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1   **Characterization of neutralizing versus binding antibodies and memory B cells in COVID-19**

2   **recovered individuals from India**

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1

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**

72

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

4

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°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°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 2.5×10^4 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)

8

179 (counted first under the "green light" setting followed by background subtraction under the

180 "red light" setting). FRNT-mNG$_{50}$ 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 ≤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

10

213   healthy individuals **(Figure 3A)** and COVID-19 recovered individuals is shown in (**Figure**

214   **3B). Figure 3C** shows FRNT-mNG$_{50}$ 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$_{50}$ 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$_{50}$ 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

13

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

291   memory B cells past recovery will be protected if they were re-exposed to SARS-CoV-2 or a

292   related virus.

302

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,

305 R.K, S.L. C.D, J.W, M.S.S, and D.S. Clinical site coordination by D.S, P.K.G, S.A, A.S and M.R.

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

Figure legends

310

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$_{50}$) 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$_{50}$ 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

17

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.

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**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$_{50}$)***
				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.
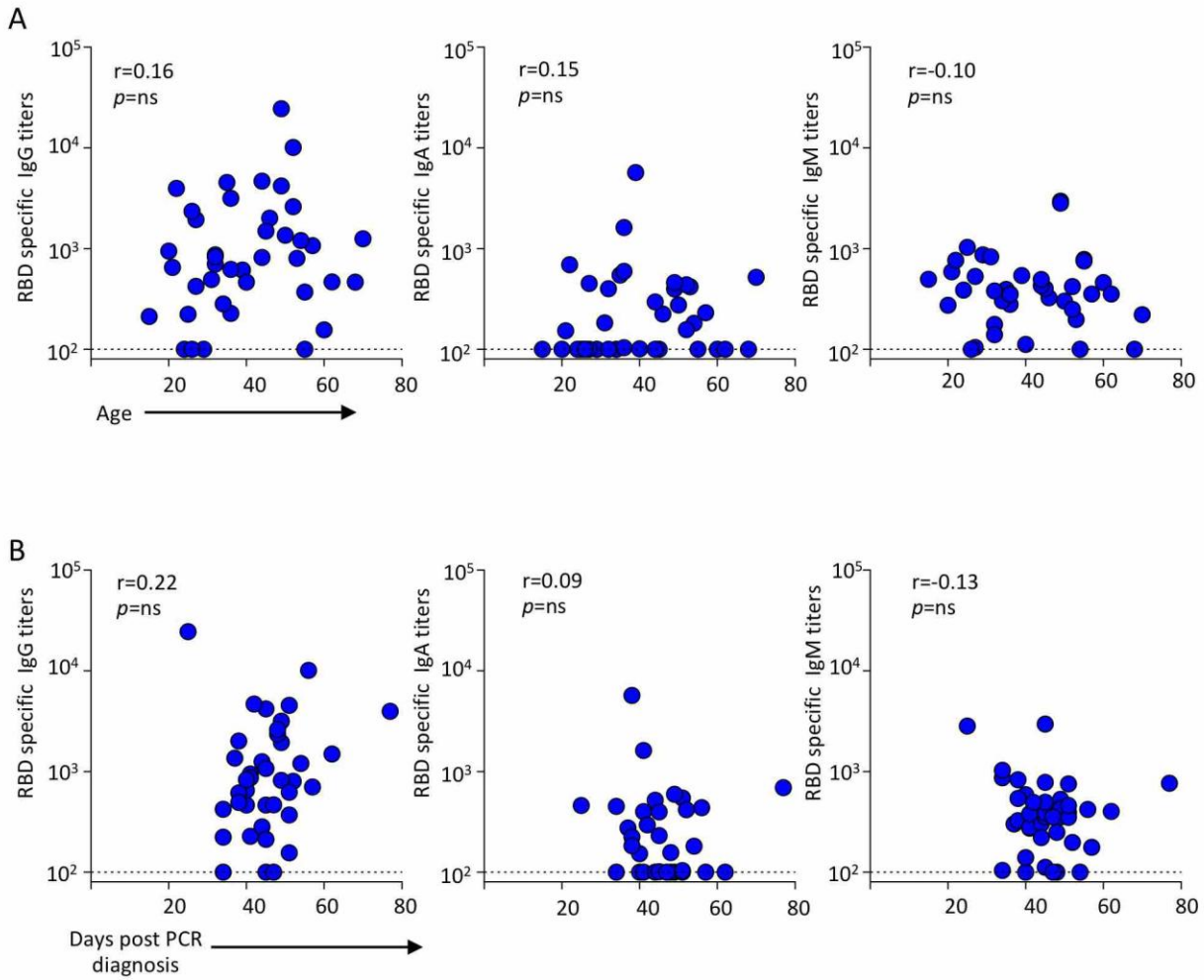
***Neutralization titres: Neutralization assay were performed using 3 fold dilution of plasma, starting at 1:20 up to 1:43740. Limit of detection for FRNT-mNG$_{50}$ is 20.
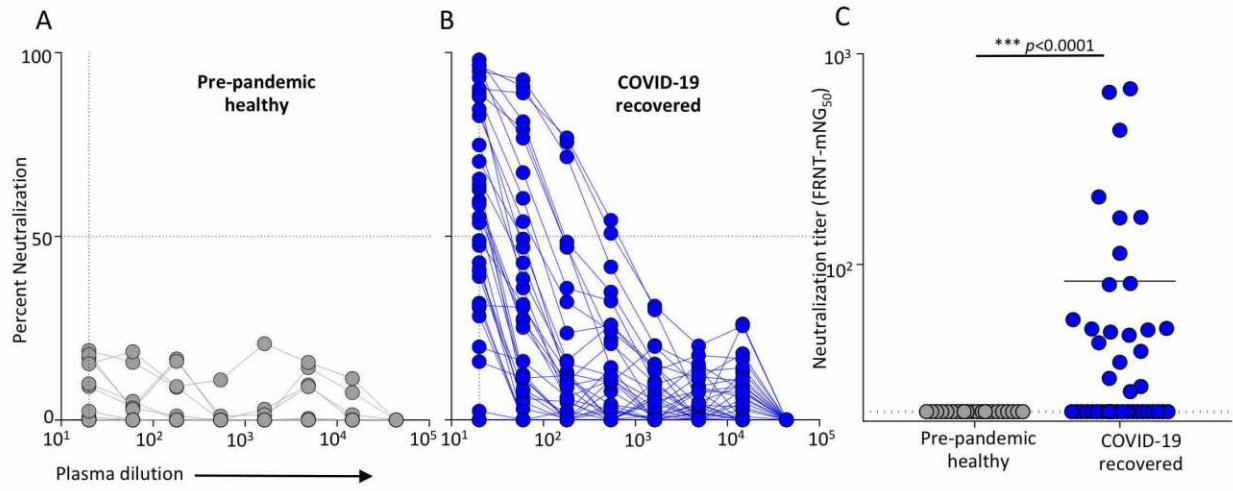
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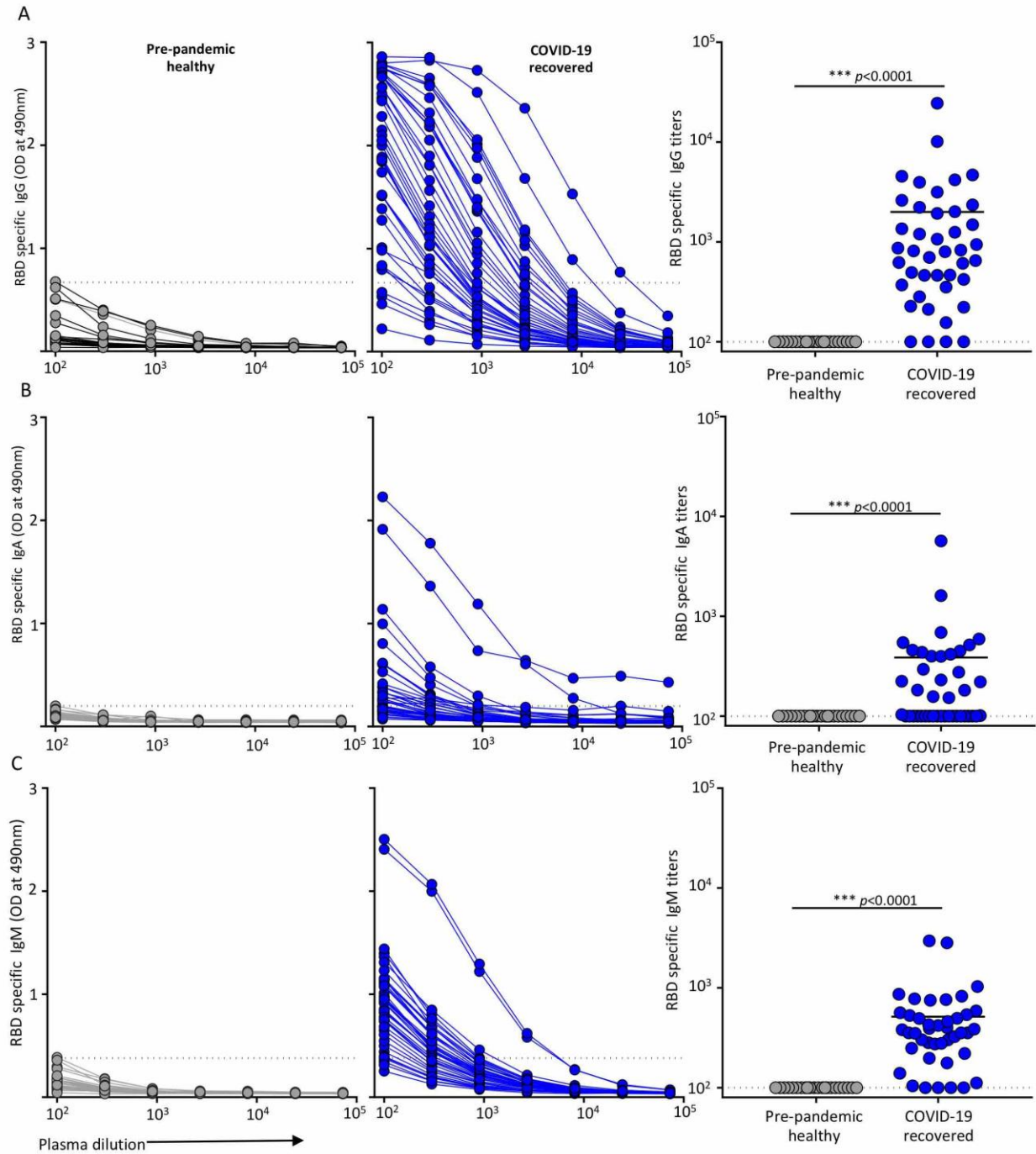
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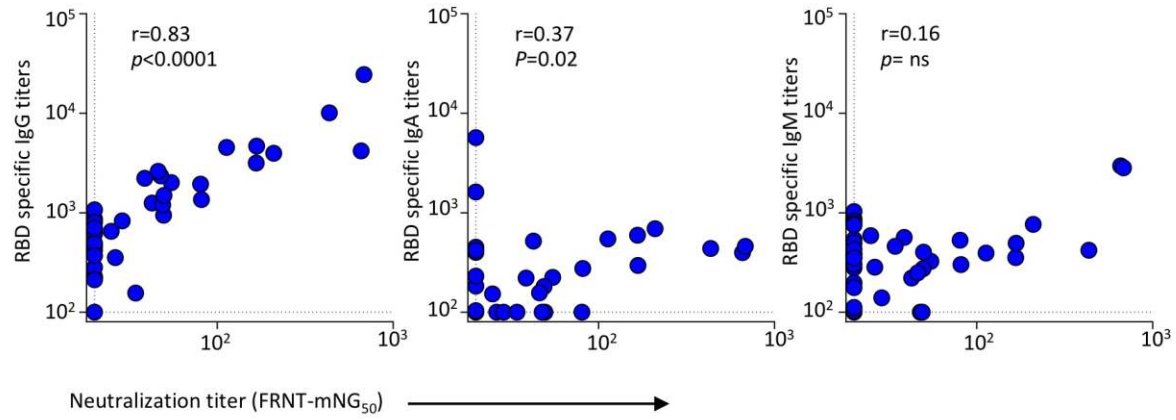570   **Figure 1**
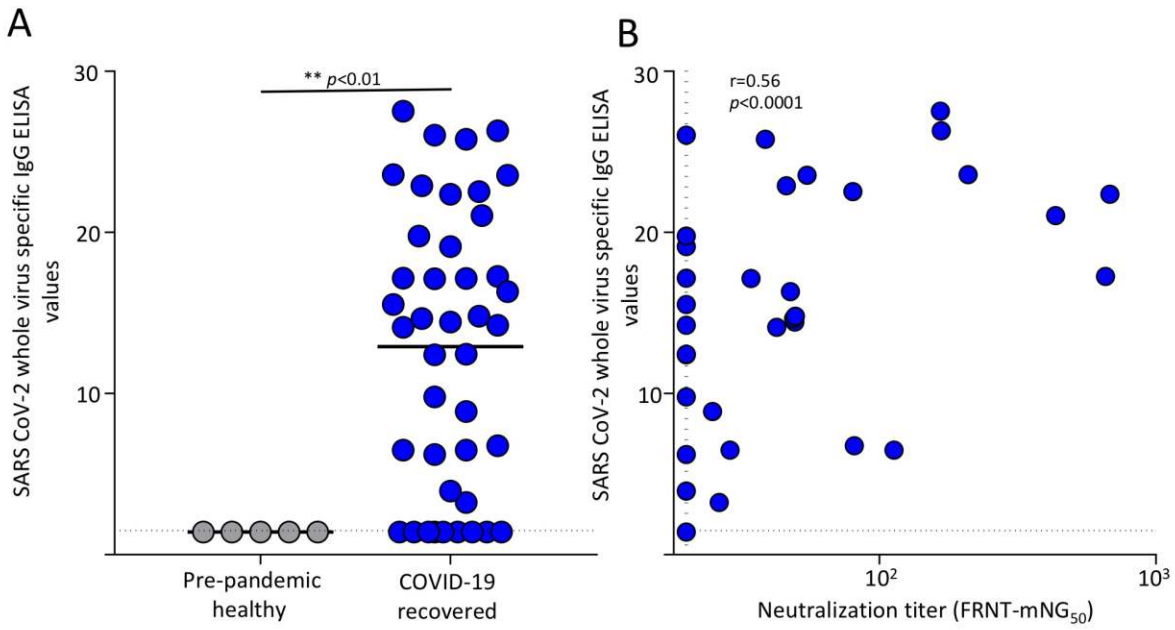571

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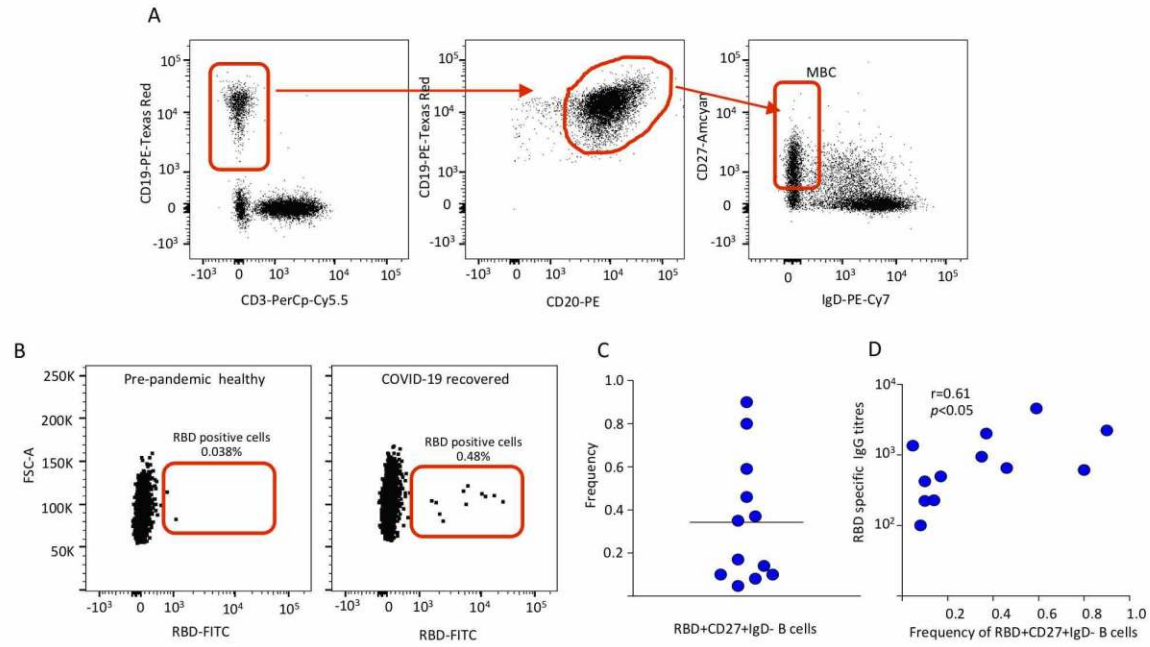**Figure 3**

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