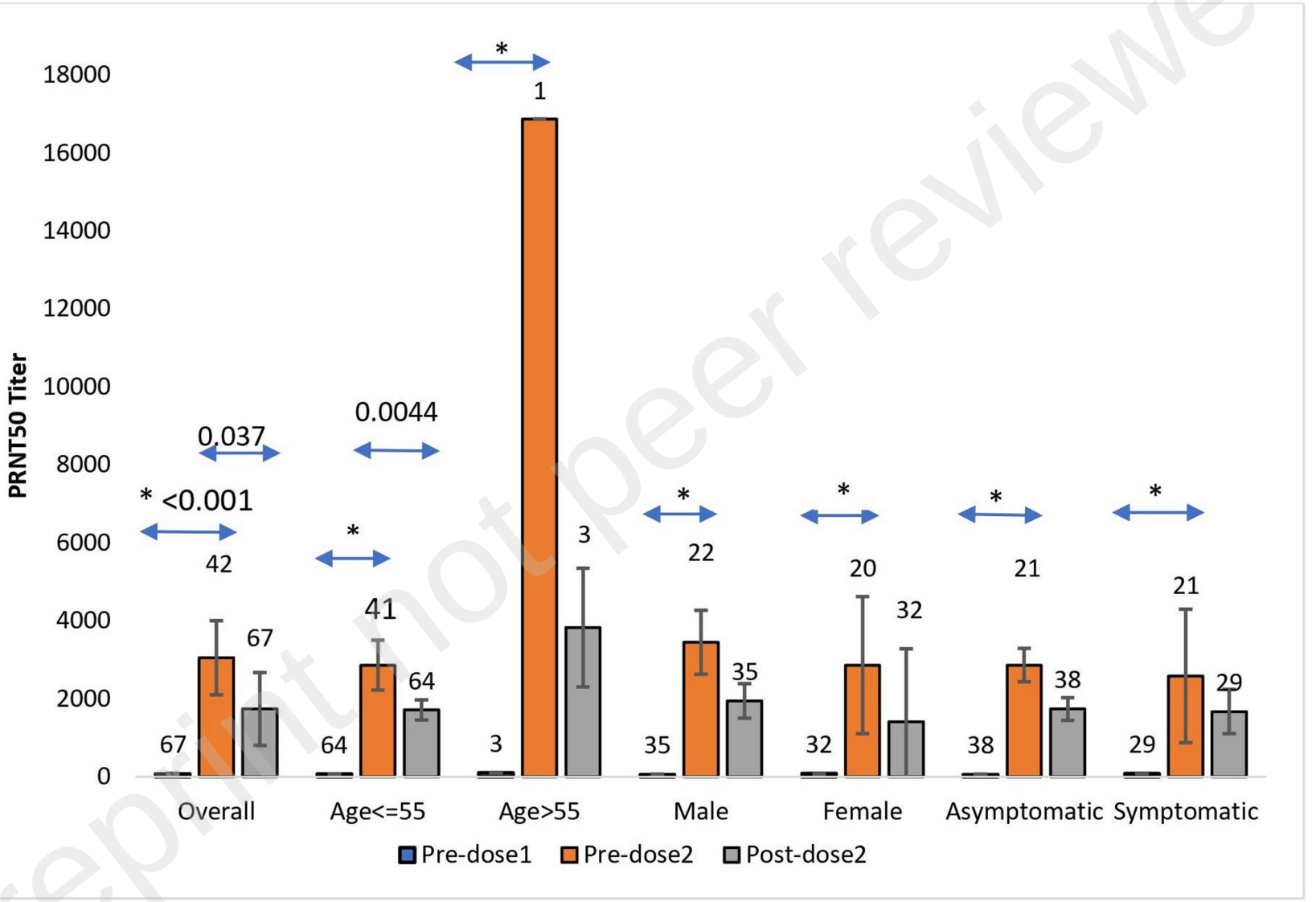
Figure-3: **T cell responses to COVISHIELD**

Spike protein peptide-specific T cell responses among (A) pre-vaccination IgG-anti-SARS-CoV-2 negative COVISHIELD recipients (n=45) and (B) pre-vaccination IgG-anti-SARS-CoV2 positive COVISHIELD recipients (n=18). Purple circles (IFN-γ ELISpot, Spot forming Units/million PBMCs), red squares (ICS, %IFN-γ+ T cells) and green triangles (ICS, %IL2+ T cells) depict corresponding values for the individual patients. Dotted lines show cut off values for each parameter. Figure 3C depicts IFN-γ ELISpot responses in 16 pre-IgG negative COVISHIELD recipients prior to post-2nd dose vaccination. ELISpot=enzyme-linked immunospot; PBMC=peripheral blood mononuclear cells; ICS= intracellular cytokine secretion.

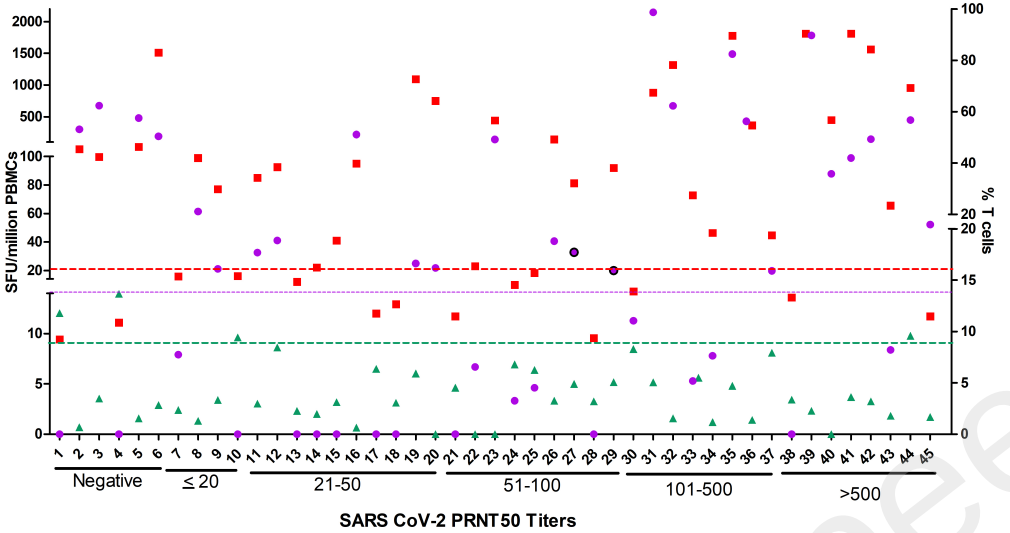
Figure-4: **T cell responses to COVAXIN**

Spike protein peptide-specific T cell responses among pre-vaccination IgG-anti-SARS-CoV-2 negative COVAXIN recipients (n=12) and one IgG-anti-SARS-CoV-2 positive. One IgG negative recipient (No 13, green rectangle) developed clinical COVID-19 post 2nd dose; sampled 3weeks post-diagnosis. Before vaccination, the pre-positive vaccinee (No 12) had PRNT titer of 119 that increased to 469 post-second dose). Purple circles (IFN-γ ELISpot, Spot forming Units/million PBMCs), red squares (ICS, %IFN-γ+ T cells) and green triangles (ICS, %IL2+ T cells) depict corresponding values for the individual patients. Dotted lines show cut off values for each parameter). ELISpot=enzyme-linked immunospot; PBMC=peripheral blood mononuclear cells; ICS= intracellular cytokine secretion.





A]



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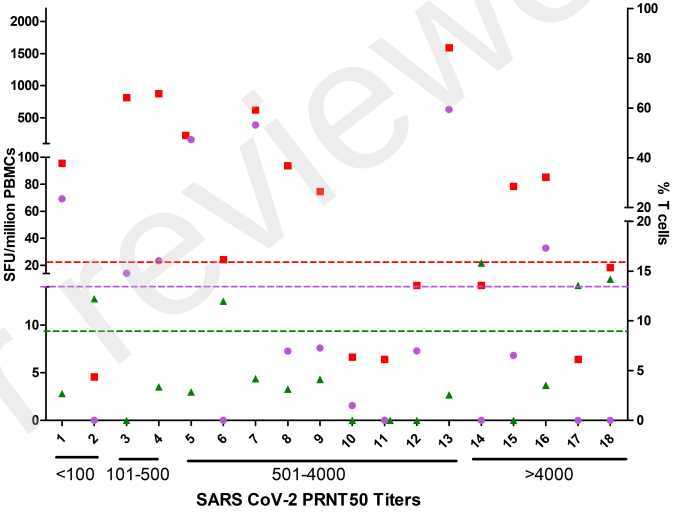


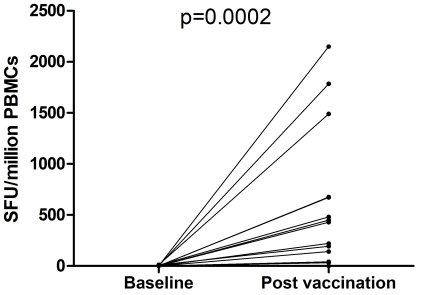
Left Y axis- SFU/million PBMC

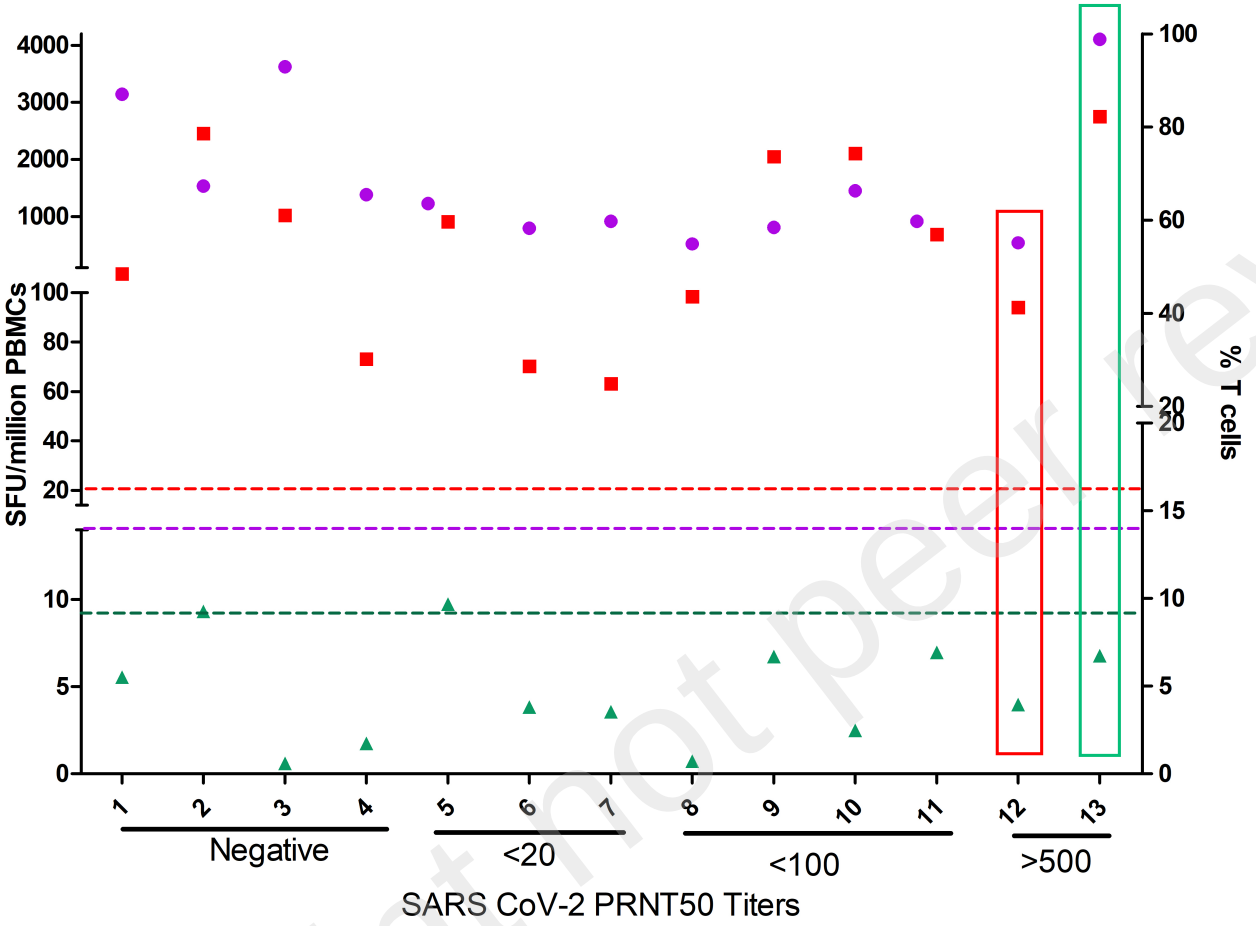
Right Yaxis- IL-2 +ve T cells

IFN-γ +ve T cells

Dotted lines depict cut off for respective parameter

C]





Left Y axis- ● SFU/million PBMC

Right Yaxis- ▲ IL-2 +ve T cells

■ IFN-γ +ve T cells

Dotted lines depict cut off for respective parameter