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ORIGINAL ARTICLE

Detection of Neutralizing Antibodies of a Recombinant Adenovirus Type-5 Vectored COVID-19 Vaccine in Healthy Adults in China

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Abstract

We conducted a descriptive analysis of the specific IgM, IgG, and Nabs test results of participants with the rAd5 vectored COVID-19 vaccine to provide reference information for the vaccine's protective efficacy. The levels of specific IgM, IgG, and Nabs antibodies in 111 participants vaccinated with the rAd5 vectored COVID-19 vaccine were measured by chemiluminescence results were compared and analyzed. We found that IgG and NAbs concentrations in all participants were inversely correlated with age, and significant differences were found between age groups. 102 (91.89%) and 95 (85.59%) participants were positive for 2019-nCoV IgG and NAbs, respectively. The IgG concentrations of participants younger than 40 years old were generally higher than that of participants older than 40 years old (P= 0.025). Similar results were found at the NAbs concentrations (P= 0.05). Our findings demonstrate that the NAbs of the rAd5 vectored COVID-19 vaccine was more than 85% in the Chinese, which provided certain reference information for the vaccine's protective efficacy.

Key words: **Neutralizing antibodies; COVID-19 vaccine; IgG; Chemiluminescence**

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused more than 13 million cases of COVID-19 worldwide, resulting in hundreds of thousands of deaths and severe economic disruption[1-5]. The current pandemic has highlighted the need for effective preventive solutions to reduce its burden and spread. Vaccination will restrict the spread of COVID-19 and reduce mortality.

Because of the rapid global spread of SARS-CoV-2 infection and the high mortality rate, a vaccine's development is an urgent task. Intensive research and development of vaccines are currently underway in China, the USA, Russia, the UK, and other countries [6-11]. According to the world health organization (WHO), 29 candidate COVID-19 vaccines based on different platforms (inactivated, viral vectored, DNA, mRNA, etc.) were currently under evaluation in clinical trials worldwide[12-17]. Feng-Cai Zhu et al[8,9]. reported the immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine (rAd5) in healthy adults in double-blind, randomized controlled phase 2 trials. The vaccine is a replication-defective rAd5 vectored vaccine expressing the spike glycoprotein of SARS-CoV-2. Denis Y Logunov et al[6]. reported the result of a rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in Russia.

The blood titer of the neutralizing antibodies (NAbs) is critical. One of the most critical indicators to evaluate the new coronavirus vaccine's effectiveness is NAbs in subjects. We detected and assessed neutralizing antibodies of the rAd5 vectored COVID-19 vaccine in 111 Han Chinese.

Materials and Methods

Study Design and Participants

The current study was performed from 12 to 24 March 2021 and was conducted in Nanjing, China. The participants were voluntarily recruited in this study. All participants provided written informed consent for the collection of samples and their subsequent analysis. We were approved by the Jinling hospital ethics committee and were conducted following the Declaration of Helsinki's tenets and its amendments.

Vaccine Information

All participants aged between 24 and 71 years, who intramuscularly injected with 0.5 ml rAd5 vectored COVID-19 vaccine in the left arm. The vaccine was developed by the Beijing Institute of Biotechnology (Beijing, China) and CanSino Biologics (Tianjin, China).

Antibody Measurements

Upon informed consent, blood was taken by vein puncture; one serum tube was collected per patient. For serum collection, tubes were centrifuged at 3000 rpm, 10 min. Blood samples were taken from all participants for antibody tests before the vaccination and days 17 and 45. 2019-nCOV antibodies IgM, IgG, and NAbs chemiluminescence immunoassay kits and iFlash 3000-A fully automatic CLIA analyzer were from Shenzhen YHLO Biotech Co, Ltd (China). The amount of anti-SARS-CoV-2 antibodies IgM, IgG, and Neutralizing antibody is positively correlated with the relative light units measured by the chemiluminescence analyzer. Cut off value proposed by the manufacturer is 10 AU/mL both for IgM, IgG, and Neutralizing antibody: hence samples with concentrations more than equal to 10 AU/mL are considered positive reactively.

Statistical Analysis

Categorical variables were summarized as counts and percentages (%). For the continuous measurement, the Kolmogorov-Smirnov test was used to evaluate distribution type. Normally distributed data were expressed as mean ± standard deviation (SD); otherwise, the continuous variables were described using median and interquartile ranges (IQR). Independent sample two-tailed t-test and Mann-Whitney test were used to compare the continuous variables for data of different groups. The frequencies of categorical variables were compared using the chi-square and Fisher's exact test as appropriate. Statistical analyses were performed with SPSS (v.20.0; SPSS Inc., Chicago, IL, USA).

Results

Patients' Characteristics

Among a total of 111 Han Chinese with subcutaneous injection rAd5 vectored COVID-19 vaccine enrolled in the study, 80 of them (72.07%) were males (Table 1). The mean age of the studied participants was 43.44 years (SD, ±10.02). More than 80 percent of participants had more than 30 days between vaccination and blood collection. The mean number of days and standard deviation from vaccination and blood collection was 33.78 days (SD, ±5.14).

Antibody Measurement

The age-dependent scatter plot of the 2019-nCoV IgG and NAbs of all participants showed that antibody levels were negatively correlated with age (Fig 1A and 1B). 102 (91.89%) and 95 (85.59%) participants were positive for 2019-nCoV IgG and NAbs, respectively. The IgG concentrations of participants younger than 40 years old (91.14±64.22 AU/mL) were generally higher than that of participants older than 40 years old (63.68±62.27 AU/mL) (*P*= 0.025). Similar results were found at the NAbs concentrations (61.19±78.29 AU/mL versus 35.95±55.94 AU/mL, *P*= 0.05, Fig 1C and 1D). We also found significant differences in IgG positive rates between age groups (*P*= 0.042). No significant association was found between SARS-CoV-2 antibody levels and gender and number of days, and standard deviation from vaccination and blood collection. The results of 2019-nCoV antibodies detection are reported in Table 1.

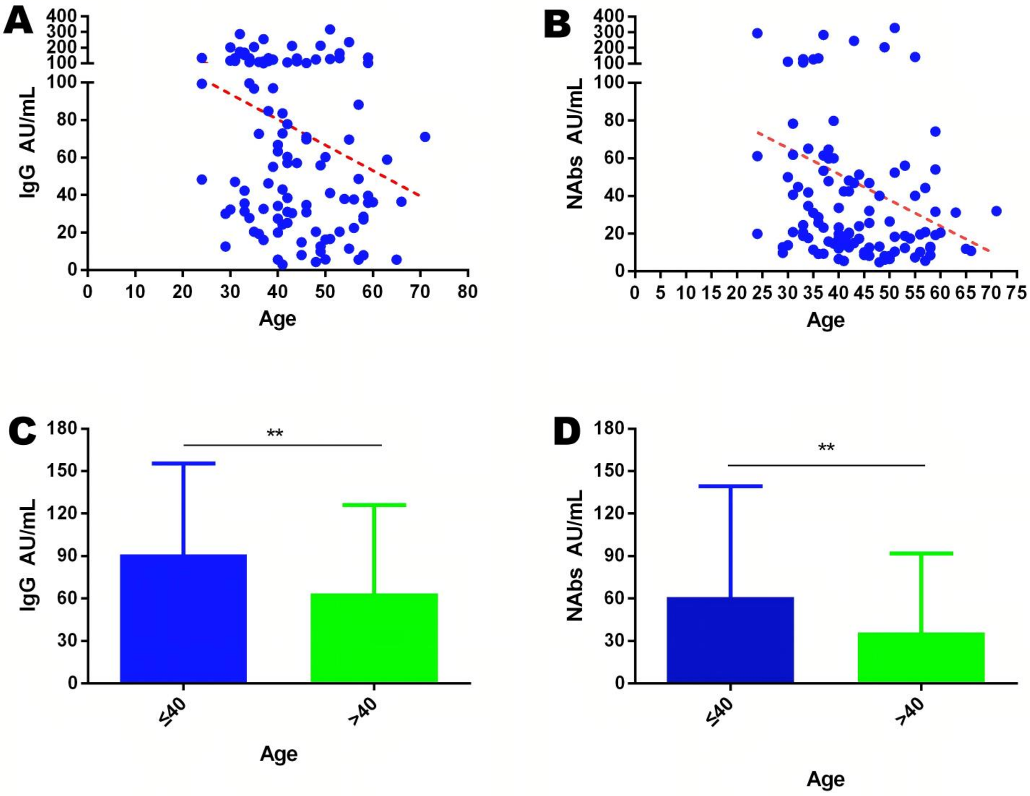

Figure 1 IgG and Nabs concentrations distribution in all participants with received rAd5 vectored COVID-19 vaccine

Table 1 the results of antibodies in all participants with rAd5 vectored COVID-19 vaccine

	All (n=111)	**Age**		***P***	**Gender**		***P***	**Number of days**		***P***
		Age≤40 (n=48)	Age>40 (n=63)		Male (n=80)	Female(n=31)		Days≤30 (n=20)	Days>30 (n=91)	
Age	43.44±10.02				43.19±9.00	43.54±10.44	0.892	45.05±11.99	43.09±9.57	0.43
≤40 no(%)	48(43.24%)									
>40 no(%)	63(57.76%)									
Gender										
Male no(%)	80(72.07%)									
Female no(%)	31(27.93%)									
Number of days	33.78±5.14	34.38±4.27	33.33±5.71	0.292	32.65±5.74	34.23±4.85	0.147			
≤30 no(%)	20(18.02%)									
>30 no(%)	91(81.98%)									
IgG (AU/mL)	75.56±64.30	91.14±64.22	63.68±62.27	**0.025**	77.76±52.27	74.70±68.59	0.823	68.54±55.98	77.10±66.17	0.593
Positive no(%)	102(91.89%)	47(97.92%)	55(87.30%)	**0.042**	73(91.25%)	29(93.55%)	0.691	17(85%)	85(93.41%)	0.212
Negative	9(8.11%)	1(2.08%)	8(12.70%		7(8.75%)	2(6.45%)		3(15%)	6(6.59%)	

no(%)			)							
IgM (AU/mL)	4.67±16.44	7.25±24.35	2.70±4.55	0.149	2.75±2.95	5.41±19.26	0.447	3.94±5.65	4.83±17.98	0.828
Positive no(%)	9(8.11%)	6(12.5%)	3(4.76%)	0.139	8(10%)	1(3.23%)	0.241	3(15%)	6(6.59%)	0.212
Negative no(%)	102(91.89%)	42(87.5%)	60(95.24%)		72(90%)	30(96.77%)		17(85%)	85(93.41%)	
NAbs (AU/mL)	46.86±67.38	61.19±78.29	35.95±55.94	**0.05**	38.21±31.46	50.22±76.85	0.402	37.52±31.54	48.92±72.91	0.496
Positive no(%)	95(85.59%)	44(91.67%)	51(80.95%)	0.111	68(85%)	27(87.10%)	0.778	18(90%)	77(84.62%)	0.535
Negative no(%)	16(14.41%)	4(8.33%)	12(19.05%)		12(15%)	4(12.90%)		2(10%)	14(15.38%)	

Number of days: The mean number of days and standard deviation from vaccination and blood collection.

Discussion

The global COVID-19 pandemic continues to expand in many countries. A protective vaccine will be required to achieve sufficient herd immunity to SARS-CoV-2 infection to control the COVID-19 pandemic ultimately[14, 16]. Unlike typical vaccine development, which often takes decades, developing a vaccine to prevent COVID-19 has become a race between humans and the virus. Many countries have accelerated clinical trials to determine an effective and safe vaccine to avoid COVID-19 and influence the current pandemic[11, 12, 16].

The NAb is specific in the serum of patients with coronavirus that can block human cells' invasion. A NAb with antiviral activity only accounts for a small part of the antibodies secreted by B cells. NAbs can bind novel coronavirus surface protein and block virus protein binding to the cell-surface-specific receptor (angiotensin-converting enzyme 2, ACE2), thus preventing the virus from invading cells. When the neutralizing antibody titer is insufficient, it may lead to the deterioration of the antibody-dependent enhanced disease. The virus binds to Fc receptors on the cell surface through antibodies and then infects more types of cells, including macrophages, leading to a disordered immune response[1-3, 10, 18-21].

In the study, we collected blood samples from 111 participants who received rAd5 vectored COVID-19 vaccine and examined 2019-nCOV antibodies IgM, IgG and Nabs. We found that IgG and NAbs concentrations in all participants were inversely correlated with age, and significant differences were found between age groups. 102 (91.89%) and 95 (85.59%) participants were positive for 2019-nCoV IgG and NAbs, respectively. The IgG concentrations of participants younger than 40 years old were generally higher than that of participants older than 40 years old (P= 0.025). Similar results were found at the NAbs concentrations (P= 0.05). These results showed that the NAbs of the rAd5 vectored COVID-19 vaccine was more than 85% in the Chinese Han population, which provided certain reference information for the vaccine's protective efficacy.

It should be noted that there were some limitations of this study. First of all, the lack of test results in the control group of healthy people in this study may lead to false-positive problems that cannot be explained. Secondly, the number of samples in this study was limited. There were some differences in the number of pieces among each subgroup, which might affect the analysis results' accuracy. Finally, this study was a cross-sectional study without dynamic tracking and analysis of participants' antibody data after vaccination, which could not reflect vaccine protection duration.

In conclusion, the findings demonstrate that the NAbs of the rAd5 vectored COVID-19 vaccine was more than 85% in the Chinese, which provided certain reference information for the vaccine's protective efficacy.

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Data availability

The data supporting the results of this study are available upon request to the corresponding author.

Declarations

We declare that there are no competing financial and non-financial interests for any of the authors.

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Author Contributions

WJJ conceived and designed the experiments. WJJ and JWP performed publication searches and selection. WJJ, QYW and YH analyzed the data. WJJ and MC prepared the figures. WJJ contributed materials/analysis tools. WJJ and XYX wrote and revised the paper. All authors reviewed the manuscript.

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