Comparative Study of Seroneutralization and Antibody Response against the N-Protein of SARS-CoV-2 (July 2020)

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Background

The antibody-mediated humoral response is crucial for preventing viral infections. A subset of these antibodies binds to the surface epitopes of viral particles, blocking the entry of the virus into the host cells. These are defined as neutralizing antibodies, which reduce viral infectivity.

The major structural proteins of SARS-CoV-2 include the spike (S) and nucleocapsid (N) proteins. The S protein is responsible for allowing viruses to enter a cell by attaching to the host cell receptor. It comprises an N-terminal subunit, S1, which includes a virus receptor binding domain (RBD) and a C-terminal subunit, S2, responsible for virus cell membrane fusion (1, 2). The N protein is delivered into the cells and expressed abundantly during infection (3, 4, 5). Both antigens show high immunogenic activity.

The N protein is highly conserved among different strains of coronaviruses (CoVs) (6, 7), but still no cross-reactivity has been demonstrated with other related CoVs. Several studies based on the N protein of SARS-CoV-2 antigens observed no cross-reactivity with the N protein of seasonal CoVs associated with either the common cold (NL63, 229E, OC43 and HK1U) or MERS. The only significant cross-reactivity was seen with SARS-CoV (8, 9). However, SARS-CoV has not been circulating in the human population since 2003, making it unlikely that antibodies to this virus are present in the population. All these features make the N protein a potential target for serodiagnosing SARS-CoV-2 infection. To date, several diagnostic methods based on the N protein have been developed to better understand the epidemiology of SARS-CoV-2.

The N protein is one of the most immunogenic antigens of SARS-CoV-2, making it a prime candidate as target in immunological assays. Eurofins INGENASA and VIROTECH Diagnostics utilized this antigen for the development of serological assays to detect total antibodies (INgezim® COVID-19 DR) or the IgG, IgA and IgM antibody subclasses (VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA). Both assays demonstrate excellent performance to identify patients with a SARS-CoV-2 infection. However, it has not been scientifically clarified if the antibody response to the N protein is connected to the ability to neutralize the virus, one important factor to mediate immunity. To provide some answers to this question, using the INgezim® COVID-19 DR assay, we compared the total antibody response against the N protein in a set of 55 serum samples, with their ability to neutralize the virus. In parallel, we analyzed a subset of sera using the VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA that is specific for IgM, IgA and IgG antibodies to the N protein.

Materials and Procedures

Samples: 31 positive sera and 24 negative sera (confirmed through various methods: PCR, symptoms, serological tests).

Procedures

1. Virus seroneutralization (VSNT)

The neutralizing antibody titer was determined in 10 serum samples. For this, a known amount of SARS-CoV-2 was incubated with different dilutions of the serum sample to determine the dilution at which cytopathic effect is observed in 50% of infected wells (MN 50%). The detailed protocol is described below:

- Cell culture medium supplemented with 1X pen / strep were added to 96-well plates (60µL/well).
- Serum samples: two-fold serial dilutions of serum samples were performed, starting at 1:5 dilution and up to 1:640
- SARS-2 was prepared in 50mL of medium with antibiotic at a concentration of 10^3 TCID50/mL and 60µL was added to each well over the serum dilutions.
- The mixture of virus and serum was incubated at 37ºC for 1 hour
- After this incubation period, the medium was removed from the cells and 100µL of fresh medium was added to the well and incubated at 37ºC for 72 hours.
- 72 hours after infection, the plate was dipped in 10% formaldehyde to fix the cells and inactivate the virus. The formaldehyde was then removed and crystal violet was added for 15 minutes to observe the cytopathic effect.
- Finally, the neutralization titer was calculated and expressed as the **serum dilution** capable of **reducing the cytopathic effect to 50%** (MN 50%).

2. Serological methods

Several commercial ELISA were used for comparison purposes: VIROTECH (VIROTECH SARS-CoV-2 IgG or IgM or IgA ELISA), and INGENASA (INgezim COVID-19 DR). All the tests were carried out according to the manufacturers' instructions for use.

Results

A total of 55 serum samples were included in the study, of which 31 were considered true positive samples according to various techniques and 24 were true negatives. These sera were tested using seroneutralization (SNT) and INgezim® COVID-19 DR. Both assays showed 100% agreement (Figure 1).

n= 55		Virus seroneutralization (VSNT)	
		Positive	**Negative**
INgezim® COVID-19 DR	**Positive**	31	0
	Negative	0	24

		95% confidence interval
Sensitivity	100%	88,7% to 100%
Specificity	100%	85,6% to 100%

Figure 1. Sensitivity and specificity of INgezim® COVID-19 DR using a neutralization assay as the reference technique

After demonstrating that the total antibody response to the N protein shows a 100% correlation with the ability to neutralize the virus, of the 55 samples, 10 specimens were further tested using the antibody class-specific assay from VIROTECH Diagnostics (Table 1). The IgG ELISA showed the highest correlation to the seroneutralization assay. Only one sample showed a discrepant result, however, this samples was highly positive on the IgM ELISA. In conclusion, combining the IgG and IgM ELISA, the correlation between neutralization and N ELISA is 100% as well.

Table 1. Antibody titer of a panel of sera using neutralization and different ELISA tests. . Blue and orange colors indicate positive and negative results respectively, keeping in mind the cut-offs of the different techniques.

SERUM REF	NT	INgezim® COVID-19 DR	VIROTECH IgM	VIROTECH IgA	VIROTECH IgG
3	1:57	1:80	< 1:100	<1:100	1:1600
4	< 1:5	< 1:5	< 1:100	<1:100	< 1:100
8	< 1:5	< 1:5	< 1:100	<1:100	< 1:100
12	<1:5	< 1:5	< 1:100	<1:100	< 1:100
15	1:34	1:80	1:100	1:400	1:800
16	1:08	1:40	1:400	<1:100	< 1:100
22	1:12	1:5	<1:100	<1:100	1:100
28	1:28	1:5	<1:100	<1:100	1:400
33	1:26	1:40	<1:100	<1:100	1:800
61-3	1:15	1:20	<1:100	<1:100	1:400

Conclusion

In summary, although it is well known that the S protein of SARS-CoV-2, as for many other CoVs, is the main target for antibody-mediated neutralization (10, 11), we observed a strong correlation between the response of antibodies to the N protein, based on INgezim® COVID-19 DR and the antibody class specific assay VIROTECH SARS-CoV-2 IgG/IgM/IgA ELISA, in comparison to the detection of SARS-CoV-2 neutralizing antibodies in patients after infection. These results therefore indicate that antibodies produced against the N protein correlate with the presence of antibodies that can neutralize the virus. For that reason, the evaluated tests not only identify people exposed to SARS-CoV-2, but could also be used to predict immunity levels and to identify potential donors for plasma therapy.

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