

Study on the Change of Neutralizing Antibody in Unpaid Blood Donors after COVID-19 Vaccine Inoculation

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Abstract: To analyze the antibody production of unpaid blood donors after COVID-19 vaccination, and provide basis for the blood donation strategy of healthy people, follow-up vaccine research and development, and COVID-19 prevention and treatment. 1) The sera of 553 unpaid blood donors were collected at the 24th, 36th, 48th and 60th weeks after COVID-19 vaccine inoculation, and the specific SARS-CoV-2 neutralizing antibody was detected by competitive ELISA. The absorbance was inversely proportional to the effective activity of anti RBD neutralizing antibody in the samples; 2) Grouping all volunteers by gender and age, t-tests and analysis of variance were used to statistically analyze the differences in antibody inhibition rates between the groups at each time point of serum collection, in order to clarify the trend of neutralizing antibody inhibition rates on viruses. The neutralizing antibody activity produced by unpaid blood donors who received two different vaccines showed the same trend of change, and the neutralizing antibody activity of the two groups of volunteers remained at a certain level 24 weeks after the last vaccination; After 36 weeks of vaccination, the neutralizing antibody activity showed a slow decrease compared to the previous time point; At the 48th week after inoculation, the neutralizing antibody activity surged to its peak. It was explored that the reason was that most unpaid blood donation volunteers were infected with COVID-19 virus due to the changes in the prevention and control policies of Chinese Mainland on COVID-19, which was equivalent to an active immune response of the body itself, leading to a rise in the neutralizing antibody activity in the body again, even more than 24 weeks after inoculation, and then gradually declined to the normal level. In addition, there was no statistically significant difference in the inhibition rate produced by volunteers of different genders and vaccinated with different types of vaccines at the same time point; However, there was a statistically significant difference in the virus inhibition rate between volunteers under 30 and over 50 years old at various time points ($p < 0.01$). Within 36 weeks after inoculation of COVID-19 vaccine, neutralizing antibodies can maintain their relative vitality against COVID-19 and have protective power against human body, and are basically not affected by age and gender. Timely vaccination against COVID-19 reduces the risk of SARS-CoV-2 infection. After receiving the COVID-19 vaccine, unpaid blood donors can donate blood at normal intervals according to the guidelines provided by relevant ministries and commissions.

Keywords: Unpaid Blood Donors, Novel Coronavirus Vaccine, Neutralizing Antibody Activity, SARS Cov-2, Virus Inhibition Rate

1. Introduction

Coronavirus is a type of positive stranded single stranded RNA virus with a capsule envelope that can infect various mammals, including humans. In 2002 and 2012, two highly pathogenic coronaviruses, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), were found to be zoonotic and cause fatal respiratory diseases, making the coronavirus a new public health problem in the 21st century[1]. At the end of 2019, a new novel coronavirus named SARS-CoV-2 appeared, causing a worldwide outbreak of viral pneumonia. Infectious pneumonia caused by SARS-CoV-2 infection, also known as coronavirus disease 2019 (COVID-19), is highly pathogenic and contagious, spreading rapidly around the world. Both the number of infected people and the spatial scope of the epidemic area are far more than SARS and MERS, posing a serious threat to global public health[2]. As of April 12, 2021, approximately 130 million people have been infected with SARS-CoV-2, distributed in 235 countries and regions worldwide, with approximately 3 million deaths. Although genetic evidence suggests that SARS-CoV-2 is a natural virus that may have

originated in animals, there is currently no conclusive conclusion on the exact time and location of the virus's first infection in humans^[3].

The interpersonal transmission of SARS-CoV-2 is mainly mediated by droplets or aerosols produced during coughing and sneezing. In the early stage of infection, the high expression of ACE2-2 in the mouth and tongue promotes the invasion of SARS-CoV-2^[4]. A study on the patients of the cruise ship Princess Diamond in COVID-19 showed that in the early stage of Covid-19 outbreak, the proportion of asymptomatic infected people was 17.9%^[5]. Therefore, accurate and rapid identification of asymptomatic infected persons is the key to SARS-CoV-2 epidemic prevention and control, and it is also the key to curb the spread of the virus to close contacts. In the research and development of drugs and vaccines for novel coronavirus epidemic, the Food and Drug Administration, FDA) clearly indicated that there is no approved therapy or drug for treating COVID-19^[6]; However, three preventive COVID-19 vaccines have been approved for marketing. Therefore, vaccination is considered to be the hope to end the global novel coronavirus epidemic. At present, COVID-19 vaccine development strategies around the world mainly include: recombinant vector vaccine, lipid nanoparticle vaccine wrapped with DNA or mRNA, inactivated virus vaccine, inactivated virus vaccine and protein subunit vaccine. By December 2020, the World Health Organization had recorded more than 214 candidate vaccines of COVID-19, of which 51 were in the clinical evaluation stage and 13 were in the phase III trial stage. Some vaccines have been approved for use in some parts of the world. Among them, there are 18 Covid-19 protein subunit vaccines, accounting for 30% of the vaccines in clinical trials, which is the largest vaccine type under research; The DNA vaccine against SARS-CoV-2 also occupies a place, and 8 kinds are undergoing clinical trials^[7]. However, the characteristics of DNA vaccine that need to enter the nucleus during its action process lead to the risk and security hidden danger of interfering with the host genome sequence, inducing autoantibodies to accelerate the progress of immune diseases, and the carrier system causing allergies; In addition, seven new RNA vaccines have entered clinical trials. RNA vaccine consists of mRNA encoding virus antigens, and human cells translate these RNA to produce antigen proteins and stimulate the immune system. This kind of vaccine usually forms a complex with other preparations (such as protamine) to improve its efficacy, which has the advantages of high adaptability to new pathogens, reappearing the natural conformation and modification of antigen proteins, and does not interact with host cell DNA, thus avoiding the risk of genome integration; The disadvantage is that foreign RNA can activate interferon-mediated antiviral immune response, leading to translation stagnation and messenger RNA degradation, thus inhibiting vaccine efficacy.

At present, the COVID-19 vaccine that has been put into use in China is mainly inactivated virus vaccine, and several vaccines can induce a large number of neutralizing antibodies in the early animal model, without causing T cell reaction. Considering the global situation, the best way to prevent SARS-CoV-2 infection until the situation of epidemic control is unclear is to wear masks, avoid crowds, and at the same time screen for anti-SARS-CoV-2 lead drugs and develop a vaccine. Tackling the epidemic remains a long-term task that requires joint efforts. The detection of neutralizing antibody is an extremely important index for the detection of antibody level after vaccination or infection. In addition, it is worth noting that at present, a large number of global research data on SARS-CoV-2 antibody level are concentrated in America or Europe, Southeast Asia with less developed economy or resources, and there is no complete monitoring data in Mediterranean region and Africa region. This study mainly investigates whether neutralizing antibodies will be produced in the body after vaccination and infection with Covid-19, and its duration. Therefore, we select unpaid blood donors from Changzhou Central Blood Station in Jiangsu Province, China for serological testing, and judge the changes of neutralizing antibodies and evaluate the antibody levels of different vaccines according to their vaccination and COVID-19 infection. At the same time, the antibody production of unpaid blood donors after vaccination with COVID-19 vaccine was analyzed, so as to provide basis for blood donation strategy, follow-up vaccine development and prevention and treatment of COVID-19 in healthy people.

2. Material and Methos

2.1 Study Design and Participants

The volunteers who participated in this study were all unpaid blood donors from Changzhou Central Blood Station, with a total of 561 people. They all completed COVID-19 vaccination (including booster shots) from October 2022 to December 2022. During the experiment, a total of 8 people lost their visits, so a total of 553 volunteers were actually included. The volunteers ranged in age from 18 to 55, including 425 males and 128 females. 508 people were vaccinated with inactivated vaccine (Vero cells) Coronavac

and 45 people with recombinant protein vaccine (CHO cells) Zifivax (ZF 2001). The vaccines before and after vaccination of each subject were all from the same manufacturer, but the batch numbers were different.

2.2 Serological Tests

Serum samples were collected at the 24th week, 36th week, 48th week and 60th week after the last vaccination, and the titer of neutralizing antibody against SARS-CoV-2 was detected. SARS-CoV-2 encodes four major structural proteins, including spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein. Among them, S protein contains a receptor binding region (RBD), and SARS-CoV-2 is attached to the target cell surface receptor-angiotensin converting enzyme 2 (ACE2) of the host respiratory tract epithelium through the RBD region. SARS-CoV-2 enters the host cell through membrane fusion after recognizing and binding ACE2 receptor, causing infection. In the second process, the body produces corresponding antibodies through immune response, among which the antibodies that can block the interaction between RBD and ACE 2 are called neutralizing antibodies. Neutralizing antibody was detected by competition ELISA (Vazyme Biotechnology Co., Ltd.). SARS-CoV-2 detected by this method is a neutralizing epitope antibody against SRBD. Preparation of the experiment: 1) Take the kit out of the cold storage environment and allow it to equilibrate to room temperature (18~28°C) for at least 30 minutes. 2) Preparation of washing liquid: 30 ml of 80× concentrated washing liquid is diluted and mixed with 2379mL of purified water for later use. 3) preparation of enzyme-labeled antigen working solution: add 100 μl of 100× concentrated enzyme-labeled antigen into 9.9mL of enzyme diluent, and mix it upside down for at least 30 times to prepare 1× enzyme-labeled antigen working solution. Note: the enzyme-labeled antigen working solution can be stored at 2~8°C for use on the same day, and the amount of working solution can be prepared according to the number of samples to be tested. Each sample or control needs 1× 100μL of enzyme-labeled antigen working solution. 4) Dilution of sample and reference substance: add 8μL of sample or reference substance to each hole of the 96-hole dilution plate provided by the detection box, and then add 72μL of sample diluent to each hole according to the dilution ratio of 1: 9 to dilute the sample or reference substance by 10 times. Experimental operation: 1) First, add 8 μL of sample/reference substance to a 96-well plate, then add 72 μL of sample diluent to achieve a 10-fold dilution of the sample. Next, add 80 μL of 1× enzyme-labeled antigen working solution to each well at a volume ratio of 1:1.2) Seal the 96-well dilution plate with a plate sealer and use a horizontal shaker to oscillate for 60 seconds to ensure thorough mixing of the liquid in the wells. 3) Incubate at 37°C for 20 minutes. 4) Carefully remove the plate sealer, then transfer 100 μL of the reaction solution from each well of the 96-well dilution plate to the corresponding well of the hACE2-coated plate. 5) Replace the plate sealer, reseal the hACE2-coated plate, and incubate at 37°C for 20 minutes. 6) Carefully remove the plate sealer and wash the plate 4 times with 350 μL of 1× washing solution per well. Note: After washing is added to the hACE2 coated board every time, it is necessary to let the washing liquid soak the hACE2 coated board for 30-60 seconds, then shake off the liquid in the board and pat it dry, and then add the washing liquid again for the next round of washing operation. After washing the plate, pat the enzyme-labeled plate dry on absorbent paper. 7) Add 100 μL of TMB substrate solution to each well, replace the plate sealer, reseal the hACE2-coated plate, and incubate at 37°C in the dark for 15 minutes to allow the reaction to proceed. 8) Carefully remove the plate sealer and add 50 μL of stop solution to each well to terminate the reaction. 9) Immediately after termination, measure the absorbance of each well at a single wavelength of 450 nm using a microplate reader. Test effect: Each test must be set up positive control and negative control, positive control OD450 less than 0.3, negative control OD450 greater than 0.1. If the outside diameter of any control does not meet the requirements, the test is invalid and needs to be retested.

Results determination: 1) Criteria for neutralizing antibody results: In a single test, the OD450 of negative reference substance and sample is used to calculate the antibody inhibition rate in the serum of the sample to be tested, and the OD450 of positive reference substance is only used to determine the effectiveness of the test. 2) The inhibition rate $\geq 20\%$ is positive; The inhibition rate of SARSCoV-2 neutralizing antibody in the test sample is negative when it is less than 20%, that is, the test sample does not contain SARSCoV-2 neutralizing antibody or the antibody is below the minimum detection limit.

2.3 Statistical Analysis

In this study, 561 unpaid blood donors were selected as volunteers in Changzhou, and all volunteers were vaccinated with COVID-19 vaccine. After eliminating the lost visitors, 553 volunteers were finally retained. All volunteers are divided into male and female groups by gender. According to age, they were

divided into four groups: < 30 years old, 30-39 years old, 40-49 years old and ≥ 50 years old. They were divided into VERO cell group and CHO cell group according to different kinds of vaccines. The software used for data processing and statistics in this paper is spss20.0 and graphpad prism9.0. When the variable is univariate, the statistical methods are t test and one-way ANOVA. When there are more than two variables, the statistical method for the data is two-factor and multifactor analysis of variance. Among them, $P < 0.05$ was considered statistically significant.

3. Results

3.1 Overall changes of neutralizing antibodies

All the 561 volunteers who participated in the study completed the vaccination including booster shots, and they were not infected with Covid-19 before and during the vaccination. However, there were 8 people who were lost during the sampling process, so in the end, a total of 553 volunteers completed the whole study. Serological sampling was taken for the first time at 24 weeks after inoculation, and sampling and testing were completed at 36 weeks, 48 weeks and 60 weeks later. The change of antibody is shown in Figure 1. At the 24th week after vaccination, the inhibition rate of neutralizing antibody in 34 volunteers was less than 20%. At the 36th week, the inhibition rate of neutralizing antibody in 48 volunteers was less than 20%. At the 48th week, the inhibition rate of neutralizing antibody in 5 volunteers was less than 20%. At the 60th week, the inhibition rate of neutralizing antibody in 6 volunteers was less than 20%. It can be seen that about half a year after inoculation, most volunteers contain a certain concentration of neutralizing antibodies (Figure 1). T-test was used to compare the inhibition rate of each time node, and it was found that the inhibition rate of each time node was statistically different ($p < 0.0001$), and the inhibition rate of the 48th week was significantly higher than that of the 36th week, because during this period, the China government adjusted its policy on COVID-19 epidemic, and all volunteers were infected with COVID-19 from December to January, 2023, which was equivalent to giving birth to an active immunization, so the concentration of neutralizing antibodies in the body surged.

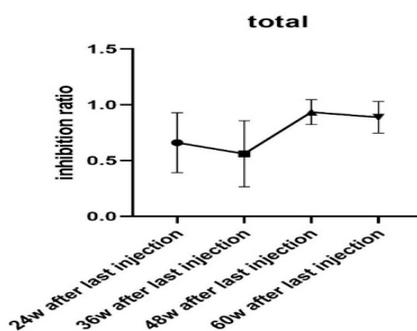


Fig.1 Inhibition rate changes at 24,36,48 and 60 weeks after serum collection

24w after last injection vs. 36w after last injection, 24w after last injection vs. 48w after last injection, 24w after last injection vs. 60w after last injection, 36w after last injection vs. 48w after last injection, 36w after last injection vs. 60w after last injection, 48w after last injection vs. 60w after last injection $p < 0.0001$, The statistical differences were respectively 0.0036, 0.0018, 0.0025, 0.0015

3.2 Comparison of the changes of neutralizing antibodies in different ages, sexes and vaccination types

In this study, all volunteers were divided into four groups according to their age, namely, under 30 years old, 30-39 years old, 40-49 years old and over 50 years old, with 113 people in each group, 171 people in each group, 175 people in each group and 76 people in each group. According to gender, they were divided into two groups, male and female, with 425 and 128 people in each group respectively. Vaccines prepared according to different inoculation methods are divided into CoronaVac and ZifiVax(ZF2001), with 508 people in each group and 45 people in each group. In subgroups of different sexes, ages and vaccinations, the trend of neutralizing antibodies is the same as that in Figure 1, and there is no statistical difference between different sexes and different kinds of vaccines at each time point ($p >$

0.05). However, the results of two-factor analysis of variance show that there are statistical differences in the inhibition rates between the < 30-year-old group and the ≥50-year-old group at each time node (p=0.0094), as shown in Figure 2.

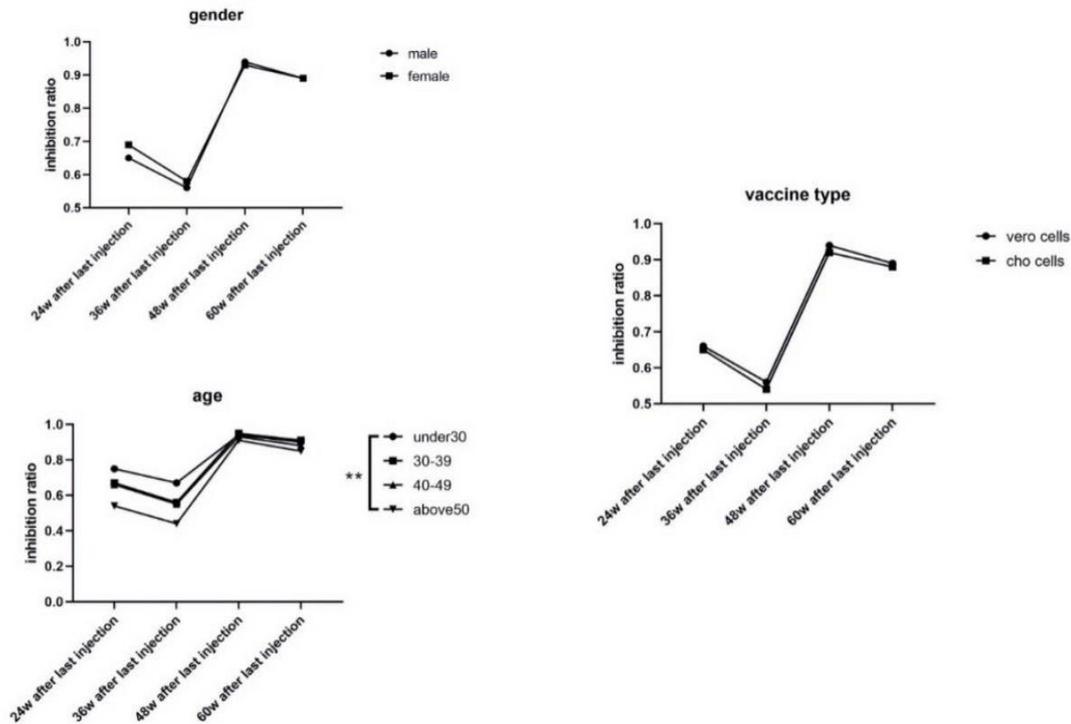


Fig.2 Comparison of the changes of neutralizing antibodies in different ages, sexes and different kinds of vaccination.

3.3 Influence of gender and age factors on volunteers vaccinated with the same vaccine

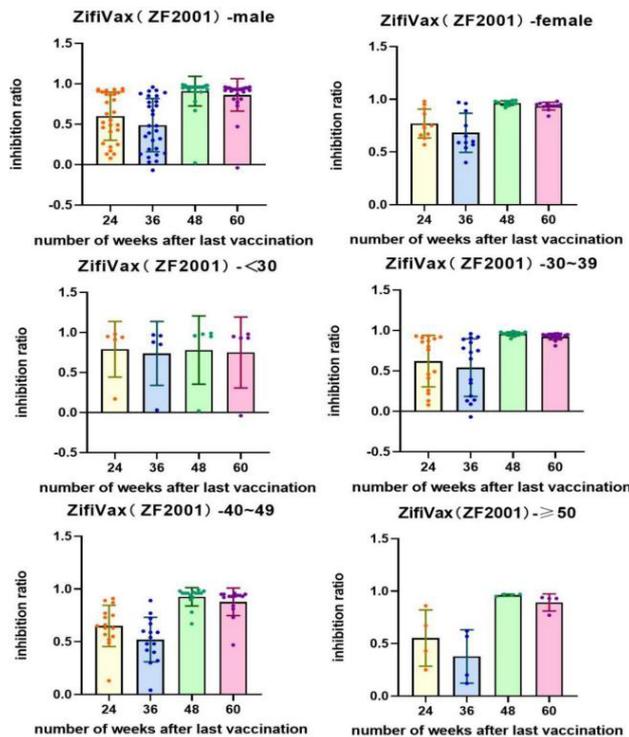
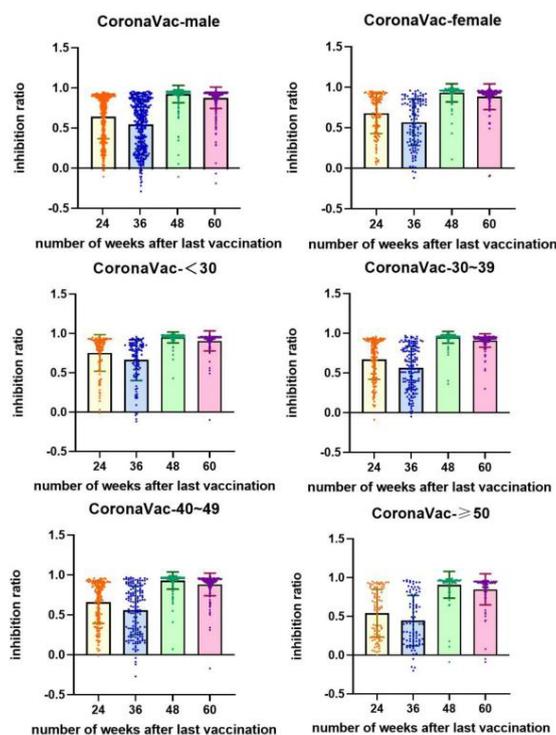


Fig.3 Comparison of neutralizing antibodies grouped by age and sex after vaccination with ZifiVax(ZF2001)

There are two kinds of vaccines vaccinated by volunteers, namely, the inactivated vaccine CoronaVac and the recombinant protein vaccine ZifiVax(ZF2001), so they are used as variables to compare the vaccination effects of different populations. The results of multivariate analysis of variance showed that there was no statistical difference in the protective effect of antibodies produced by people of different sexes and ages after inoculation with ZifiVax(ZF2001) ($p > 0.05$), as shown in Figure 3.

However, for people vaccinated with CoronaVac vaccine, gender and age have influenced the protective effect of the vaccine to varying degrees, as shown in Figure 4.



male:24 vs. female:48, $p=0.007$;male:24 vs. female:60, $p=0.0129$; male:36 vs. female:48, $p=0.0026$;
male:36 vs. female:60, $p=0.004$; male:48 vs. female:24, $p=0.0074$; male:48 vs. female:36, $p=0.0027$;
male:60 vs. female:24, $p=0.0138$; male:60 vs. female:36, $p=0.0042$. <30 vs. ≥ 50 , $p=0.0064$

Fig.4 Comparison of neutralizing antibodies grouped by age and sex after vaccination with Vero cell vaccine

4. Discussion

At the end of 2019, a coronavirus named SARS-CoV-2 spread rapidly around the world, and was officially declared as an epidemic by the World Health Organization in March 2020, which caused widespread concern in the international community and caused serious consequences for global health and economy. The research of Garcia-Beltran et al shows that the serum SARS-CoV-2 specific antibody and neutralizing titer of COVID-19 patients are important predictive indexes for judging the severity of the disease and whether reinfection can be prevented. Therefore, knowing the titers, titers and long-term trends of neutralizing antibodies against SARS-CoV-2 in vivo, whether infected with Covid-19 or vaccinated against Covid-19, is very effective in reducing the risk of reinfection and autoimmune protection. After the specific neutralizing antibody binds to the corresponding virus, the virus can't be adsorbed on sensitive cells and lose its infection ability, so the titer of neutralizing antibody in serum is an important evaluation standard to evaluate whether the body can resist SARS-CoV-2 infection.

The genome of SARS-CoV-2 contains 29,903 bases and encodes about 9,860 amino acids. Among the virus-related proteins encoded by SARS-CoV-2, S protein is the key protein to mediate virus invasion into host cells. S protein is a kind of homotrimer I fusion glycoprotein, which contains two domains (S1 and S2) with different functions. S1 domain is exposed on the protein surface and contains receptor binding domain, RBD), because it can selectively bind to receptors on target cells.

Therefore, the tendency and pathogenicity of the virus can be determined to a certain extent.S2 domain is located in the transmembrane region of virus shell, which plays a certain role in the

conformational recombination of virus and promotes the fusion of virus and host cells. At the same time, there is a multi-base cleavage site between S1 and S2, which enables the virus to invade the host cell through enzyme digestion. Based on the above facts, this study selected 561 unpaid blood donors who had been vaccinated with COVID-19 vaccine for 60 weeks to detect neutralizing antibodies.

Neutralizing antibody only detects the antigen epitope of SARS-CoV-2 invading host, that is, the antigen epitope of SRBD protein. The immune response after vaccination with COVID-19 vaccine involves two processes: cellular response (producing different T cell lineages, interferon and interleukin) and IgG antibody targeting virus antigen (including protein S), which is triggered by the first process. Generally speaking, these antibodies can still be detected within about 6 months, and then they will be reduced by 5-10 times. In this study, volunteers were vaccinated two vaccines with different preparation principles. Inactivated vaccine is a kind of vaccine preparation method with long development time and relatively mature and comprehensive technology. Through some reasonable physical and chemical means, the pathogen loses its pathogenicity, but still maintains its immunogenicity, thus stimulating the body to produce corresponding specific antibodies. Its advantages are good safety and convenient preparation. The main preparation principle of recombinant protein vaccine is to combine the pathogen target antigen with an expression vector protein by genetic engineering technology, purify the target antigen after it is expressed in large quantities, and stimulate the body to produce immune response after it is injected into the human body. In the preparation process, adjuvant is often needed to expand the immune effect. Twenty-four weeks after inoculation, the inhibition rate of neutralizing antibody of 25 voluntary blood donors was less than 20%, that is, it was judged that the sample did not contain SARS-CoV-2 or the antibody was below the minimum detection limit. Bioinformatics research shows that, the secondary spatial structure of SRBD protein is mainly irregular curl, accompanied by a certain β -fold and β -turn, and α -fold accounts for the lowest proportion. Therefore, in the irregular spatial fold, the main epitope may be folded or covered, so that it cannot be detected by binding with antibodies, which leads to the titer of neutralizing antibodies in some volunteers being undetectable. However, most volunteers are still positive for neutralizing antibody serology, which is consistent with most global research results, indicating that racial differences have no substantial impact on the humoral response of the vaccine. At the 36th week after inoculation, the activity of neutralizing antibody showed a downward trend, and the inhibition rate of neutralizing antibody of 67 voluntary blood donors was less than 20%. This result showed that neutralizing antibody could still maintain a certain activity and protect more people within nearly one year after inoculation. At the 48th week after that, the activity of neutralizing antibody increased significantly. The retrospective reason was that Chinese mainland's policy towards Covid-19 changed, and most people were infected with Covid-19, which was equivalent to an active immunization. Therefore, the activity of neutralizing antibody reached its peak after rehabilitation, even exceeding the activity 24 weeks after vaccination. At the 60th week after inoculation, the activity of neutralizing antibody decreased, still higher than that at the 24th week after inoculation, and then showed a normal trend. There are statistical differences between all the above comparisons. It has been reported that the relationship between neutralizing antibody titer and protection is analyzed by using the data of vaccination and recovery period research. It shows that if the neutralizing antibody level reaches 20.2% of the serum level of recovered people after vaccination, there is a 50% probability of avoiding symptomatic infection. When the neutralizing antibody level reaches 3% of the serum level of the recovered person, there is a 50% probability to avoid serious illness after infection. In addition, the vaccine can also make the human body produce cellular immunity and form corresponding immune memory. This experiment can effectively provide some illustrative data, and provide a certain basis for the government to formulate the corresponding vaccination booster time, so that the neutralizing antibody can be maintained at a relatively high level and protect the human body. The number of antibodies is an important basis for strengthening vaccination, and it has an extremely important reference value for the formulation of vaccine policy. It is particularly important to control the timing of vaccination in susceptible populations.

Our data comparative study also found that there was no difference in immune effect between two different vaccines in different ages and sexes, so it can be said that there was no significant difference in neutralizing antibodies produced in the population as long as COVID-19 vaccine with booster was injected. However, among the volunteers injected with the same vaccine, the people injected with CHO vaccine ZifiVax(ZF2001) did not have statistical differences in antibodies due to gender and age, but the volunteers injected with inactivated CoronaVac had statistical differences in gender and age. However, due to the limitation of the number of specimens, whether this conclusion is universal still needs a lot of experimental data and long-term observation to prove, and it cannot be judged that a certain vaccine has a better advantage in inducing neutralizing antibodies.

Due to the limitations of the study, the first one does not include the corresponding data of non-Asians,

the elderly and the immunocompromised, and the detection degree of the second kit will also have a certain impact on the experimental results. Therefore, whether the above conclusions are universal or not needs a lot of data and long-term observation to be confirmed.

To sum up, the neutralizing antibody can still maintain a certain activity 36 weeks after vaccination, which is of great significance to protect people's lives, health and safety. At the beginning of 2021, the General Office of the National Health and Wellness Commission and the Health Bureau of the Logistics Support Department of the Central Military Commission jointly issued the Notice on Printing and Distributing the Guidelines for the Prevention and Control of COVID-19 Epidemic in Blood Stations, which made the latest provisions on the follow-up blood donation time after vaccination with COVID-19 vaccine. The notice pointed out that COVID-19 inactivated vaccine vaccinators can donate blood 48 hours after vaccination; Those who receive other types of vaccines (not including live attenuated vaccines) can donate blood 14 days from the date of vaccination. This study also revealed that the unpaid blood donors can donate blood normally according to the interval of the document guidelines after being vaccinated with COVID-19 vaccine.

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