Antibody profiles in mild and severe cases of COVID-19

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To the Editor:

The ongoing COVID-19 pandemic has spread to more than 200 countries and territories. As of 13 May 2019, there are >4 million confirmed cases and about 7% of these patients have died from the infection. Nucleic acid amplification tests are the methods of choice for detecting COVID-19 patients at early disease onset. Several serological tests have been developed for detecting SARS-CoV-2-specific antibodies for clinical research (1, 2). Farnsworth and Anderson have recently highlighted the limitations of SARS-CoV-2 antibody testing for routine clinical application due to limited data regarding its utility (3). Here, we report the use of antibody tests to study severe and mild COVID-19 cases.

192 RT-PCR confirmed COVID-19 patients admitted to the First Affiliated Hospital of Nanchang University, China were studied. Patients who had one of the following conditions at the time of, or following admission were classified as severe cases: 1) respiratory distress (≥30 breaths/min), 2) oxygen saturation at rest ≤93%, 3) PaO_2/FiO_2 ratio ≤300 mmHg or 4) severe complications (e.g. respiratory failure, requirement of mechanical ventilation, septic shock and/or non-respiratory organ failure). Amongst these 192 patients, 83 (43%) were classified as severe cases. Single or serial serum samples (N=1,019; sampling period: 01/29/2020-03/28/2020) collected from these 192 patients were tested for SARS-CoV-2 spike protein receptor binding domain (RBD)-specific IgM or total antibodies (IgA/IgG/IgM) using two commercial microparticle chemiluminescence immunoassays (Wantai). In addition 144 control sera collected in the same period were tested. All control samples were negative in the IgM assay and 98.6% (142/144) were negative in the total antibody assay (**Fig 1A and 1B**).

We first stratified our results according to the date of disease onset and disease severity. The IgM antibody responses of mild and severe cases within the first 6 days of disease onset were not statistically different from each other (**Fig 1A**; $P>0.05$). Interestingly, the IgM profile of mild cases was found to be statistically different from the severe cases thereafter. Severe cases had significantly higher IgM titers than mild cases after Day 6 post-onset (**Fig 1A**). The mean IgM titer of severe cases peaked at about day 21 post-symptom onset, whereas the mild cases did not have such a sharp peak over time. We noted that only 46.9% of all tested mild case samples were IgM positive, whereas 85.7% of all tested severe case samples were IgM positive. From samples taken within Days 7-12 post-onset, the IgM positive rate of severe cases was higher than that of mild cases ($P=0.013$; chi-square test). Additionally, all severe patients were IgM positive from Days 13 to 18 post-symptom onset, whereas only 57% of mild patients were positive in this period.

The titers of severe cases for the total antibody test were also statistically higher than those of mild cases from days 7-42 post-symptom onset (**Fig 1B**). Over 99% of severe patients from day 13 of disease onset or beyond were positive by the total antibody test. The overall positivity rate of severe cases (98.7%) was higher than that of mild cases (83.0%) ($P<0.00001$; chi-square test). These results are similar to those seen above for the IgM antibody test, suggesting that COVID-19 patients are more likely to mount robust antibody responses in severe cases. In addition, unlike the IgM profile as described above, the total antibody titers of both severe and mild cases remained at high levels until the end of our study period.

We noted that several mild cases were serologically negative in our assays. We further analyzed data from 35 patients with multiple serial samples (N > or = 3) in whom there was at least 1 sample collected before Day 19. Strikingly, 34.3% (12/35) and 14.3% (5/35) of studied mild patients were consistently serologically negative for IgM and total antibody (Data not shown), respectively. In addition, those who were negative in the total antibody test were also negative in the IgM test. By contrast, all severe patients (N=42) with multiple serial samples had at least 1 positive sample in these tests.

In this study, we observed that patients with severe COVID-19 are more likely to mount robust antibody responses than those with mild cases. Our results agree with those reported by Zhou and colleagues (4). It is not known whether the enhanced antibody responses are associated with the immunopathology observed in severe COVID-19 cases (5). Owing to the limitation of our assays, the antibody profiles of IgG and IgA in the studied patients could not be determined. Nonetheless, it is interesting to note that there were several mild COVID-19 cases that failed to develop antibodies against the RBD-domain of the spike protein. These results might have implications for clinical diagnosis, serological surveillance and control policies (e.g. immunity passport) for COVID-19. It is not known whether these mild cases can develop antibodies against other epitopes of SARS-CoV-2. Further characterization of this group of mild cases is warranted.

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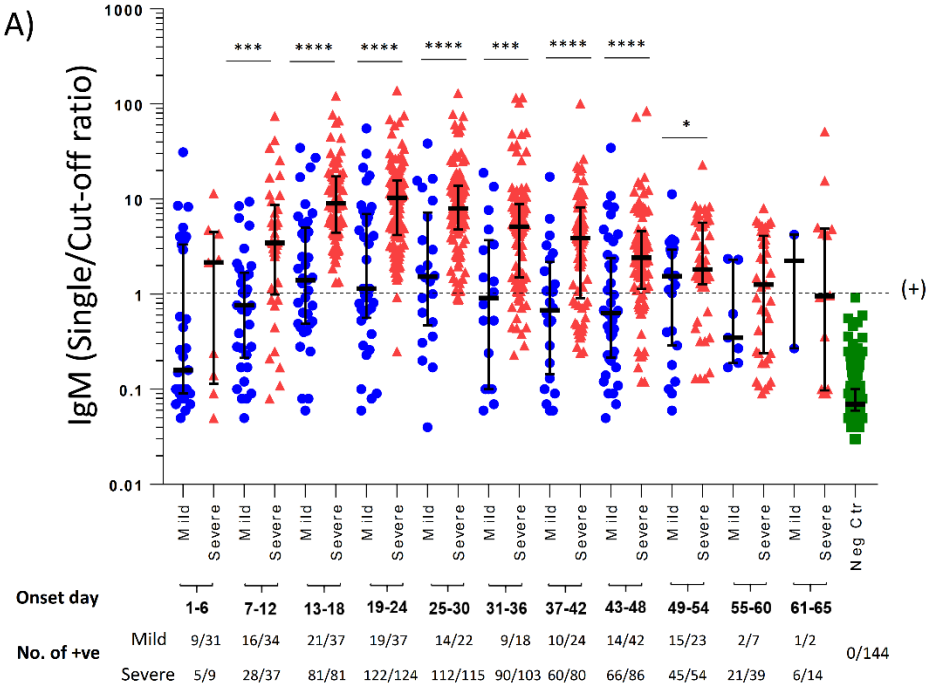
Figure legend

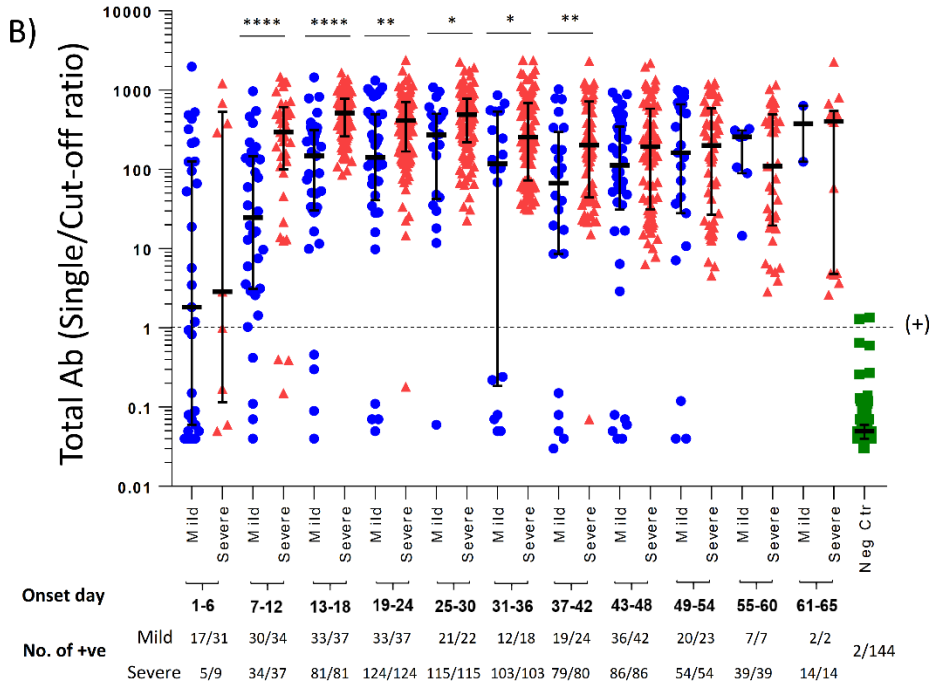
Figure. Antibody profiles of all COVID-19 patients. The levels of IgM (panel A) and total Ab (panel B) specific for the SARS-CoV-2 spike protein receptor binding domain (RBD) from patients at different periods after disease onset are shown (where day 1: the first day symptoms begin). The cut off value of positivity for each assay is indicated by a dotted line. The numbers of tested cases in severe and mild groups are indicated. Control serum samples (green; N=144) were collected from individuals who were not known to have COVID-19-like symptoms, but none of these donors were screened for SARS-CoV-2 by RT-PCR. Mann-Whitney test: ****P<0.0001, ***P<0.001, **P<0.01; *P<0.05.

A)



Onset day	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54	55-60	61-65	
No. of +ve Mild	9/31	16/34	21/37	19/37	14/22	9/18	10/24	14/42	15/23	2/7	1/2	0/144
Severe	5/9	28/37	81/81	122/124	112/115	90/103	60/80	66/86	45/54	21/39	6/14	

B)



Onset day	1-6	7-12	13-18	19-24	25-30	31-36	37-42	43-48	49-54	55-60	61-65	
No. of +ve Mild	17/31	30/34	33/37	33/37	21/22	12/18	19/24	36/42	20/23	7/7	2/2	2/144
Severe	5/9	34/37	81/81	124/124	115/115	103/103	79/80	86/86	54/54	39/39	14/14	