

# Characterization of neutralizing versus binding antibodies and memory B cells in COVID-19 recovered individuals from India

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## 34 Running title:

## 35 Humoral and B cell memory in COVID-19 recovered individuals from India

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48 **Abstract**

49

50 India is one of the countries most affected by the recent COVID-19 pandemic.  
51 Characterization of humoral responses to SARS-CoV-2 infection, including immunoglobulin  
52 isotype usage, neutralizing activity and memory B cell generation, is necessary to provide  
53 critical insights on the formation of immune memory in Indian subjects. In this study, we  
54 evaluated SARS-CoV-2 receptor-binding domain (RBD)-specific IgG, IgM, and IgA antibody  
55 responses, neutralization of live virus, and RBD-specific memory B cell responses in pre-  
56 pandemic healthy versus convalescent COVID-19 individuals from India. We observed  
57 substantial heterogeneity in the formation of humoral and B cell memory post COVID-19  
58 recovery. While a vast majority (38/42, 90.47%) of COVID-19 recovered individuals  
59 developed SARS-CoV-2 RBD-specific IgG responses, only half of them had appreciable  
60 neutralizing antibody titers. RBD-specific IgG titers correlated with these neutralizing  
61 antibody titers as well as with RBD-specific memory B cell frequencies. In contrast, IgG titers  
62 measured against SARS-CoV-2 whole virus preparation, which includes responses to  
63 additional viral proteins besides RBD, did not show robust correlation. Our results suggest  
64 that assessing RBD-specific IgG titers can serve as a surrogate assay to determine the  
65 neutralizing antibody response. These observations have timely implications for identifying  
66 potential plasma therapy donors based on RBD-specific IgG in resource-limited settings  
67 where routine performance of neutralization assays remains a challenge.

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71 **Importance**

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73 Our study provides an understanding of SARS-CoV-2-specific neutralizing antibodies,  
74 binding antibodies and memory B cells in COVID-19 convalescent subjects from India. Our  
75 study highlights that PCR-confirmed convalescent COVID-19 individuals develop SARS-CoV-  
76 2 RBD-specific IgG antibodies, which correlate strongly with their neutralizing antibody  
77 titers. RBD-specific IgG titers, thus, can serve as a valuable surrogate measurement for  
78 neutralizing antibody responses. These finding have timely significance for selection of  
79 appropriate individuals as donors for plasma intervention strategies, as well as determining  
80 vaccine efficacy.

81 **Introduction**

82

83 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus responsible for  
84 the coronavirus disease 2019 (COVID-19) pandemic, emerged as a grave public health threat  
85 beginning in December 2019(1), paralyzing daily lives and causing economic downturns in  
86 many parts of the world. Currently, India is one of the countries most affected with more  
87 than 3 million COVID-19 confirmed cases and 60,000 associated deaths (2).

88

89 Intense efforts are underway to develop vaccines and antiviral therapeutics (3-11). These  
90 efforts require a detailed understanding of immune correlates of protection, formation of  
91 immune memory, and durability of these responses. Additionally, infusion of plasma derived  
92 from COVID-19 recovered individuals is also being explored as a treatment strategy (12-20).  
93 All these efforts require a detailed understanding of humoral immunity, immunoglobulin  
94 isotype usage and neutralizing activity following recovery from SARS-CoV-2 infection.  
95 Moreover, given that many of the SARS-CoV-2 neutralizing epitopes are located in the viral  
96 receptor binding domain (RBD) of the Spike (S) protein (21-29), it is important to evaluate  
97 the relationship between RBD-specific IgG titers and neutralizing antibody responses.

98

99 In this study, we evaluated IgG, IgA, IgM, neutralizing antibodies and memory B cell  
100 responses in PCR-confirmed COVID-19 convalescent subjects. Our results show that while a  
101 vast majority (38/42, 90.47%) of COVID-19 recovered individuals developed SARS-CoV-2  
102 RBD-specific IgG responses, we were able to detect appreciable levels of neutralizing  
103 antibody responses in only half of the convalescent subjects. Neutralizing responses

104 correlated closely with RBD-specific IgG titers, but weakly with IgG titers measured against  
105 crude virus concentrate using a commercial ELISA kit. Taken together, these findings suggest  
106 that despite significant inter-individual variation in the RBD-specific IgG titers and  
107 neutralizing antibodies, RBD-specific IgG titers can serve as a valuable and robust surrogate  
108 measurement for neutralizing antibody responses. These observations not only provide a  
109 glimpse of humoral immune responses in COVID-19 recovered individuals from India, but  
110 also have timely implications for identifying potential plasma therapy donors using on RBD-  
111 specific IgG ELISA's in India where routine performance of neutralization assays remains a  
112 challenge.

113 **Methods**

114

115 ***Subject recruitment***

116 COVID-19 recovered individuals were recruited at Shaheed Hasan Khan Mewati Government  
117 Medical College, Nuh, Haryana, India, Super Specialty Pediatric Hospital and Post Graduate  
118 Teaching Institute, Noida and ICMR-National Institute of Malaria Research, New Delhi. The  
119 Institutional ethical boards approved the study. Informed consent was obtained prior to  
120 inclusion in the study. All subjects (mean age 39.4 years, range 15 – 70 years) were SARS-  
121 CoV-2 PCR positive at the time of initial diagnosis, and were PCR negative when recruited for  
122 this study at 3.6 – 12 weeks post initial diagnosis (**Table 1**). Samples collected from healthy  
123 adult blood bank donors in the year 2018 are included as pre-pandemic controls.

124 ***SARS-CoV-2 specific PCR***

125 SARS-CoV-2 specific rRT-PCR was performed as per the Indian government guidelines for  
126 COVID-19 diagnosis. Nasopharyngeal and throat swabs were collected in viral transport  
127 medium (VTM) (HiMedia, #AL 167)) and transported to the testing laboratory maintaining  
128 cold chain. All the samples were subjected to the first line screening assay or the 'e' gene  
129 assay as per the guidelines (30). Samples reactive by the first line assay were subjected to  
130 the RdRp gene assay (Invitrogen SuperScript™ III Platinum® One-Step Quantitative Kit (Cat.  
131 No.11732088). Samples reactive for both the genes were labeled positive, while samples  
132 reactive to 'e' gene only were considered indeterminate and were subjected to repeat  
133 sampling. The same protocol was used to verify that the subjects were PCR negative at the  
134 time of recruitment for this study.

135 **SARS-CoV-2 RBD-specific direct ELISA**

136 Recombinant SARS-CoV-2 RDB gene was cloned, expressed, purified and standard direct  
137 ELISAs were performed as previously described (31). Briefly, purified RBD was coated on  
138 MaxiSorp plates (Thermo Fisher, #439454) at a concentration of 1 ug/mL in 100 uL  
139 phosphate-buffered saline (PBS) at 4<sup>o</sup>C overnight. The plates were washed extensively with  
140 PBS containing 0.05% Tween-20. Three-fold serially diluted plasma samples were added to  
141 the plates and incubated at room temperature for 1hr. After incubation, the plates were  
142 washed and the SARS-CoV-2 RBD specific IgG, IgM, IgA signals were detected by incubating  
143 with horseradish peroxidase (HRP) conjugated - anti-human IgG (Jackson ImmunoResearch  
144 Labs, #109-036-098), IgM (Jackson ImmunoResearch Labs, #109-036-129), or IgA (Jackson  
145 ImmunoResearch Labs, #109-036-011). Plates were then washed thoroughly and developed  
146 with o-phenylenediamine (OPD) substrate (Sigma, #P8787) in 0.05M phosphate-citrate  
147 buffer (Sigma, #P4809) pH 5.0, containing with 0.012% hydrogen peroxide (Fisher Scientific,  
148 #18755) just before use. Absorbance was measured at 490 nm.

149 ***Enumeration of SARS-CoV-2 RBD-specific memory B cells***

150 Purified RBD protein (100 ug) was labeled with Alexa Fluor 488 using microscale protein  
151 labeling kit (Life Technologies, #A30006) as per manufacturer's protocol. PBMC's were  
152 stained with RBD-Alexa Fluor 488 for 1 hour at 4<sup>o</sup>C, followed by washing with PBS containing  
153 0.25% FBS, and incubation with efluor780 Fixable Viability (Live Dead) dye (Life  
154 Technologies, #65-0865-14) and anti-human CD3, CD19, CD27, CD38 and IgD antibodies (BD  
155 Biosciences) for 30 minutes. Cells were washed twice with FACS buffer and acquired on BD  
156 LSR Fortessa X20. Data was analyzed using FlowJo software 10. SARS-CoV-2 RBD-specific

157 memory B cells were identified in cells positive for CD19, CD20, CD27 that were negative for  
158 IgD and CD3.

159

160 ***IgG ELISA for SARS-CoV-2 whole virus preparation***

161 SARS-CoV-2 antigen specific IgG was detected using a commercially available assay (COVID-  
162 Kavach ELISA tests kit, Zydus diagnostics), which measures responses to antigen  
163 concentrated from gamma-irradiated SARS-CoV-2-infected tissue culture fluid as per the  
164 manufacturer's instructions (32, 33).

165 ***SARS-CoV-2 neutralization assay***

166 Neutralization titers to SARS-CoV-2 were determined as previously described (31). Briefly  
167 infectious clone of the full-length mNeonGreen SARS-CoV-2 (2019-nCoV/USA\_WA1/2020)  
168 was used to test heat-inactivated COVID-19 convalescent samples and healthy donor  
169 samples (pre-pandemic). Heat-inactivated serum was serially diluted three-fold in duplicate  
170 starting at a 1:20 dilution in a 96-well round-bottom plate and incubated between 750 FFU  
171 of ic-SARS-CoV-2-mNG for 1 h at 37°C. This antibody-virus mixture was transferred into the  
172 wells of a 96-well plate that had been seeded with Vero-E6 cells the previous day at a  
173 concentration of  $2.5 \times 10^4$  cells/well. After 1 hour, the antibody-virus inoculum was removed  
174 and 0.85% methylcellulose in 2% FBS containing DMEM was overlaid onto the cell  
175 monolayer. Cells were incubated at 37°C for 24 hours. Cells were washed three times with  
176 1XPBS (Corning Cellgro) and fixed with 125 µl of 2% paraformaldehyde in PBS (Electron  
177 Microscopy Sciences) for 30 minutes. Following fixation, plates were washed twice with 1x  
178 PBS and imaged on an ELISPOT reader (CTL Analyzer). Foci were counted using Viridot (34)

179 (counted first under the “green light” setting followed by background subtraction under the  
180 “red light” setting). FRNT-mNG<sub>50</sub> titers were calculated by non-linear regression analysis  
181 using the 4PL sigmoidal dose curve equation on Prism 8 (Graphpad Software).  
182 Neutralization titers were calculated as 100% x [1- (average foci in duplicate wells incubated  
183 with the specimen) ÷ (average number of foci in the duplicate wells incubated at the highest  
184 dilution of the respective specimen)].

185 ***Statistical analysis***

186 Statistical analysis was performed using GraphPad prism 8.0 software. Non-parametric t test  
187 (Mann-Whitney) was used to calculate the differences between groups. Non-parametric  
188 Spearman’s correlation coefficient (r) was used to calculate correlation between groups.  
189 A *p* value of  $\leq 0.05$  was considered as significant.

190

191 **Results**

192

193 ***SARS-CoV-2 RBD-specific humoral immunity in COVID-19 recovered individuals.***

194 The demographic profile of COVID-19 recovered individuals recruited for this study is shown  
195 in **Table 1**. All subjects were at least 3.6 weeks past their initial SARS-CoV-2 positive  
196 diagnosis. RBD-specific ELISA curves for IgG, IgA and IgM at different dilutions of plasma in  
197 pre-pandemic healthy versus COVID-19 recovered individuals are shown in **Figure 1**. RBD-  
198 specific responses were highly elevated in COVID-19 recovered individuals as compared to  
199 pre-pandemic healthy controls (**Figure 1A,B,C, left versus middle panels**). Titers of IgG,  
200 IgA and IgM in the COVID-19 recovered individuals showed substantial inter-individual  
201 variation (**Figure 1 A, B, C, right panel**) - with IgG endpoint titers ranging from below  
202 detection to 24484 (2000 $\pm$ 619); IgA titers from below detection to 5686 (386 $\pm$ 136) and IgM  
203 titers from below detection to 2958 (515 $\pm$ 90). Four individuals had undetectable RBD-  
204 specific IgG and IgA titers. One of these individuals was also below detection for IgM (**Table**  
205 **2**). Inter-individual heterogeneity was not related to the age of the individuals (**Figure 2A**)  
206 or the number of days that elapsed between PCR confirmation of infection and sample  
207 collection (**Figure 2B**).

208

209 ***SARS-CoV-2 specific neutralizing titers in COVID-19 recovered individuals.***

210 To assess plasma neutralizing titers from COVID-19 convalescent individuals, we performed  
211 a live virus neutralization assay using a focus-reduction neutralization mNeonGreen (FRNT-  
212 mNG) assay (31). The neutralizing activity at different dilutions of plasma for pre-pandemic

213 healthy individuals (**Figure 3A**) and COVID-19 recovered individuals is shown in (**Figure**  
214 **3B**). **Figure 3C** shows FRNT-mNG<sub>50</sub> titers calculated based on the plasma dilution that  
215 neutralized 50% of the virus. While all pre-pandemic healthy individuals had undetectable  
216 FRNT-mNG<sub>50</sub> titers, only half of the COVID-19 recovered individuals showed 50% or more  
217 neutralization even at a 1:20 dilution of plasma. Similar to RBD-specific IgG titers, the FRNT-  
218 mNG<sub>50</sub> titers were heterogeneous with the latter reaching titers as high as 682 (**Figure 3C**).  
219

220 Previous studies in other viral infections have shown that all three antibody isotypes (IgG,  
221 IgA and IgM) can potentially neutralize (35-39). We next determined if any correlation exists  
222 between SARS-CoV-2 neutralizing titers and RBD-specific IgG, IgA, IgM binding antibody  
223 titers. We observed a positive correlation ( $r=0.83$ ;  $p<0.001$ ) between SARS-CoV-2  
224 neutralizing titers and RBD-specific IgG titers (**Figure 4, left graph**) but not with IgA  
225 (**Figure 4, middle graph**) or IgM titers (**Figure 4, right graph**).  
226

227 Plasma infusion therapy has recently been started in India as an intervention therapy for  
228 COVID-19. For this, plasma donors are being typically identified by the presence of IgG to  
229 SARS-CoV-2 by commercial ELISA tests (40). One of these tests detects IgG towards viral  
230 antigens concentrated from gamma-irradiated SARS-CoV-2-infected tissue culture fluid (32,  
231 33). It was therefore of interest to examine the correlation between neutralization titers and  
232 IgG responses measured using this test. We observed that, of the 42 COVID-19 recovered  
233 individuals tested, 33 were IgG positive whereas 9 were below the assay cut off (**Figure 5A**).  
234 Of the 9 individuals that were below cut off, 4 also tested negative by the RBD-specific IgG  
235 ELISA (**Table 2**). All of the samples from the pre-pandemic healthy individuals were below

236 the limit of detection using both the ELISA methods. Most importantly, the IgG values  
237 obtained by whole virus-based ELISA did not show as robust a correlation ( $r=0.56$ ) with  
238 neutralizing antibody titers (**Figure 5B**) as compared to those observed with RBD-specific  
239 IgG titers ( $r=0.83$ ) (**Figure 4, left graph**).

240

241 ***Characterization of RBD-specific memory B cells in COVID-19 recovered individuals.***

242 While circulating neutralizing antibodies help prevent re-infection by viruses, memory B  
243 cells allow for rapid production of new antibodies in case of re-infection. To address whether  
244 the COVID-19 recovered individuals generated memory B cells, we enumerated RBD-specific  
245 memory B cells using fluorescently-conjugated RBD antigen. An example of the flow  
246 cytometric gating strategy and RBD staining among the gated memory B cells is shown in  
247 **Figure 6A and 6B.** **Figure 6C** shows the frequency of RBD-specific memory B cells in a  
248 subset of the individuals where sufficient PBMCs were available. Though we found that there  
249 was substantial inter-individual variation in the frequency of SARS-CoV-2 RBD-specific  
250 memory B cells, their frequencies modestly correlated with RBD-specific IgG titers.

251 **Discussion**

252

253 Our study provides a detailed understanding of humoral immunity and memory B cells in  
254 COVID-19 recovered individuals from India. We examined SARS-CoV-2 neutralizing  
255 antibodies, IgG, IgM, IgA and memory B cells in pre-pandemic healthy versus COVID-19  
256 recovered individuals and further evaluated inter-individual variation and relation among  
257 these.

258

259 Our correlative analysis of RBD-specific IgG binding titers with neutralizing antibody titers  
260 and memory B cells has important implications for not only identifying potential donors for  
261 plasma therapy but also for understanding humoral and cellular memory post COVID-19.  
262 Though current plasma therapy guidelines in India do not consider neutralizing antibody  
263 titers, United States Food and Drug Administration (FDA) guidelines recommend, when  
264 available, a neutralizing titer of 1:160 or 1:80 to be used for identifying potential plasma  
265 donors (41). Our correlation analysis shows that RBD-specific titers of more than 3668 can  
266 provide a suitable surrogate for identifying the individuals with neutralizing titers of above  
267 1:160 and RBD-specific IgG titers 1926 for neutralizing titers of 1:80. Though larger scale  
268 studies are needed to establish robustness, these observations have timely implications to  
269 identify potential plasma therapy donors.

270

271 Our study raises important questions on formation of protective immune memory after  
272 recovering from COVID-19. We found that nearly half of the COVID-19 recovered individuals  
273 did not induce 50% neutralizing titers even at 1:20 dilution of plasma. This raises the

274 question of whether these individuals with low neutralizing antibodies also differ in  
275 formation of cellular immune memory. Our data show that individuals with low neutralizing  
276 antibodies indeed had lower memory B cells. Given that T cells may also contribute to COVID-  
277 19 protection, studies are needed to understand whether these individuals may also differ  
278 in the generation of memory CD8 and CD4 T cells (42-44).

279

280 The reason why only half of the COVID-19 recovered individuals developed appreciable  
281 levels of neutralizing antibody titers requires further investigation. This may be related to  
282 inter-individual differences in human immune responses associated with the expected  
283 heterogeneity in initial viral inoculum(45), initial viral loads (46-48), incubation period (49),  
284 host genetic factors (50-52) and disease severity (53, 54). This is consistent with previous  
285 studies that show relatively higher neutralizing antibodies in COVID-19 hospitalized patients  
286 during the acute febrile phase, or in recovered individuals that were previously hospitalized  
287 with severe COVID-19 disease (53, 54). It is noteworthy that the COVID-19 recovered  
288 individuals from our study had mild to moderate symptoms during the initial diagnosis. In  
289 light of these studies, our findings warrant future studies to seek an understanding of  
290 whether the individuals that have generated low or no neutralizing antibodies, IgG titers or a  
291 memory B cells past recovery will be protected if they were re-exposed to SARS-CoV-2 or a  
292 related virus.

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302

303 **Author Contributions**

304 Experimental work, data acquisition and analysis of data by K.N, K.G, S.K, E.S.R, V.V.E., K.F,  
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306 Conceptualization and implementation by A.S, R.A, K.M, A.C. Manuscript writing by A.C and  
307 K.M. All authors contributed reviewing and editing the manuscript.

308

309 **Figure legends**

310

311 ***Figure 1: Evaluation of SARS-CoV-2 RBD specific IgG, IgA and IgM antibody responses.***

312 **(A)** RBD-specific IgG, **(B)**, RBD-specific IgA; **(C)**, RBD-specific IgM. Left, pre-pandemic  
313 healthy (n=22), middle COVID-19 recovered (n=42); right, endpoint titers. ELISA cutoff  
314 values are calculated using the average plus 3 standard deviations of the 22 healthy controls  
315 at 1:100 dilution (shown as a dotted line). The unpaired analysis was done using non-

316 parametric Mann-Whitney-U test.  $p \leq 0.05$  was considered significant. Assay cutoff value is  
317 marked with dotted line.

318 **Figure 2. Correlation of age and day post initial diagnosis of COVID-19 recovered**  
319 **individuals with SARS-CoV-2 IgG, IgM and IgA titers.**

320 **(A).** Age versus IgG (left, n=42), IgA (middle, n=42) or IgM (right, n=42) titers. **(B).** Time post  
321 initial diagnosis versus IgG (left, n=42), IgA (middle, n=42) or IgM (right, n=42) titers.  
322 Correlations were calculated by Spearman's correlation coefficient  $r$ .  $p \leq 0.05$  is considered  
323 significant. Note that none of the data sets above reached significant values of correlation.

324

325 **Figure 3. Evaluation of SARS-CoV-2 neutralizing antibodies in COVID-19 recovered**  
326 **individuals.**

327 SARS-CoV-2 neutralizing activity at indicated dilutions of plasma is shown in pre-pandemic  
328 healthy (n=22, in grey) **(A)** and in COVID-19 recovered individuals (n=42, in blue) **(B)**.  
329 Dotted line represents the plasma dilution that leads to 50% neutralization. **(C)** Scatter plot  
330 shows neutralization titers (FRNT-mNG<sub>50</sub>) in pre-pandemic healthy (n=22) and COVID-19  
331 recovered (n=42) individuals. The unpaired analysis was done using non-parametric Mann-  
332 Whitney-U test.  $p \leq 0.05$  was considered significant. Limit of detection is marked with a  
333 dotted line.

334

335 **Figure 4. Correlation analysis of SARS-CoV-2-specific antibody responses versus**  
336 **neutralization titers.**

337 Correlation analysis shows FRNT-mNG<sub>50</sub> titers (x-axis) versus RBD-specific IgG (Left), IgA  
338 (middle) and IgM (right) titers on y-axis in COVID-19 recovered individuals (n=42, blue  
339 dots). Correlation analysis was performed by log transformation of the endpoint ELISA titers  
340 followed by linear regression analysis. Correlations were calculated by Spearman's  
341 correlation coefficient  $r$ .  $p \leq 0.05$  was considered significant. Dotted line on x-axis and y-axis  
342 indicate limit of detection.

343

344 **Figure 5. Correlation analysis of SARS-CoV-2 whole virus specific IgG versus neutralizing**  
345 **titers.**

346 **(A).** Scatter plots shows SARS-CoV-2 whole virus specific IgG measured using measured  
347 using commercial kit (Zydus diagnosis, Covid Kavach) in pre-pandemic healthy (n=5) and  
348 COVID-19 recovered (n=42). The unpaired analysis was done using non-parametric Mann-  
349 Whitney-U test.  $p \leq 0.05$  was considered significant. **(B).** Correlation analysis of SARS-CoV-2  
350 whole virus antigen specific IgG ELISA kit values (y-axis) versus neutralizing titers (x-axis)  
351 in COVID-19 recovered individuals (n=42). Correlations were calculated by Spearman's  
352 correlation coefficient  $r$ .  $p \leq 0.05$  was considered significant. Dotted line on x-axis indicate  
353 limit of detection and on y-axis assay cut off.

354

355 **Figure 6. SARS-CoV-2 RBD-specific memory B cell analysis in COVID-19 recovered**  
356 **individuals.**

357 **(A)** Gating strategy used to identify memory B cells. **(B)** SARS-CoV-2 RBD-specific memory  
358 B cells on gated total memory B cells that were CD19 positive, CD20 high, IgD negative and  
359 CD27 high is shown. **(C)** Frequency of RBD-specific memory B cells of the total memory B  
360 cells in the COVID-19 recovered individuals (n= 13). **(D)** Correlation analysis shows  
361 frequency of RBD-specific memory B cells (x-axis) and the RBD-specific IgG titers (y-axis) in  
362 COVID-19 recovered individuals.

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**Table 1. COVID-19 recovered individuals characteristics (n=42)\***

Age in years	Mean (Range)	39.4 (15-70)
Males/Females		38/4
Days post PCR diagnosis	Mean (Range)	47.3 (25-84)

\*COVID-19 recovered individuals were recruited at Shaheed Hasan Khan Mewati Government Medical College, Nuh, Haryana, India. Super Speciality Paediatric Hospital and Post Graduate Teaching Institute, Noida and ICMR-National Institute of Malaria Research, New Delhi. All subjects were SARS-CoV-2 PCR positive at the time of initial diagnosis and were PCR negative when recruited for this study at 4.8 – 11 weeks post initial diagnosis.

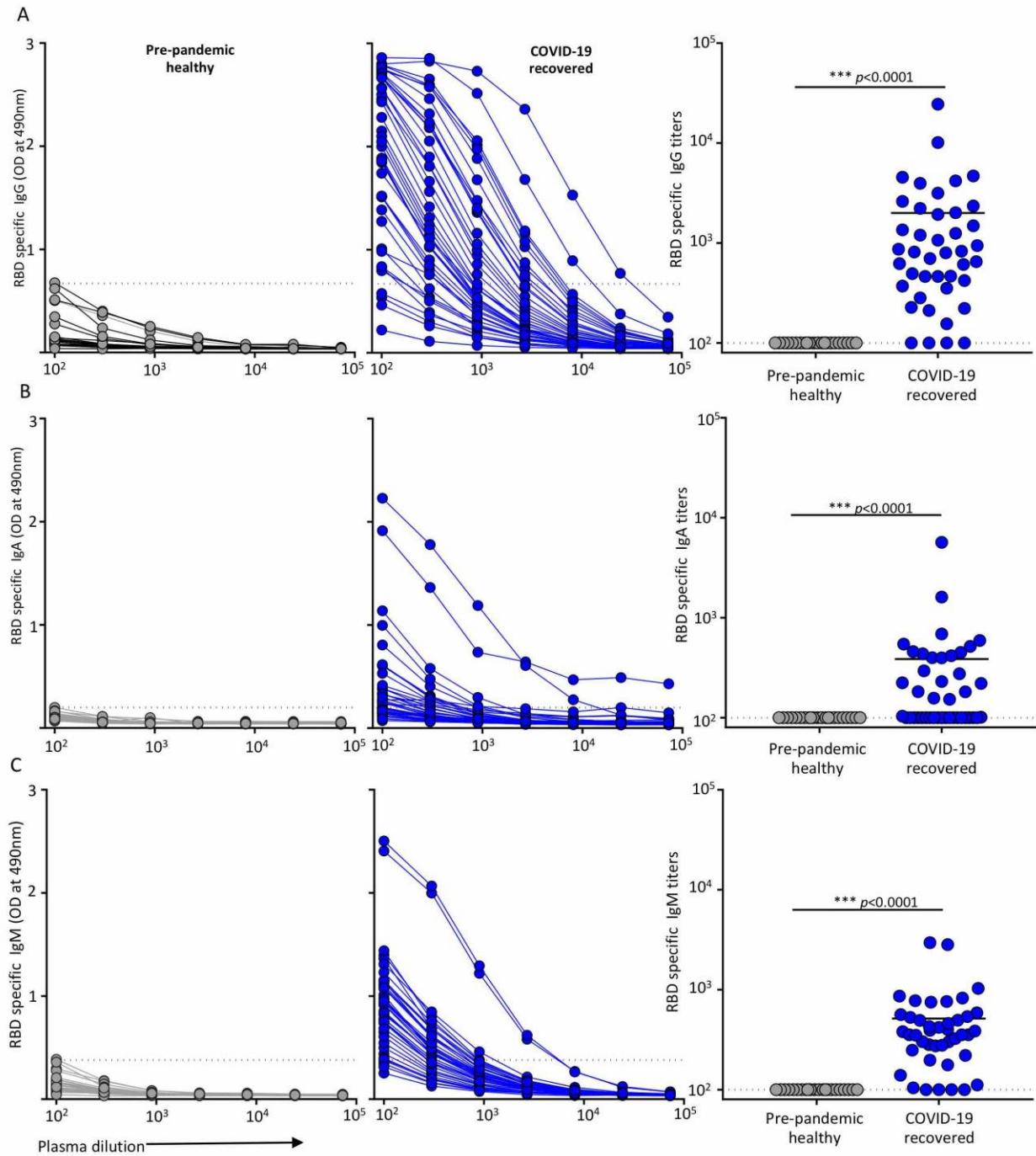
**Table 2. Individual characteristics of the COVID-19 recovered subjects**

Subject number	Age	Gender (Male,M Female,F)	Days Post PCR Diagnosis	SARS CoV-2 RBD specific Immunoglobulin titers*			SARS Cov-2 whole Virus specific IgG ELISA values**	Neutralization titer (FRNT-mNG <sub>50</sub> )***
				IgG	IgM	IgA		
1	23	M	84	2220	565	220	26	39
2	22	F	84	354	283	<100	3	26
3	68	M	40	464	<100	<100	19	<20
4	35	M	51	4547	393	545	6	113
5	50	M	37	1354	301	275	7	81
6	29	M	34	<100	866	<100	<1.5	<20
7	27	M	34	422	104	450	<1.5	<20
8	25	M	34	222	1031	<100	26	<20
9	21	M	40	650	588	153	9	25
10	39	M	38	612	539	5686	12	23
11	46	M	38	2011	325	224	24	55
12	31	M	38	494	828	183	10	<20
13	20	M	41	944	274	<100	14	49
14	36	M	41	228	279	1614	<1.5	<20
15	34	M	44	282	302	<100	4	<20
16	70	M	44	1250	220	518	14	43
17	40	M	45	464	112	101	16	<20
18	32	M	41	867	381	399	<1.5	<20
19	57	M	45	1069	354	231	<1.5	<20
20	27	F	49	1935	528	<100	23	80
21	36	M	49	3156	355	593	28	166
22	24	M	45	<100	387	<100	<1.5	<20
23	55	F	45	<100	778	<100	<1.5	<20
24	15	M	45	212	496	<100	<1.5	<20
25	49	M	45	4183	2958	397	17	657
26	26	M	48	2352	<100	<100	16	48
27	54	F	54	1202	<100	182	15	49
28	53	M	52	799	197	417	12	<20
29	52	M	48	2611	249	157	23	46
30	45	M	62	1490	401	<100	15	50
31	52	M	56	10127	421	437	21	434
32	26	M	47	<100	<100	<100	<1.5	<20
33	32	M	57	701	177	<100	14	<20
34	44	M	49	815	428	<100	20	<20
35	32	M	40	829	140	<100	6	29
36	44	M	42	4685	494	295	26	167
37	22	M	77	3954	764	690	24	209
38	49	M	25	24484	2828	459	22	682
39	55	M	51	371	753	<100	17	<20
40	36	M	51	621	350	104	17	<20
41	60	M	51	156	459	<100	17	34
42	62	M	47	467	354	<100	6	<20

\*ELISA end point titre limit of detection is 100.

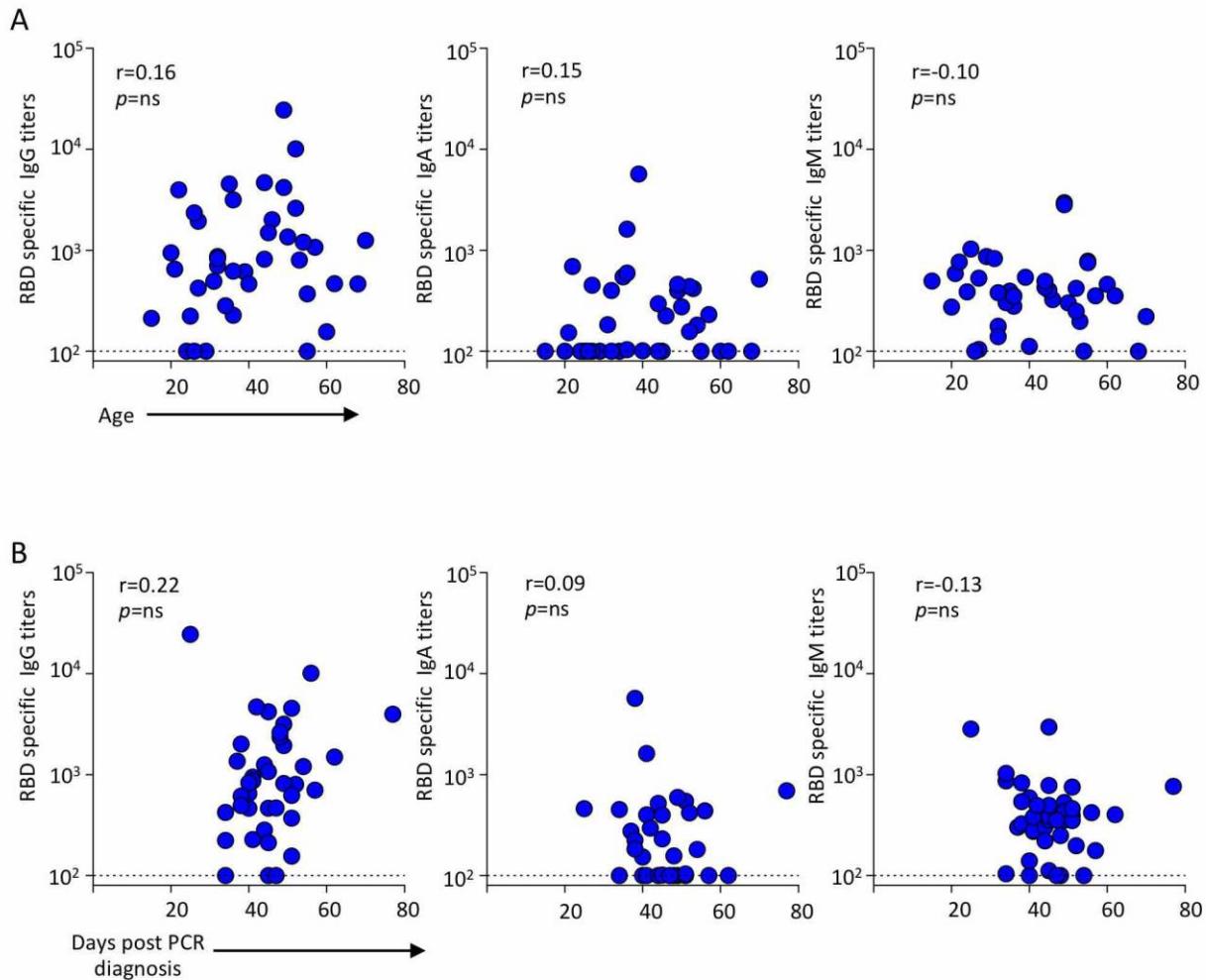
\*\*ELISA was performed with a commercial kit (Covid Kavach, Zydus) using 1:100 dilution of plasma as per by the manufacturer's recommendation. Assay cut off is 1.5.

\*\*\*Neutralization titres: Neutralization assay were performed using 3 fold dilution of plasma, starting at 1:20 up to 1:43740. Limit of detection for FRNT-mNG<sub>50</sub> is 20.



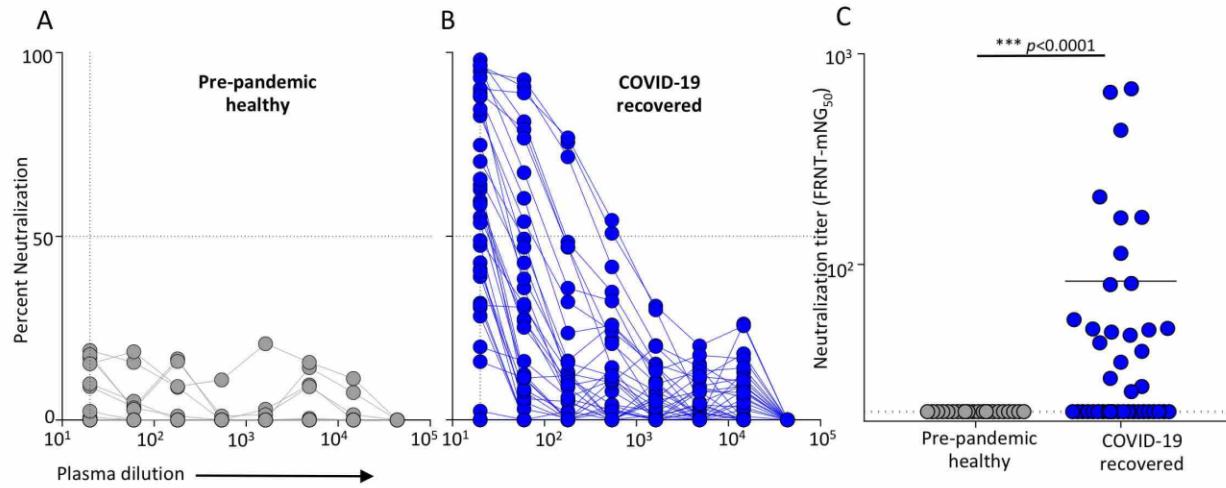
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**Figure 1**



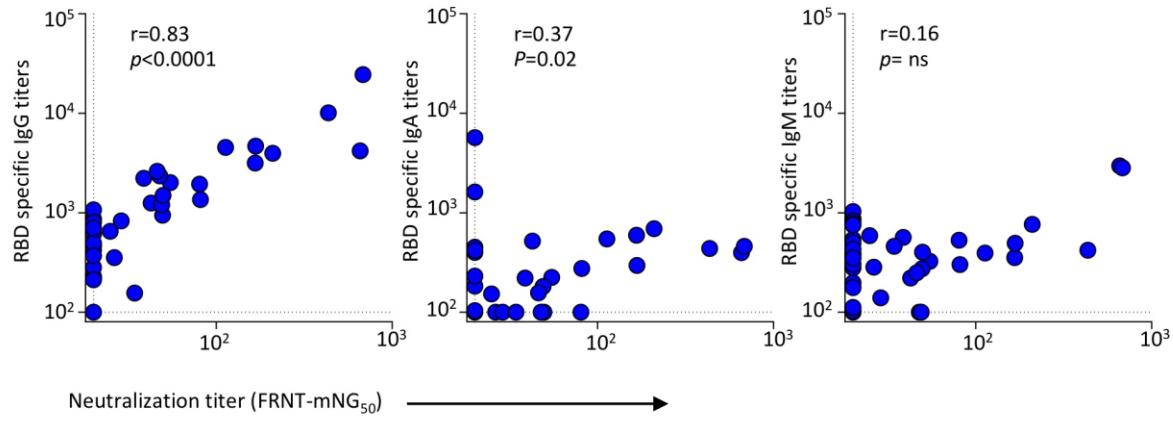
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**Figure 2**



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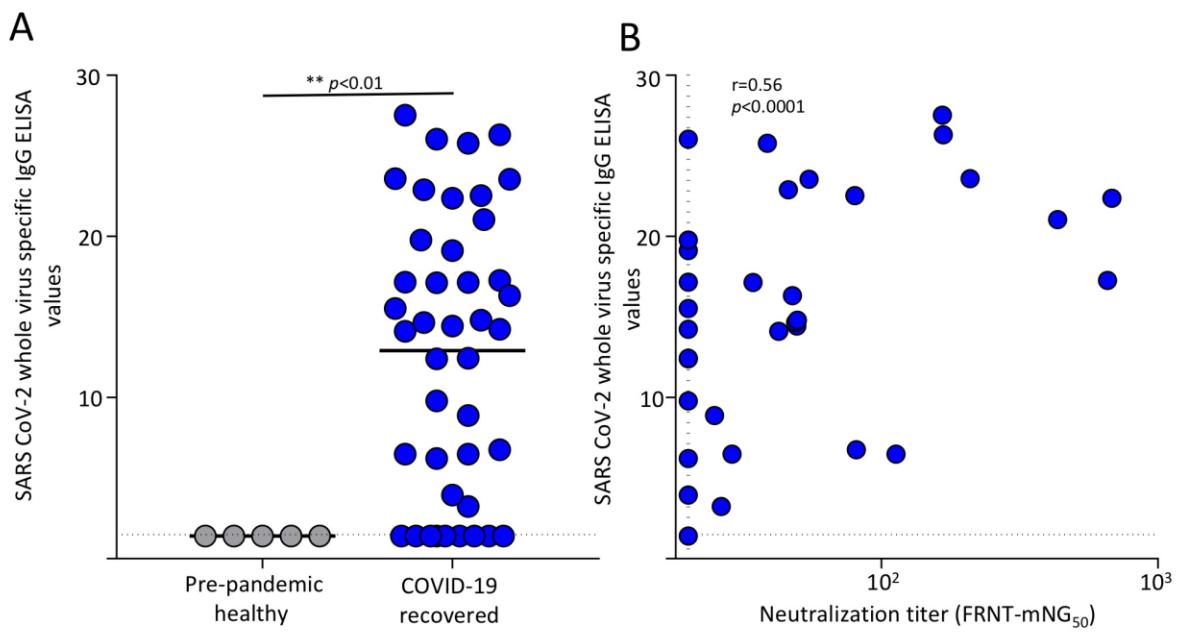
**Figure 3**



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**Figure 4**

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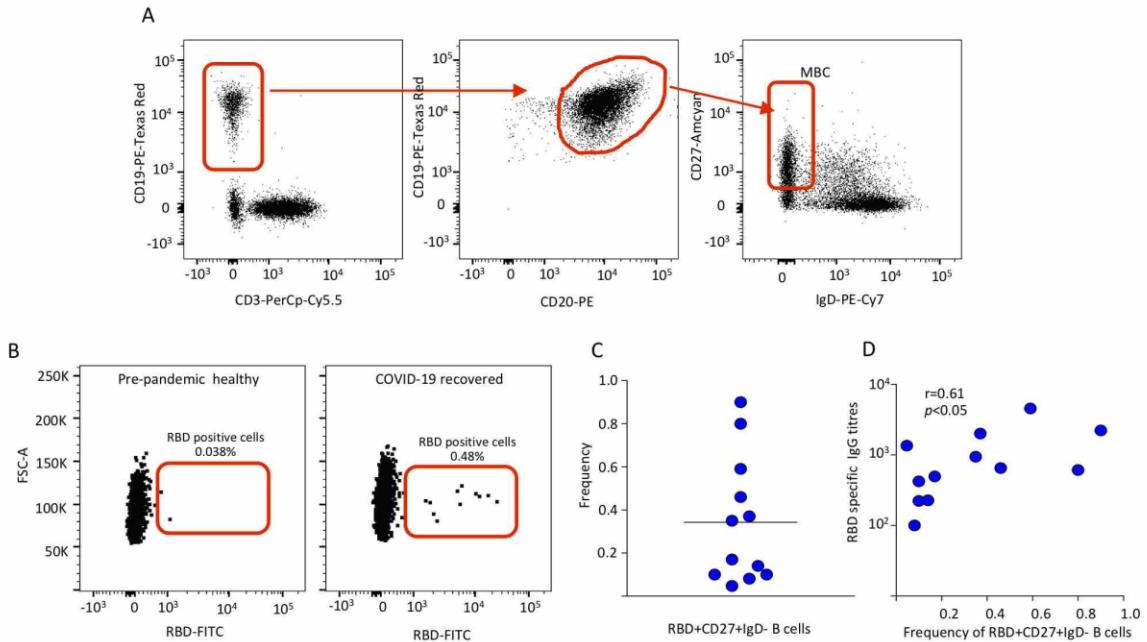
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**Figure 5**



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**Figure 6**