Twelve-month specific IgG response to SARS-CoV-2 receptor-binding domain among COVID-19 convalescent plasma donors in Wuhan

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Abstract

To investigate the duration of humoral immune response in convalescent coronavirus disease 2019 (COVID-19) patients, we conducted a 12-month longitudinal study through collecting a total of 1,782 plasma samples from 869 convalescent plasma donors in Wuhan, China and tested specific antibody response. The results show that positive rate of IgG antibody against receptor-binding domain of spike protein (RBD-IgG) to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the COVID-19 convalescent plasma donors exceeded 70% for 12 months post diagnosis. RBD-IgG kinetics displayed a gradually downward trend, the titer started to stabilize after 9 months and decreased by 68.1% compared with the 1st month. Moreover, male plasma donors produced more RBD-IgG than female plasma donors and patient age positively correlated with the RBD-IgG titer. A strong positive correlation between RBD-IgG and neutralizing antibody titers was also identified. This study is essential for understanding SARS-CoV-2-induced immune memory to develop vaccine and therapeutics.

Introduction

Since the emergence of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, the virus has spread rapidly and globally, leading to a pandemic outbreak. As of February 19, 2021, SARS-CoV-2 has infected more than 109 million people worldwide, with the death toll exceeding 2.4 million, and approximately 364,000 newly diagnosed cases are still daily reported (https://covid19.who.int/, accessed February 19, 2021).

Several SARS-CoV-2 vaccines have been approved worldwide, but their longevity of immune protection is still uncertain. Evaluating the durability of the immune response, especially humoral immune response, induced by SARS-CoV-2 is essential to understand the pathogenesis of SARS-CoV-2 and predict the longevity of its vaccine protection, which further facilitates the urgent development of vaccine or therapeutics [1]. In the patients infected with severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), the specific antibodies against SARS-CoV-1 can last for an average of 2 years, with the positive rate and titer of SARS-CoV-1-specific neutralizing antibodies significantly reduced at the third year. Therefore, SARS patients may become susceptible to the same virus 3 years after recovered from the infection [2], highlighting the importance of evaluating the durability of the humoral immune response to SARS-CoV-2.

The antibody responses against SARS-CoV-2 in humans are induced by some viral proteins, including spike glycoprotein (S protein) and nucleocapsid protein,

among which S protein can induce neutralizing antibodies that are indispensable for viral neutralization and elimination, through blocking viral binding with host cells [1]. Similar as SARS-CoV-1, SARS-CoV-2 enters host cells via the binding with S protein to angiotensin-converting enzyme 2 (ACE2) [3], which is expressed on the surface of human alveolar epithelial cells, small intestinal epithelial cells, endothelial cells, and arterial smooth muscle cells [4]. SARS-CoV-2 S protein has an approximate size of 180 kDa, consisted of S1 and S2 subunits, the former of which contains ACE2 receptor binding domain (RBD, amino acid residues 331-524) [5]. Anti-RBD IgG (RBD-IgG) titers have been shown to be strongly and positively correlated with virus neutralization [6]. Although highly homologous amino acid sequences are shared between the RBD regions of SARS-CoV-2 and SARS-CoV-1, the plasma of convalescent SARS patients or SARS-CoV-1 RBD monoclonal antibodies could not neutralize SARS-CoV-2, indicating the limited cross-neutralization protection between these two viruses [5, 7]. Nevertheless, successful convalescent plasma therapy for COVID-19 patients has been reported: The symptoms of 10 severe COVID-19 patients who received 200 mL of convalescent plasma containing high-titer neutralizing antibody were significantly improved or even completely disappeared within 3-7 days [8].

Furthermore, it has been reported that most COVID-19 patients could produce virus-specific IgM, IgA, and IgG antibodies within a few days after infection [1]. According to a longitudinal study, though both IgM and IgA antibodies are produced

early within one week after symptom onset, IgM reaches the peak at the 10th-12th days but the level subsequently decreases after 18 days, while IgA response persists at a higher level for a longer time period, reaching the peak at the 20th-22nd days [9]. On the contrary, the level of IgG antibody keeps increasing for 3 weeks after symptom onset, declines after 8 weeks, while remains detectable over 8 months [1, 10, 11]. However, the antibody response and neutralizing activity in COVID-19 convalescent patients up to 12 months are still unclear. For preventing and controlling SARS-CoV-2, as well as the vaccine development, the duration of functional neutralizing antibody response after individual infection with SARS-CoV-2 and the protective immunity for reinfection are necessary to be investigated by a long-term study.

Therefore, we aimed to investigate the RBD-IgG response of convalescent COVID-19 patients for up to 12 months. In this study, a total of 1,782 convalescent plasma samples from 869 COVID-19 convalescent plasma donors were tested for the presence and titers of RBD-IgG, which was proved to be positively associated with neutralizing antibody titers. In addition, influences of other factors (gender, age, and blood type) on the kinetics of RBD-IgG responses were analyzed. Our finding is critical for assessing the durability of the protective immunity induced by COVID-19 vaccines and predicting the future trend of COVID-19 pandemic.

Results

Sustained RBD-IgG responses to SARS-CoV-2 RBD. In order to study the SARS-CoV-2 RBD-IgG responses in convalescent COVID-19 patients, 1,782 plasma samples obtained from 869 COVID-19 convalescent plasma donors in Wuhan were analyzed. All the samples were collected within 12 months after diagnosis. The presence and titers of RBD-IgG in the plasma samples were tested by using a CE-marked SARS-CoV-2 IgG enzyme-linked immunosorbent assay (ELISA) kit [12]. Figure 1A shows the positive rate of RBD-IgG in the convalescent plasma donors at different time points within 12 months after diagnosis. Specifically, a total of 390 plasma samples from plasma donors at the 1st and 2nd months after diagnosis (defined as the early stage following diagnosis) were tested, and the positive rate of SARS-CoV-2 RBD-IgG was 94.6% (the RBD-IgG titer was 1:80 or higher). At the 6th and 7th months after diagnosis (defined as the middle stage following diagnosis) and the 11th and 12th months after diagnosis (defined as the late period following diagnosis), the positive rates of RBD-IgG were 89.4% and 81.4%, respectively. The titers of RBD-IgG were categorized as <1:80, 1:80 (the actual value range is greater than or equal to 80 but less than 160), 1:160 (the actual value range is greater than or equal to 160 but less than 320), 1:320 (the actual value range is greater than or equal to 320 but less than 640), 1:640 (the actual value range is greater than or equal to 640 but less than 1280), 1:1280 (the actual value range is greater than or equal to 1280 but less than 2560), and ≥1:2560. Titers less than 80 were considered as negative, 1:80-1:160 as

low titers, 1:320-1:640 as moderate titers, and 1:1280 and ≥ 1:2560 as high titers [13]. As shown in Figure 1B, the proportion of higher titers displayed a time-dependent downward trend, while the proportion of negative titers showed the opposite. Specifically, the proportions of plasma samples with moderate and high titers of RBD-IgG were 72.6%, 41.3%, and 27.2% at the early, middle, and late stages following diagnosis, respectively (Supplemental Table 1). It should be noted that even at the early stage following diagnosis, the titers of RBD-IgG in 5.4% of the convalescent plasma donors were at an undetectable level (<1:80) (Supplemental Table 1), which might be attributed to the failure of antibody production during infection or the low sensitivity of the method for antibody detection. As expected, at the late stage following diagnosis, the proportion of negative titers increased to 18.6% (Supplemental Table 1).

Longitudinal analysis of RBD-IgG responses. We further determined the kinetics of RBD-IgG in COVID-19 plasma donors within 12 months after diagnosis. Monthly RBD-IgG titers were calculated and shown as geometric mean titers (GMT). As displayed in Figure 2A, the RBD-IgG titers in convalescent plasma donors decreased within 12 months. After 9 months, the RBD-IgG titers began to stabilize at a GMT of approximately 200. The RBD-IgG titer at the 12th month following diagnosis was 69.86% lower (a GMT of 189) than that at the 1st month following diagnosis (Figure 2A). As aforementioned, the antibody response dramatically augmented at the initial

stage after SARS-CoV-2 infection, followed by gradual declining [1, 10, 11]. Since the plasma samples were collected after the discharge of the plasma donors from hospital (no less than 3 weeks after symptom onset), the period of antibody generation and expansion during the initial stage of onset might not be included.

In addition, we further analyzed the kinetics of RBD-IgG in 14 COVID-19 convalescent plasma donors who repeatedly donated plasma for 3 or more than 3 times. Consistent with the general kinetics of RBD-IgG for COVID-19 convalescent plasma donors, the titers of individual plasma clearly displayed a downward trend within approximately 300 days (Figure 2B).

In order to evaluate the stability of RBD-IgG titers in COVID-19 convalescent plasma donors after a long period of time, we re-collected the plasma from 237 donors, who presented different titer levels of RBD-IgG at the early stage following diagnosis, during the 10th and 11th months after diagnosis. It shows that the plasma donors with higher initial titers remained faster RBD-IgG attenuation (Figure 3A). Interestingly, after a long period of time, the RBD-IgG titers of the plasma donors with higher initial titers were still higher than those with lower initial titers. From the 1st-2nd month to the 10th-11th months, the RBD-IgG titer decreased by 70.0% to an average GMT of 183 in the 237 plasma donors (Figure 3A). Additionally, we analyzed the changes of RBD-IgG titers in plasma donors stratified by the initial titers from the early stage to the 10th and 11th months. As shown in Figure 3B and Supplemental Table 2, 51.67% of the low titer population turned into negative after a

long period of time, while 60.75% of the moderate titer population and 54.29% of the high titer population decreased by one titer grade. Nevertheless, 11.67% of the low titer population and 1.87% of the moderate titer population increased by one titer level after a long period of time (Figure 3B; Supplemental Table 2).

Influences of other factors on RBD-IgG responses. In addition, we also examined the influences of gender, age, and blood types of the plasma donors on their RBD-IgG responses. The early, middle, and late stages following diagnosis were analyzed for the generation and durability of RBD-IgG (Figure 4). The average titer of RBD-IgG in the plasma samples from male plasma donors was significantly higher than that from females within 12 months, especially at the early stage following diagnosis ($p<0.0001$); however, the significant difference of RBD-IgG titers between male and female plasma donors was compromised at the middle and late stages following diagnosis. Furthermore, we found that the RBD-IgG titers were consistently and positively correlated with the age of the plasma donors, displaying an elevated RBD-IgG titer along with an increased patient age (Figure 4B). The abovementioned correlations between RBD-IgG titer and patient gender/age were consistent with the findings from several previous studies [1, 14, 15].

Zhao et al. reported that during the outbreak of the epidemic in Wuhan, 1,775 COVID-19 patients exhibited a distribution of 37.75%, 26.42%, 25.80%, and 10.03% for A, B, O and AB blood types, respectively; the distribution in 3,694 normal people

in Wuhan was 32.16%, 24.90%, 33.84%, and 9.10% for A, B, O and AB blood types, respectively [16]. These results indicated that individuals with A-blood type were associated with a higher risk of infection, while those with O-blood type were associated with a lower risk of infection and COVID-19 severity [16]. However, in our study, negligible difference in RBD-IgG titers was found among the COVID-19 plasma donors of different blood types (Figure 4C).

Association between neutralizing antibody titers and RBD-IgG titers. In order to confirm the correlation between RBD-IgG titers and the neutralizing activity of convalescent plasma samples, we conducted plaque reduction neutralization test (PRNT) on Vero cells to define neutralizing antibody titers using an isolated viral strain [17]. The associations between the titers of PRNT (ID50) and RBD-IgG (dilution quantitative) based on a total of 150 COVID-19 convalescent plasma samples collected at the 1st-3rd months after diagnosis were analyzed. As shown in Figure 5, the PRNT titers were positively correlated with the RBD-IgG titers ($r=0.703$, $p<0.0001$).

Discussion

The overall immunity to SARS-CoV-2, including the durability of immunity against the virus and vaccine-induced protective immunity, has not been fully understood. Neutralizing antibody response is crucial for the elimination of cytopathic viruses as well as preventing the reinfection of the virus [18]. The eliminating ability of neutralizing antibodies against SARS-CoV-2 in humans has been confirmed by clinical studies [8], whereas their protective effectiveness against the viral reinfection has only been proved based on animal models [19].

Effective vaccination is well known as the safest path towards herd immunity [1, 20]. Most COVID-19 vaccine candidates employ same antigen(s) of original SARS-CoV-2, implying that the type and kinetics of neutralizing antibody response induced by vaccination are similar to those induced by the original live virus [21]. On the other hand, it has been reported that IgA and IgM antibodies are produced and reduced at the early stage of infection [9], raising concerns on the sustainable neutralizing activity of the patients' plasma. As a consequence, the long-term study of neutralizing antibody response in convalescent patients is essential to offer powerful support on developing COVID-19 vaccines and therapeutics.

At present, the longest observation period for SARS-CoV-2 antibody reaction kinetics is only several months [13, 22, 23, 24, 25], whereas no study has been carried out for more than 1 year time period. To investigate the persistence of protective immunity against SARS-CoV-2, we completed a year-long kinetic analysis on SARS-CoV-2

RBD-IgG response in 1,782 convalescent plasma samples obtained from 869 COVID-19 plasma donors, and assessed the constant influences of patient gender, age, and blood types on RBD-IgG response kinetics. Based on the fact of no further coronavirus disease outbreaks in Wuhan until the end point of plasma collection in this study, the 12-month immune response against SARS-CoV-2 in the COVID-19 plasma donors included in the present study is considered as primary immune response.

According to our findings, the RBD-IgG response in more than 70% COVID-19 convalescent plasma donors could persist for at least 12 months, indicating that vaccination can effectively restrict the spread of SARS-CoV-2. We will also examine the changes of RBD-IgG titers over a longer period of time. Compared with the titer obtained at the 1st month, the RBD-IgG titer decreased by 69.86% at the 12th month. Moreover, the proportion of the plasma donors whose RBD-IgG titers remained at or above the moderate titer at the late stage following diagnosis was 27.2% (Supplemental Table 1).

Furthermore, we found that although the RBD-IgG titer gradually decreased over time within 12 months, the RBD-IgG titer was stabilized at a GMT of approximately 200 after 9 months following diagnosis. Considering that the half-life of IgG is around 21 days [26], the sustained persistence of RBD-IgG titer over time is probably produced by long-lived bone marrow plasma cells (BMPCs), which serve as the main source of protective antibodies [27]. In a 7-month study with 73 mild COVID-19 patients

243 participation, S protein-specific long-lived BMPCs were detected in the COVID-19
244 convalescent patients at the 7th months following diagnosis [27].

245 We also evaluated the stability of RBD-IgG with different titers (stratification
246 was based on the titer value at the early stage following diagnosis) after a long time
247 period (the 10th and 11th months). Although more rapid attenuation of RBD-IgG was
248 observed in the plasma donors with high titers, after a long time period, the RBD-IgG
249 in plasma donors with higher initial titers remained to be higher than those with lower
250 initial titers. Interestingly, we found that the RBD-IgG titers significantly increased in
251 11.67% of the low-titer population and 1.87% of the moderate-titer population during
252 the 10th-11th months, which might be attributed to delayed seroconversion in a small
253 number of plasma donors. Similar phenomena have also been reported by Icahn
254 School of Medicine at Mount Sinai [13, 28].

255 Understanding the influences of factors in patient population, including gender,
256 age, and blood type, on RBD-IgG response is critical to prevent SARS-CoV-2
257 infection, which can provide potential explanations for clinical symptoms. Therefore,
258 we further correlated such factors of the COVID-19 plasma donors with their
259 RBD-IgG titers, and found that the titer of RBD-IgG in male plasma donors was
260 significantly higher than that in female plasma donors at the early stage following
261 diagnosis. Consistent with previous reports, the positive correlation between age and
262 RBD-IgG titer in a population of plasma donors aged 18 to 55 years indicates that
263 elder plasma donors might develop antibody response against SARS-CoV-2 more

effectively than younger ones [14]. Nevertheless, no significant correlation was identified between the blood type of the patients and their RBD-IgG titers, indicating that although individuals with A blood type are associated with higher risk for SARS-CoV-2 infection, the susceptibility to SARS-CoV-2 is not related to RBD-IgG titers.

Last but not least, we performed PRNT on Vero cells to test the neutralizing antibody titers from 150 COVID-19 convalescent plasma samples, followed by analyzing the correlation between RBD-IgG and neutralizing antibody titers. Consistent with previous report [6], our data showed a strong positive correlation between these two types of titers (Figure 5).

In conclusion, this 12-month longitudinal study demonstrates that despite of the downward trend of RBD-IgG response kinetics in COVID-19 convalescent plasma donors, over 70% plasma donors persist to produce RBD-IgG at detectable levels for longer than 1 year post diagnosis, which stably remain at a GMT of approximately 200. In addition, the RBD-IgG titers of male plasma donors are higher than those of female plasma donors at the initial stage of infection, meanwhile, age is positively correlated with the RBD-IgG titers. Furthermore, we confirmed the positive association between RBD-IgG and neutralizing antibody titers. Overall, this study provides long-term strong support for the duration of protection by neutralizing antibodies in COVID-19 plasma donors, indicates the potential to prevent

284 SARS-CoV-2 reinfection, and illustrates the role of neutralizing antibodies in clinical

285 research and development evaluation of vaccines.

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Methods

Donors for convalescent plasma transfusion. From February 1, 2020 to January 10, 2021, 869 COVID-19 convalescent plasma donors in Wuhan, China were recruited, and 1,782 convalescent plasma samples were collected. Donors all met the criteria for release of isolation and discharge from hospital according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 4 and subsequent versions)", released by the National Health Commission & State Administration of Traditional Chinese Medicine. The donors were between 18 and 55 years old, with no history of blood-borne diseases, and screened by clinicians following blood donation standards. The interval between two plasma collections was no less than 14 days.

As shown in Figure 6A, the average age of the donors was 38.21 years old (95% CI: 37.67-38.75). The proportion of plasma Rh-positive blood types (a total of 834 donors with blood type recorded) of A, B, O, and AB was 34.89%, 27.46%, 29.38%, and 8.27%, respectively (Figure 6B).

Plasma preparation and quality control. Plasma preparation was conducted in Wuhan according to the "Clinical Treatment Scheme of Plasma in Recovery Period of Recovered Patients with New Crown Pneumonia (Trial Version 2)", released by the National Health Commission & State Administration of Traditional Chinese Medicine.

Plasma collection was carried out at least 3 weeks after the first symptom onset of patients.

Detection of SARS-CoV-2 RBD-IgG. CE-marked coronavirus IgG antibody detection kit (batch number: NCOG20200808B) from Beijing WanTai Biological Pharmacy Enterprise Co., Ltd. (Beijing, China) was used to test the titer of RBD-IgG. COVID-19 convalescent plasma (2020021702) was used as the reference standard and 2-fold serially diluted with WanTai kit sample dilution buffer at 6 concentrations (1:10, 1:20, 1:40, 1:80, 1:160, and 1:320). The acceptable criteria for linearity of the standard curve included: the R^2 value > 0.99; the recovery rate of each standard dilution was within 20% of the target value. The properly diluted samples were added into RBD antigen-coated plates, and subsequent operations were carried out according to the instructions of the kit. The absorbance was read at 450 nm using SpectroMAX plus384 (Molecular Devices, Silicon Valley, CA, United States), with the standard curve drawn by SoftMax5.2 software.

Plaque reduction neutralization test (PRNT). Neutralizing titers were defined on Vero cells by PRNT. PRNT was performed in accordance with the method established previously [17]. Briefly, each individual serum samples were serially diluted and 1-h incubated with SARS-CoV-2 at 37°C. The serum-virus mixture was added into pre-cultured Vero E6 cell monolayers for 1-h incubation at 37°C under 5% CO_2.

Then, the cell monolayer was incubated for 3 days. Antibody titer was defined as the highest serum dilution that induced ⩾50% (PRNT50) reduction in viral plaque numbers.

Statistical analyses. GraphPad Prism 9.0 was used for statistical analysis. As for GMT calculation, the value of RBD-IgG titer in negative reaction (<1:80) was set to 1. The influences of gender and blood type on RBD-IgG titer were analyzed by nonparametric t-test for inter-group difference and by Mann-Whitney test for difference between groups. Unpaired t-test with Welch's correction was adopted for further analysis when significant difference was found between groups. Neutralizing antibody titers were Log_4-transformed by and RBD-IgG titers were Log_2-transformed. Linear regression analysis and Spearman's correlation were used to examine the association between neutralizing antibody titers and RBD-IgG titers. A P-value less than 0.05 was considered significant.

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Author contributions: XMY, HCY, DXF, YLH and DBZ conceived and designed the study. CSL, DY and HL contributed to implementation and management of the study. XXZ revised the study design and critically reviewed the manuscript. FPL, HHG, MM, YG, RZ and HRN collected the donor samples under the supervision of XW, DMD, YX and TD. ZJZ and LZL performed ELISA experiments under the supervision of YH. XZ, YZ and KD performed ABO Blood group tests under the supervision of JHY. JL performed PRNT experiments under the supervision of ZJW and KD. LF, FFW, SLY and YP analyzed and interpreted the data. JZW and YZ drafted the manuscript. TJL generated and finalized the figures under the supervision of HL. All authors reviewed the manuscript and provided critical feedback on the manuscript.

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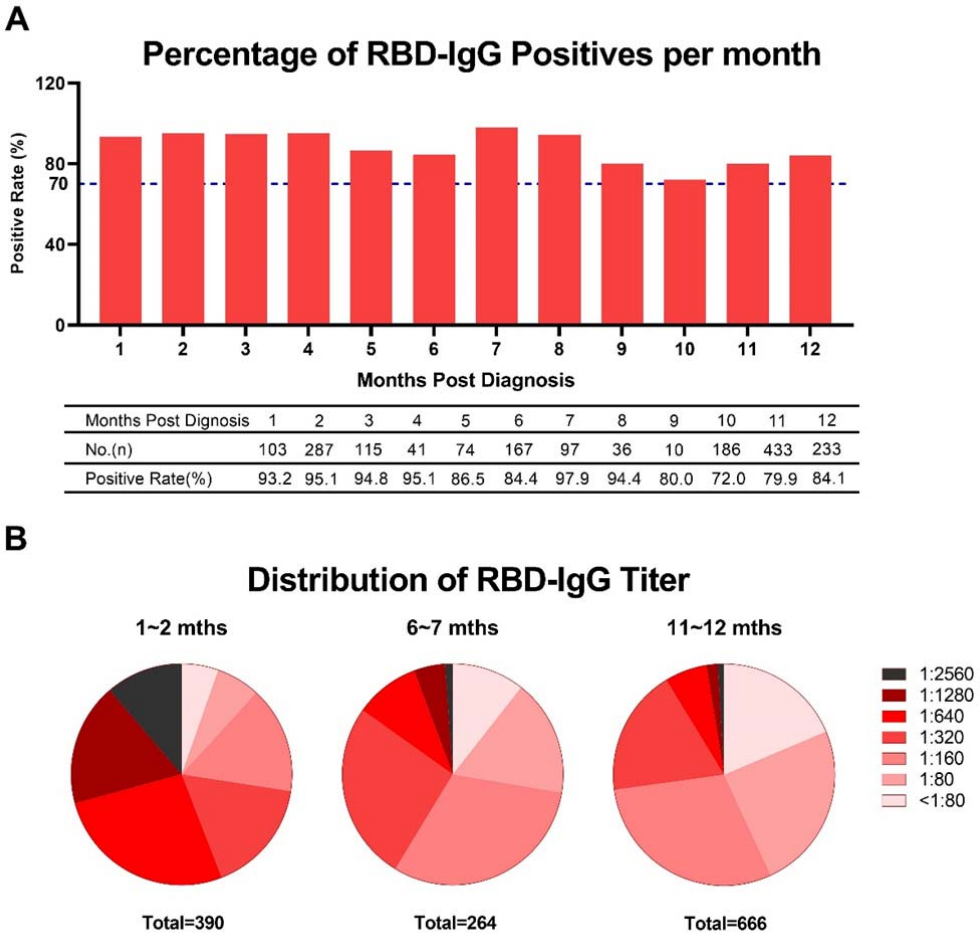
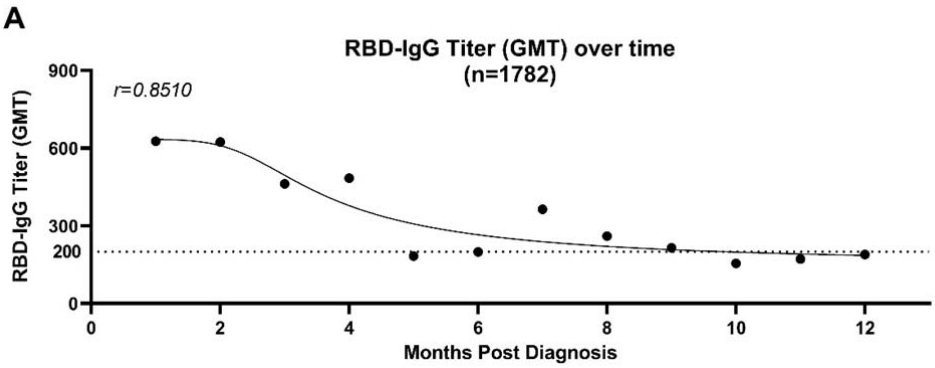
Figures and figure legends

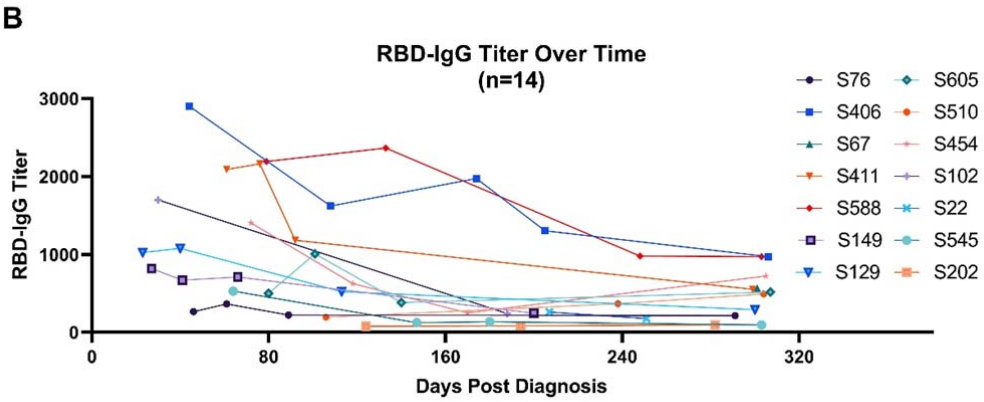


Months Post Dignosis	1	2	3	4	5	6	7	8	9	10	11	12
No.(n)	103	287	115	41	74	167	97	36	10	186	433	233
Positive Rate(%)	93.2	95.1	94.8	95.1	86.5	84.4	97.9	94.4	80.0	72.0	79.9	84.1

Fig. 1 RBD-IgG titers against SARS-CoV-2 over times. (A) Percentage changes of positive RBD-IgG. **(B)** Changes of RBD-IgG titers distribution. Titers less than 80 were considered as negative.



Months Post Dignosis	1	2	3	4	5	6	7	8	9	10	11	12
No. (n)	103	287	115	41	74	167	97	36	10	186	433	233
RBD-IgG Titer (GMT)	627	624	462	484	183	199	364	260	215	155	172	189



437

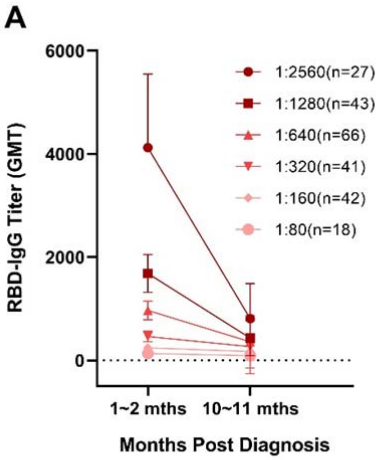
438 **Fig. 2 Kinetics of SARS-CoV-2 RBD-IgG responses. (A)** RBD-IgG kinetics curve of

439 1,782 plasma samples within 12 months. Monthly titer represents the GMT titer of all

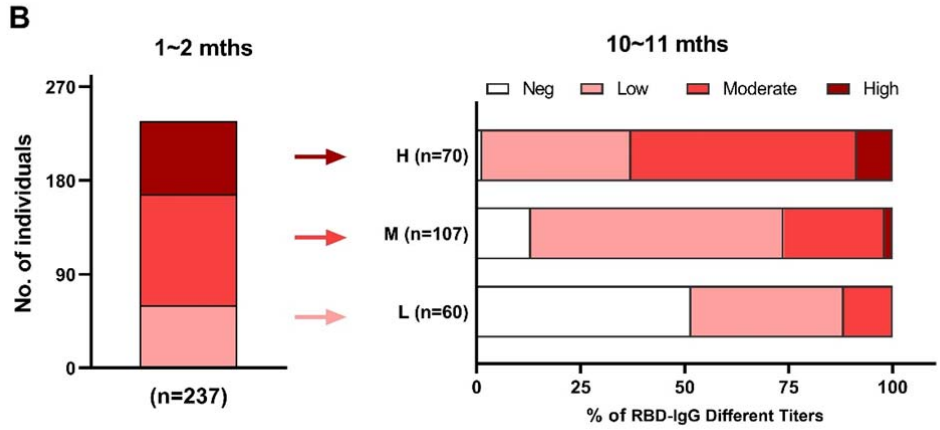
440 plasma samples collected in each individual month. **(B)** The RBD-IgG titers change in

441 14 COVID-19 convalescent plasma donors with continuous multiple oars.

442



Groups (Initial Titer)	No. (n)	1~2 mths (GMT)	10~11 mths (GMT)	Decreased (%)	Neg (%)
1:2560	27	1:3916	1:567	85.5%	3.7%
1:1280	43	1:1646	1:345	79.0%	0.0%
1:640	66	1:947	1:247	73.9%	7.6%
1:320	41	1:451	1:154	65.8%	22.0%
1:160	42	1:237	1:103	56.5%	45.2%
1:80	18	1:129	1:69	46.5%	66.7%
Total	237	1:610	1:183	70.0%	19.4%



443

444 **Fig. 3 Stability of RBD-IgG titers over time. (A)** Changes of different RBD-IgG

445 titers over time. A total of 237 COVID-19 convalescent plasma donors with different

446 titer levels of RBD-IgG at the early stage who had donated plasma again at the 10th and

447 11th months, stratified with titers at the early stage following diagnosis, and the decline

448 in titers and the proportion of plasma donors turning into negative were calculated. **(B)**

449 Change of RBD-IgG titers in plasma donors with low, moderate, or high titers after a

450 long period of time.

451

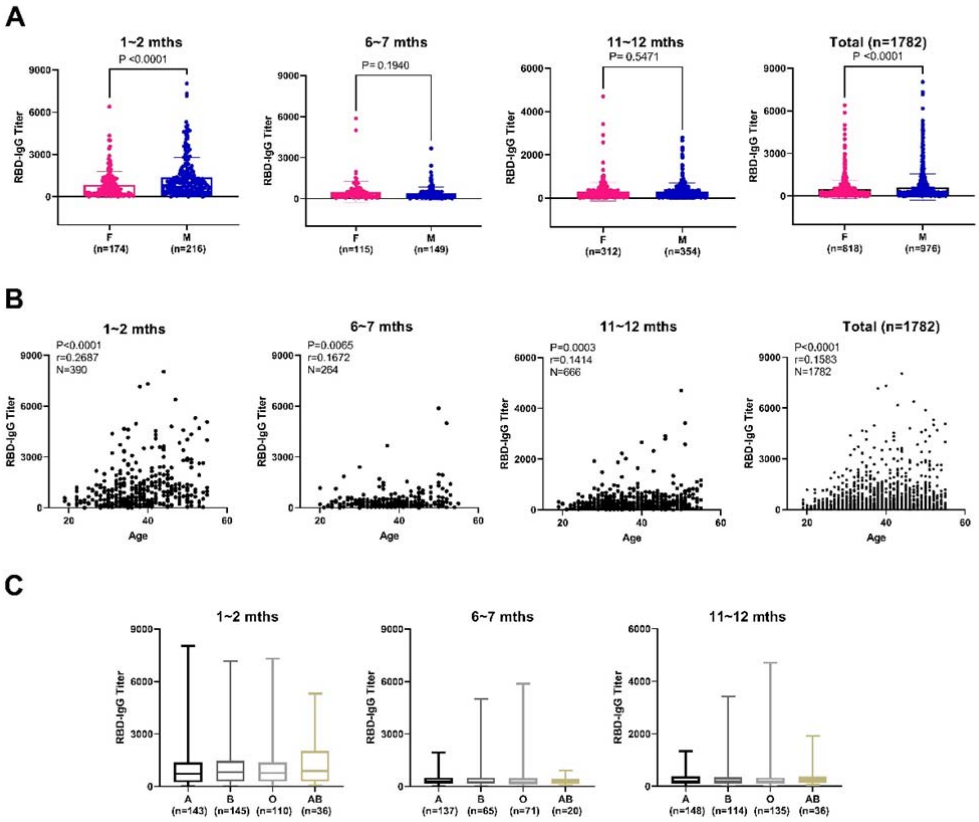
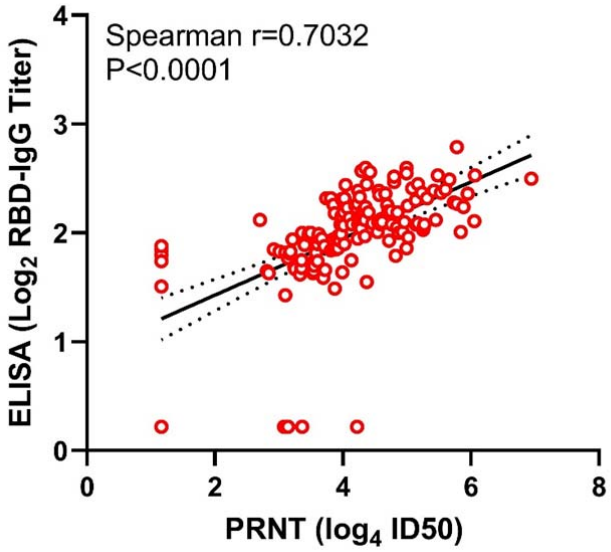


Fig. 4 Influences of gender (A), age (B), and blood types (C) on RBD-IgG response in COVID-19 plasma donors at different stages following diagnosis. Age has a positive correlation with the RBD-IgG titer, while insignificant difference was found between blood types of the plasma donors and the RBD-IgG titers.



458

459 **Fig. 5 Neutralizing activity of plasma samples positively correlated with**

460 **RBD-IgG titers.** Neutralizing antibody titers were transformed by Log_4 and RBD-IgG

461 titers were transformed by Log_2. The association between neutralizing antibody titers

462 and RBD-IgG titers was assessed by linear regression analysis and Spearman's

463 correlation.

464

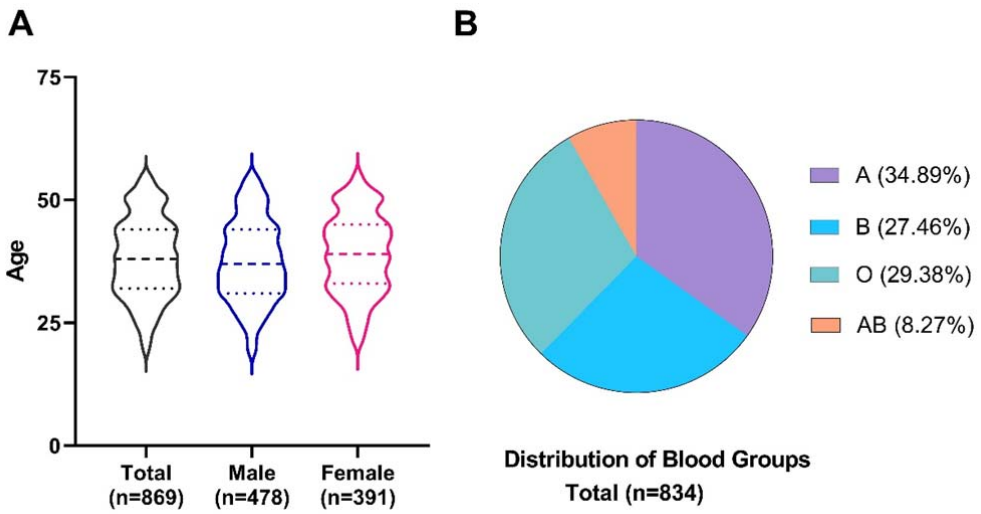
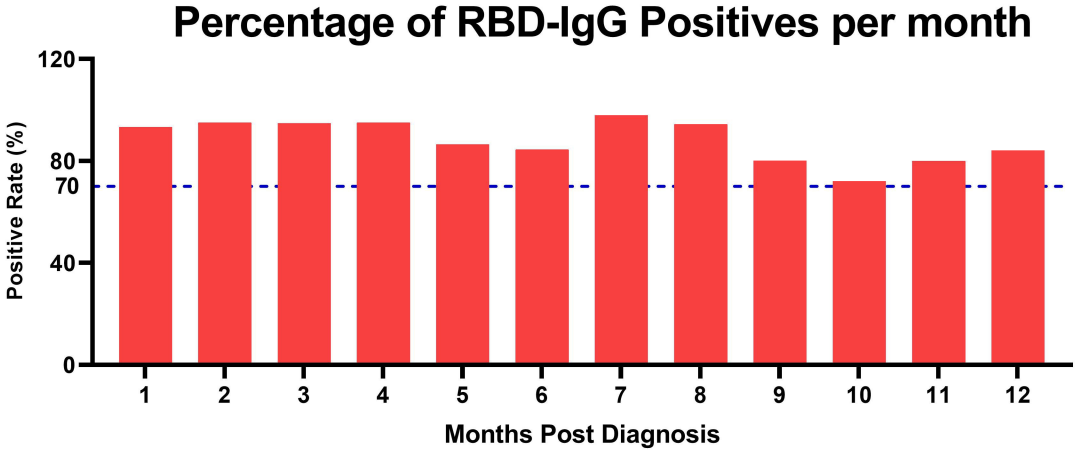


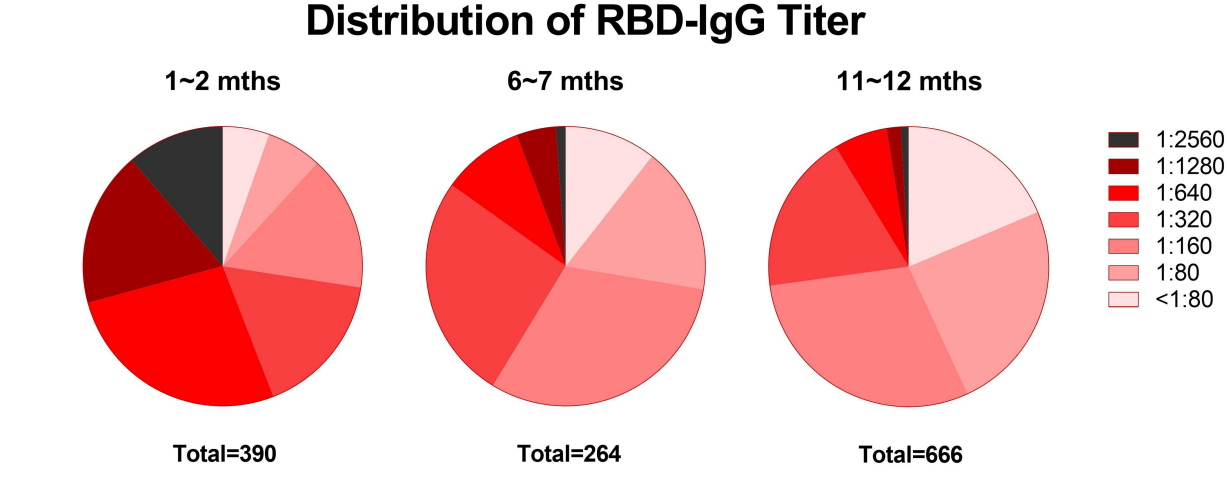
Fig. 6 Distribution of age, gender (A), and blood types (B) in COVID-19 convalescent plasma donors recruited in this study.

A

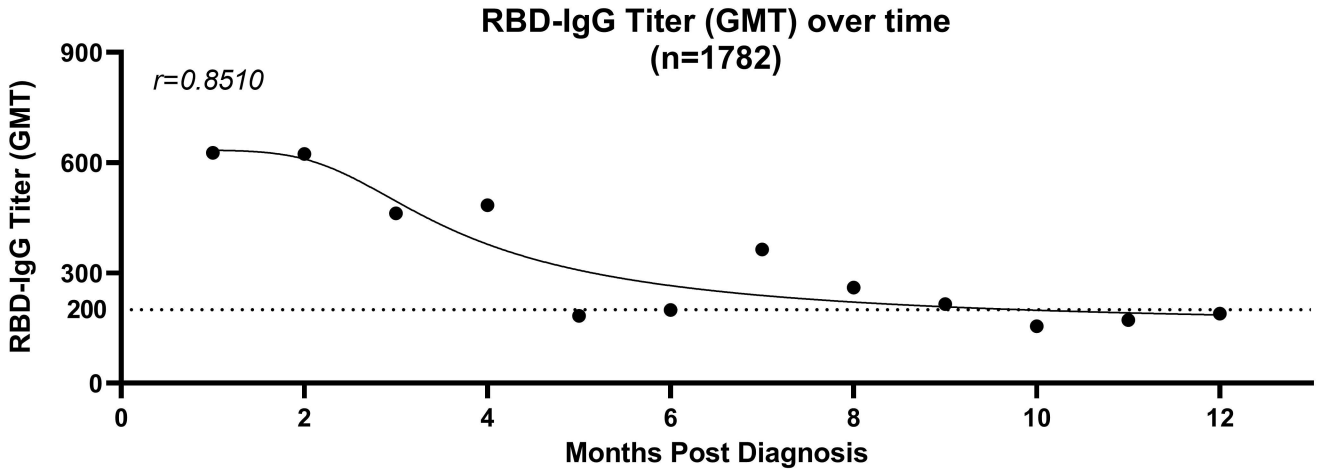


Months Post Dignosis	1	2	3	4	5	6	7	8	9	10	11	12
No.(n)	103	287	115	41	74	167	97	36	10	186	433	233
Positive Rate(%)	93.2	95.1	94.8	95.1	86.5	84.4	97.9	94.4	80.0	72.0	79.9	84.1

B



A



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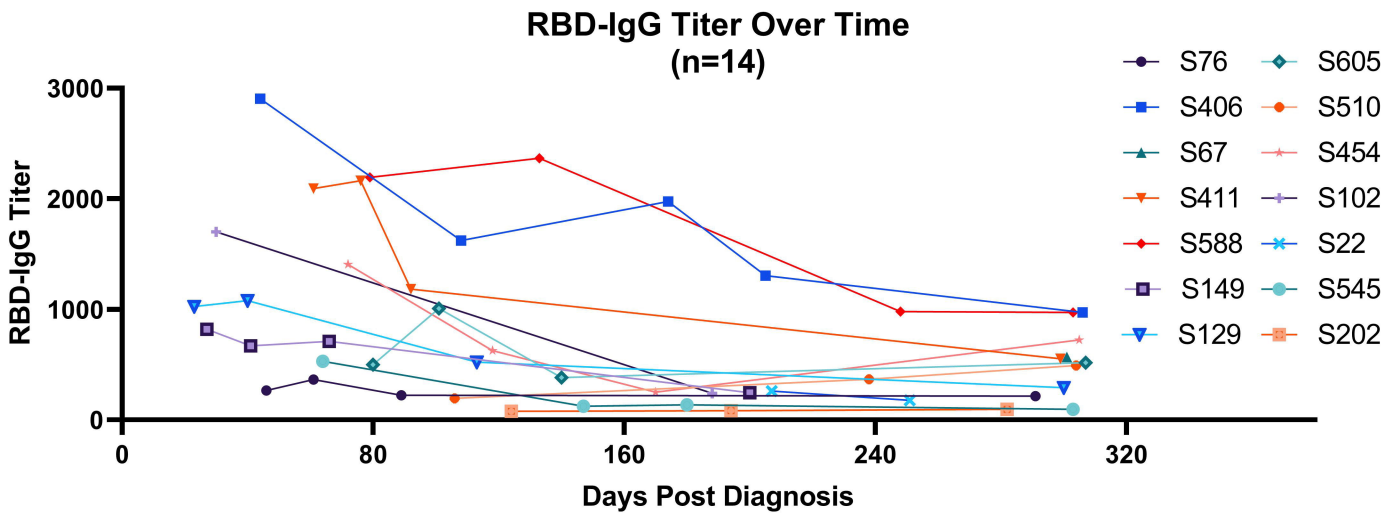
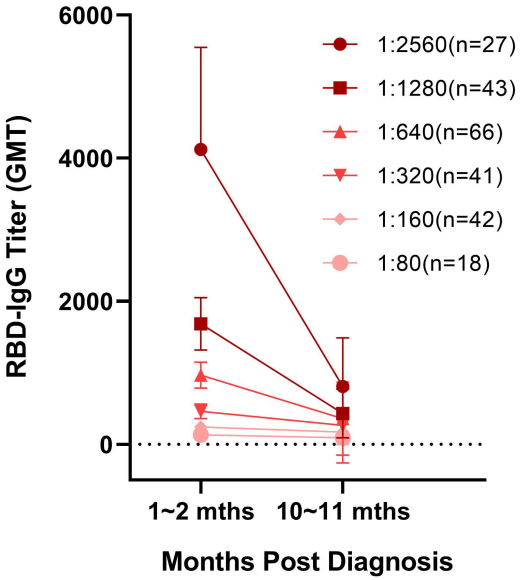
B



A



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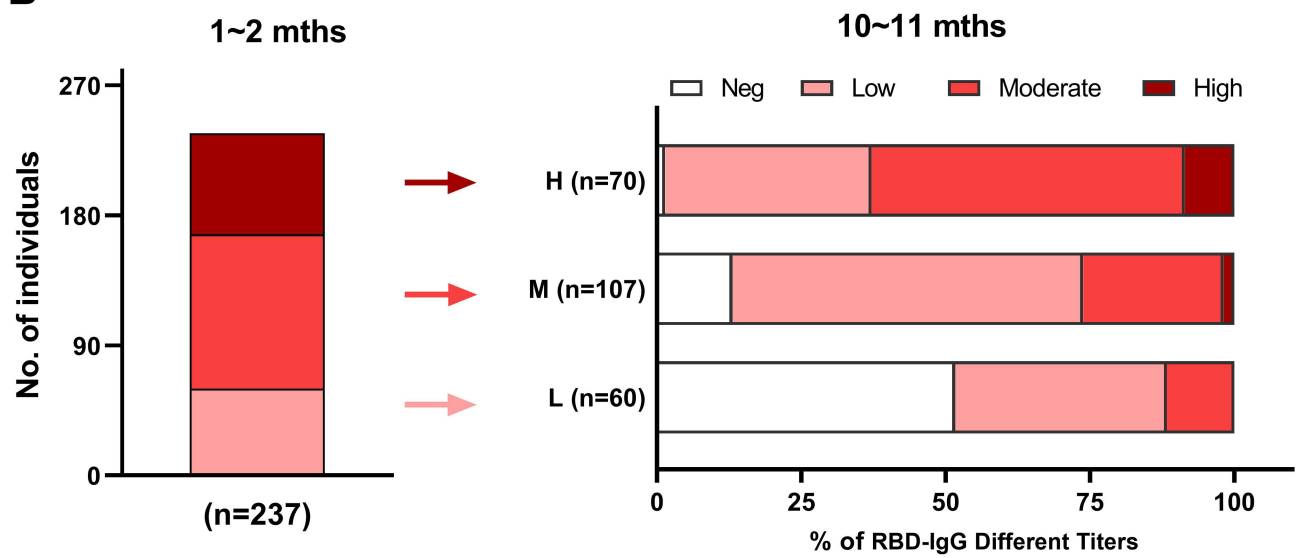
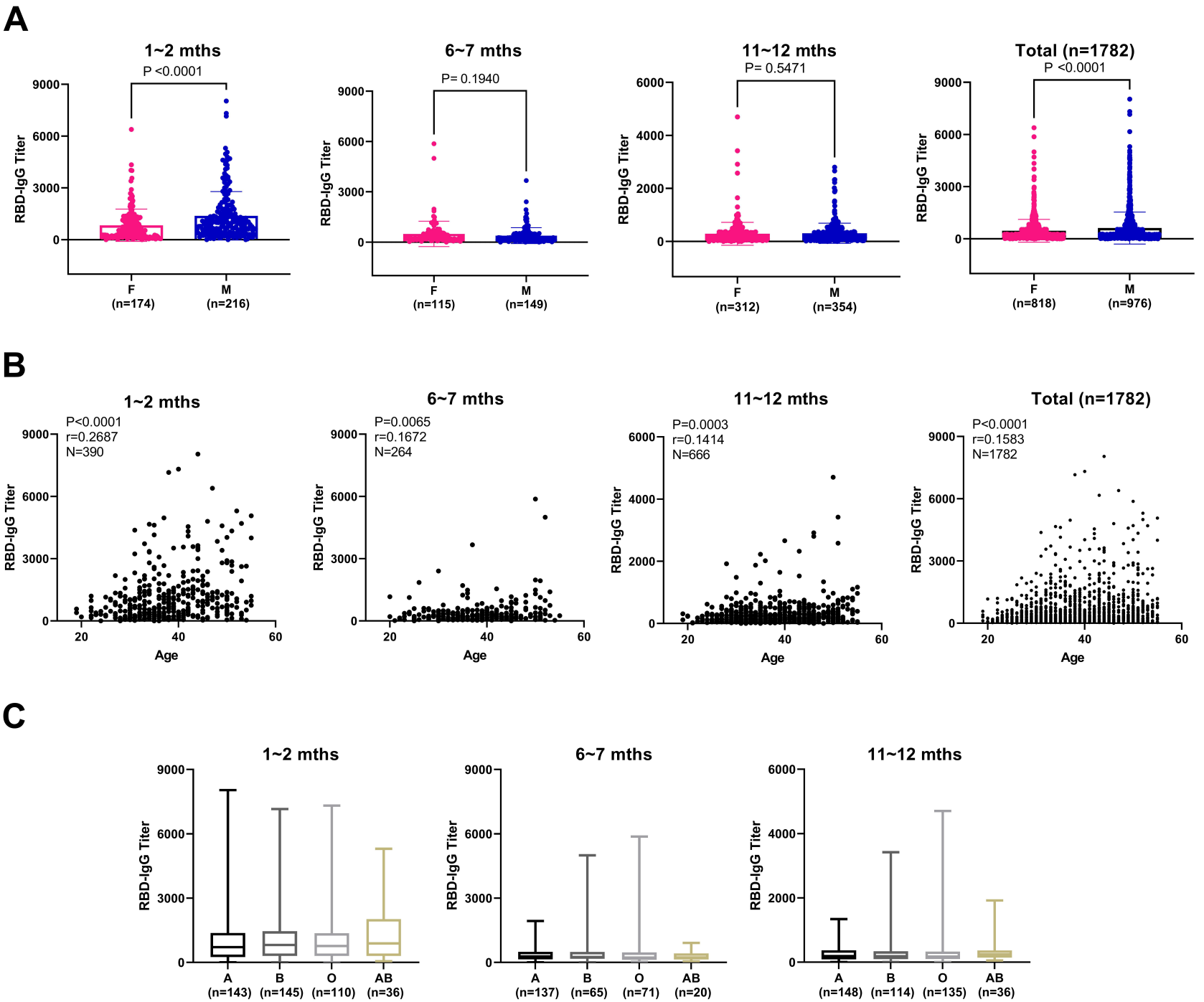
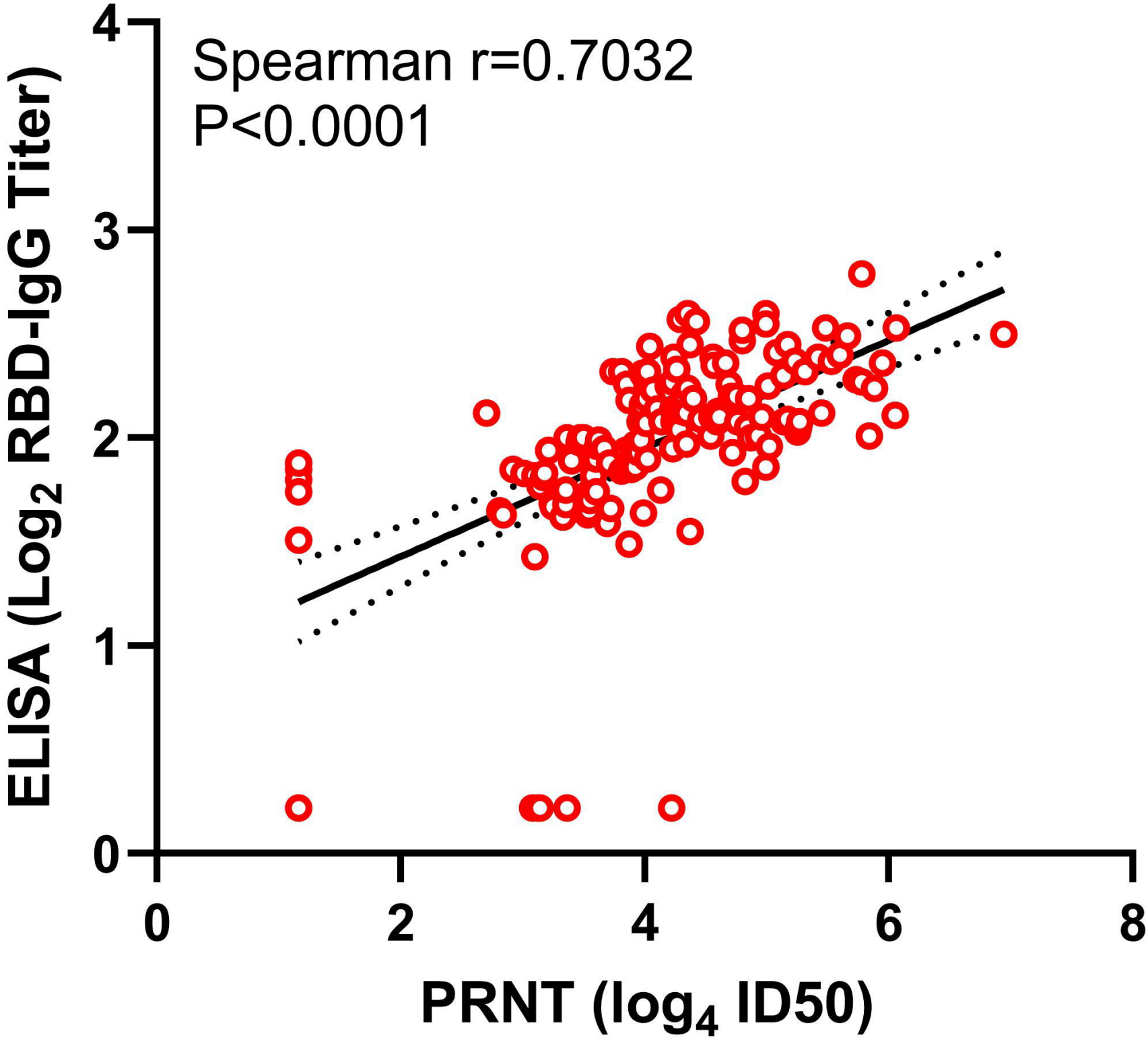
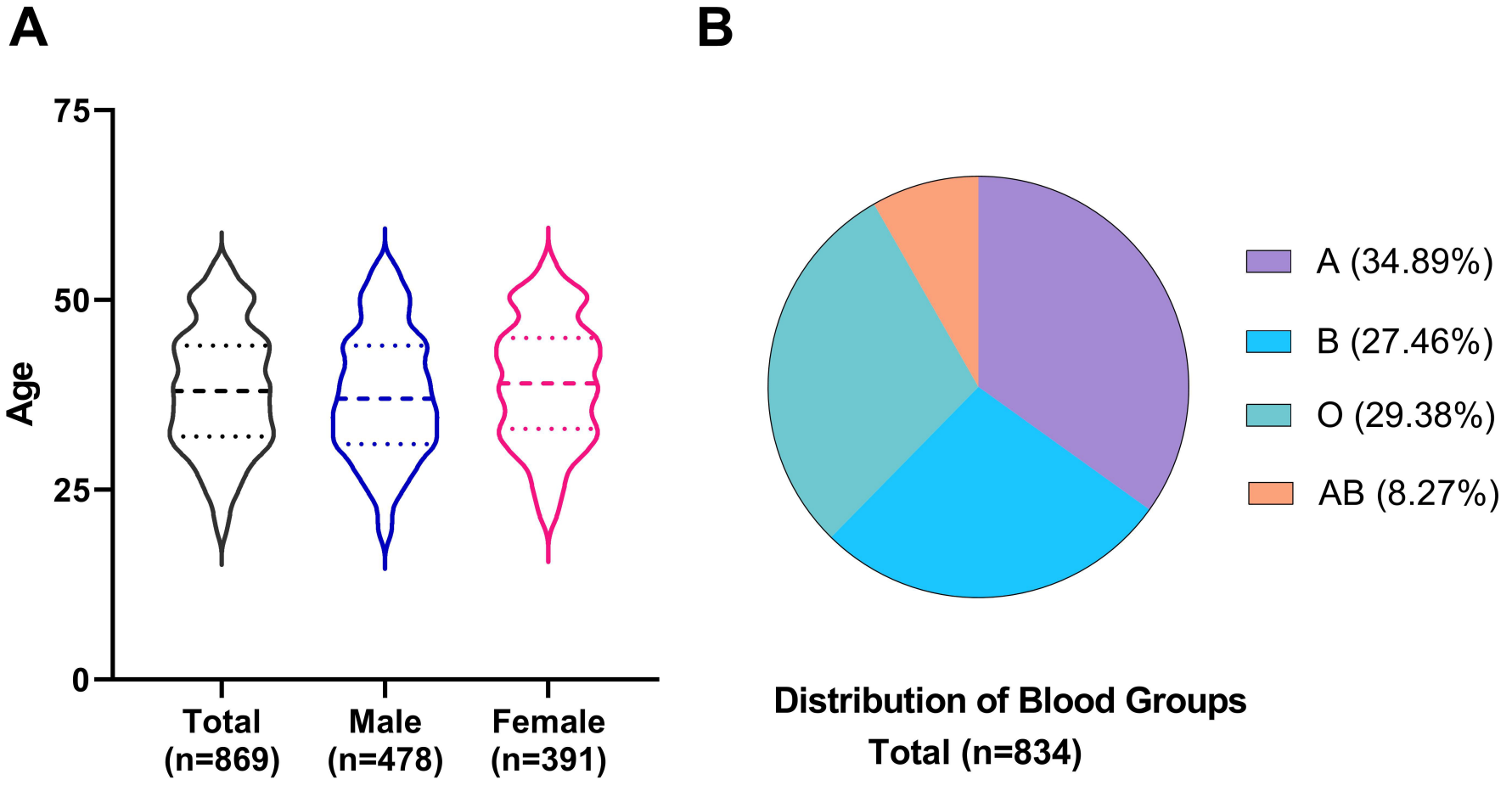
Total	237	1:610	1:183	70.0%	19.4%

B




A
1~2 mths
P <0.0001
RBD-IgG Titer
F (n=174)
M (n=216)
6~7 mths
P= 0.1940
F (n=115)
M (n=149)
11~12 mths
P= 0.5471
F (n=312)
M (n=354)
Total (n=1782)
P <0.0001
F (n=818)
M (n=976)
B
1~2 mths
P<0.0001
r=0.2687
N=390
6~7 mths
P=0.0065
r=0.1672
N=264
11~12 mths
P=0.0003
r=0.1414
N=666
Total (n=1782)
P<0.0001
r=0.1583
N=1782
Age
C
1~2 mths
A (n=143)
B (n=145)
O (n=110)
AB (n=36)
6~7 mths
A (n=137)
B (n=65)
O (n=71)
AB (n=20)
11~12 mths
A (n=148)
B (n=114)
O (n=135)
AB (n=36)


Spearman r=0.7032
P<0.0001
ELISA (Log2 RBD-IgG Titer)
PRNT (log4 ID50)
0
1
2
3
4
0
2
4
6
8


A
75
50
25
0
Age
Total
(n=869)
Male
(n=478)
Female
(n=391)
B
A (34.89%)
B (27.46%)
O (29.38%)
AB (8.27%)
Distribution of Blood Groups
Total (n=834)