improved, but COVID-19 has become a pandemic and has spread rapidly across the whole world. Although the disease was first reported in China, the source of the virus has not been confirmed. Many issues regarding prevention, control, and treatment of the disease need to be addressed by medical scientists and sociologists worldwide.

As a doctor infected with this highly infectious disease, B. Q. sincerely exhort that all of us stay in awe of the virus but do not despair or panic. We should not only maintain proactive health and hygiene regiments, but also cooperate and study actively to fully understand and defeat COVID-19.

Notes

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Whole Nucleocapsid Protein of Severe Acute Respiratory Syndrome Coronavirus 2 May Cause False-Positive Results in Serological Assays

To the Editor—The nucleocapsid protein (NP) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the widely used antigens in serodiagnostics of the novel coronavirus disease 2019 (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 the *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 of 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 NP of severe acute respiratory syndrome (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 > 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 immunoglobulin G (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 the eighth 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 cutoff. On the other hand, FL-NP ELISA exhibited false negatives in COVID-19 sera with the low sensitivity cutoff 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 immunoglobulin M 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 the second week onward while FL-NP ELISA at the same cutoff would have falsely reported these as negative (Figure 1C). Detection of antiviral 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

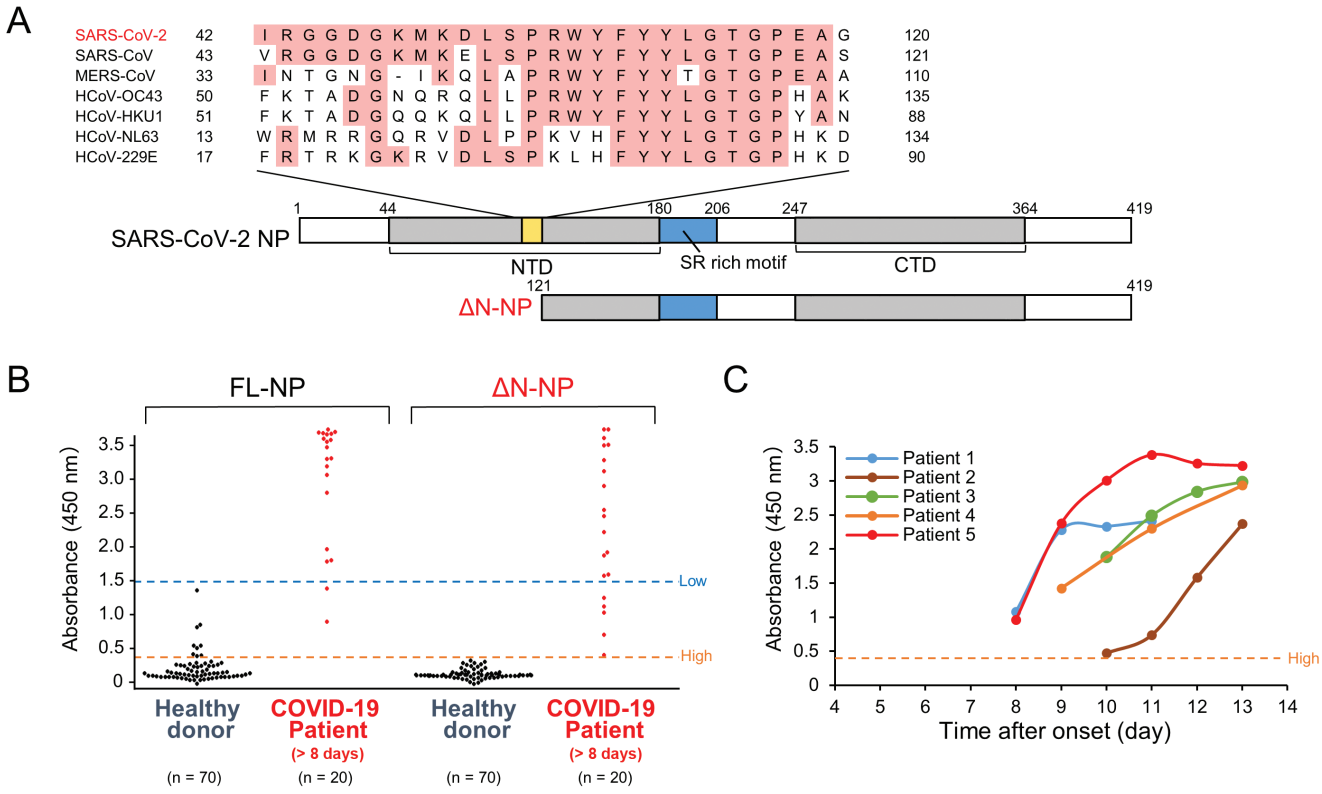

Figure 1. N-terminally truncated nucleocapsid protein (ΔN-NP) of severe acute respiratory syndrome coronavirus 2 as a serodiagnostic marker of coronavirus disease 2019 (COVID-19). *A*, Multiple sequence alignment of the conserved residues in nucleocapsid protein (NP) of closely related human coronaviruses and diagrammatic representation of the ΔN-NP construct. *B*, Comparison of sensitivities of immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) using whole/full-length NP and ΔN-NP as antigen. Seventy 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. *C*, Time course profiling of serum IgG against ΔN-NP using sera of 5 COVID-19 patients. Abbreviations: ΔN-NP, N-terminally truncated nucleocapsid protein; COVID-19, coronavirus disease 2019; CTD, C-terminal domain, FL-NP, whole/full-length nucleocapsid protein; HCoV, human coronavirus; High, higher sensitivity cutoff line; Low, lower sensitivity cutoff line; MERS-CoV, Middle East respiratory syndrome coronavirus; NP, nucleotide protein; NTD, N-terminal domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SR, serine-arginine.

high-sensitivity diagnostic assays for COVID-19.

Notes

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