Whole nucleocapsid protein of SARS-CoV-2 may cause false positive results in serological assays

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TO THE EDITOR –

The nucleocapsid protein (NP) of SARS-CoV-2 is one of the widely used antigens in serodiagnostics of the novel Coronavirus disease (COVID-19). We appreciate Guo and colleagues for shedding new light on the humoral response profile of COVID-19 [1]. They have generated recombinant whole nucleocapsid protein (rNP) of SARS-CoV-2 using *Escherichia coli* expression system and used it to develop an enzyme linked immunosorbent assay (ELISA) to detect anti–SARS-CoV-2 antibodies in plasma. The rNP ELISA was negative in the plasma of all the 285 non COVID-19 individuals tested. The authors have also shown that rNP does not have cross reactivity with antibodies of other common Coronaviruses; NL63, 229E, OC43, and HKU1. However, we have observed controversial findings and suggest the advantage of N-terminally truncated nucleocapsid protein (ΔN-NP).

Although the amino acid sequences of the entire nucleocapsid proteins (NP) of SARS-CoV and other common Coronaviruses are dissimilar to that of SARS-CoV-2 [1], the conserved residues at the N-terminal domain of NP show a high degree of similarity (Figure-1A). This was pointed out by Yu et al in SARS-CoV, who observed cross reactivity of whole NP with other Coronaviruses and encountered high rates of false positivity while testing healthy donor sera [2]. They had overcome this problem by using ΔN-NP which was devoid of the homogenous conserved residues at the N-terminal region [2,3].

Since SARS-CoV-2 NP has over 90% homology to SARS-CoV NP [1] and their conserved residues are almost identical (Figure-1A), we expected the possibility of cross reactions with whole/full length NP (FL-NP). We expressed both FL-NP and ΔN-NP of SARS-CoV-2 in wheat germ cell free protein production system [4] and designed an IgG detection ELISA to compare their performance. Upon testing sera collected from 70 healthy donors before the outbreak of COVID-19, FL-NP showed higher false positivity than ΔN-NP at any level of optimized sensitivity in 1:100 diluted sera (Figure-1B). We then tested 20 COVID-19 positive sera collected on or after 8th day of symptom onset and compared it against the 70 healthy controls. The ΔN-NP ELISA was positive in all COVID-19 patients and did not show false positives in the controls at any cut-off. On the other hand, FL-NP

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ELISA exhibited false negatives in COVID-19 sera with the low sensitivity cut-off value set to eliminate false positives in healthy donor samples.

We gather that Guo et al might have used a lower sensitivity cutoff to avoid false positives in their rNP ELISA. But by doing so, early diagnosis COVID-19 can be missed because the antibodies are still on the rise. Both IgM and IgG appear early in the second week after symptom onset and peaks after the third week [5]. In the time-course analysis, the ΔN-NP ELISA detected the lower level of antibodies that began to increase from second week onwards while FL-NP ELISA at the same cut-off would have falsely reported these as negative (Figure-1C).

Detection of anti-viral antibodies can be used both for diagnosis and population surveillance, ideally with the former possessing high sensitivity and the latter high specificity. We aimed to enhance the sensitivity to detect the low level of IgG in patients during relatively earlier stages of the disease. With higher sensitivity, FL-NP based ELISA gives a higher background but this occurs to a much lesser extent with ΔN-NP. We presume this is probably due to the cross reactivity of FL-NP with antibodies to other human coronaviruses and this should be studied further. We conclude that ΔN-NP is better suited than FL-NP to develop high sensitivity diagnostic assays for COVID-19.

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NOTES

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Conflict of interests

The authors have no conflicts of interest directly relevant to the content of this article. Y.Y. is a current employee of Kanto Chemical Co., Inc.

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Figure legend

N-terminally truncated nucleocapsid protein (ΔN-NP) of SARS-CoV-2 as a serodiagnostic marker of COVID-19.

A. Multiple sequence alignment of the conserved residues in NP of closely related human coronaviruses and diagrammatic representation of the ΔN-NP construct. NTD: N-terminal domain, CTD: C-terminal domain, SR: Serine-Arginine.

B. Comparison of sensitivities of IgG ELISA using FL-NP and ΔN-NP as antigen. 70 healthy donor sera collected before COVID-19 outbreak and sera of 20 COVID-19 patients collected on or after 8 days of symptom onset were subjected to ELISA. High: Higher sensitivity cut off line, Low: Lower sensitivity cut off line.

C. Time course profiling of serum IgG against ΔN-NP using sera of five COVID-19 patients.

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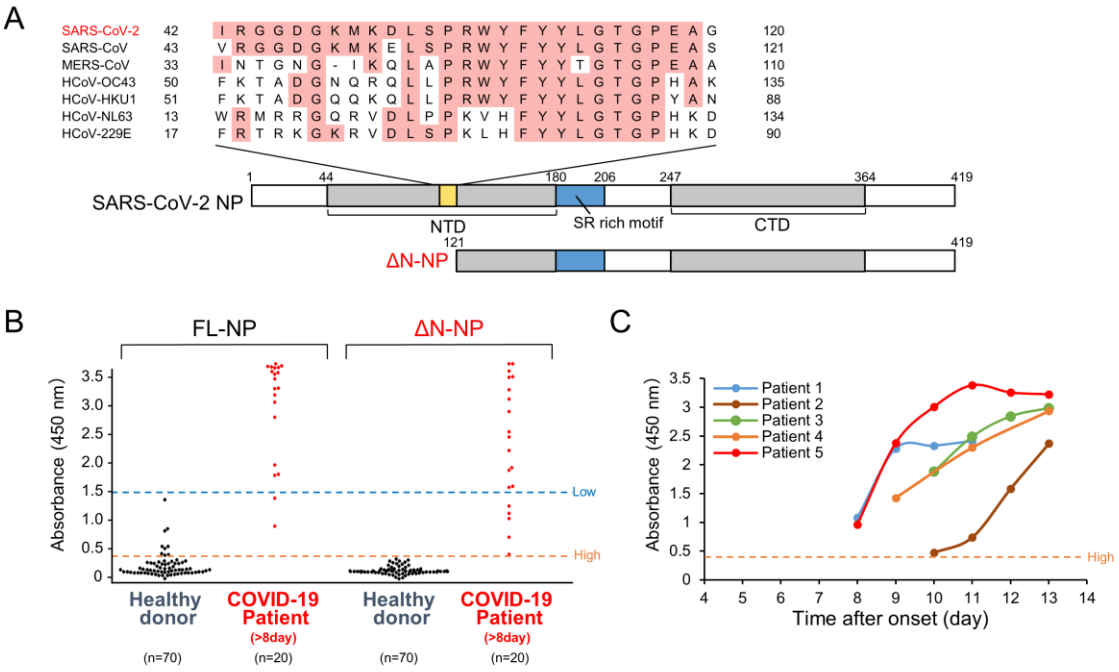
Figure 1


A
SARS-CoV-2 42 I R G G D G K M K D L S P R W Y F Y Y L G T G P E A G 120
SARS-CoV 43 V R G G D G K M K E L S P R W Y F Y Y L G T G P E A S 121
MERS-CoV 33 I N T G N G - I K Q L A P R W Y F Y Y T G T G P E A A 110
HCoV-OC43 50 F K T A D G N Q R Q L L P R W Y F Y Y L G T G P H A K 135
HCoV-HKU1 51 F K T A D G Q Q K Q L L P R W Y F Y Y L G T G P Y A N 88
HCoV-NL63 13 W R M R R G Q R V D L P P K V H F Y Y L G T G P H K D 134
HCoV-229E 17 F R T R K G K R V D L S P K L H F Y Y L G T G P H K D 90
SARS-CoV-2 NP
1
44
180
206
247
364
419
NTD
SR rich motif
CTD
ΔN-NP
121
419
B
FL-NP
ΔN-NP
Absorbance (450 nm)
Low
High
Healthy donor
COVID-19 Patient (>8day)
(n=70)
(n=20)
C
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Time after onset (day)