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1 # A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients

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30 **Abstract**

31 **Background** The outbreak of the recently emerged novel corona virus disease 2019
32 (COVID-19) poses a challenge for public health laboratories. We aimed to evaluate
33 the diagnostic value of serological assay for SARS-CoV-2.

34 **Methods** A newly-developed ELISA assay for IgM and IgG antibodies against N
35 protein of SARS-CoV-2 were used to screen the serums of 238 admitted hospital
36 patients with confirmed or suspected SARS-CoV-2 infection from February 6 to
37 February 14, 2020. SARS-CoV-2 RNA was detected by real time RT-PCR on
38 pharyngeal swab specimens.

39 **Findings** Of the 238 patients, 194 (81.5%) were detected to be antibody (IgM and/or
40 IgG) positive, which was significantly higher than the positive rate of viral RNA
41 (64.3%). There was no difference in the positive rate of antibody between the
42 confirmed patients (83.0%, 127/153) and the suspected patients (78.8%, 67/85) whose
43 nucleic acid tests were negative. After the patients were defined to the different stages
44 of disease based on the day when the test samples were collected, the analysis results
45 showed that the antibody positive rates were very low in the first five days after initial
46 onset of symptoms, and then rapidly increased as the disease progressed. After 10
47 days, the antibody positive rates jumped to above 80% from less than 50%. On the
48 contrary, the positive rates of viral RNA kept above 60% in the first 11 days after
49 initial onset of symptoms, and then rapidly decreased. In addition, half of the
50 suspected patients with symptoms for 6-10 days were detected to be antibody positive.

51 **Interpretation** The suspected patients were most likely infected by SARS-CoV-2.

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52 Before the 11th day after initial onset of symptoms, nucleic acid test is important for
53 confirmation of viral infection. The combination of serological assay can greatly
54 improve the diagnostic efficacy. After that, the diagnosis for viral infection should be
55 majorly dependent on serological assay.

56 **Keywords.** SARS-CoV-2; diagnosis; serological assay; nucleic acid test

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58 **Introduction**

59 A novel betacoronavirus [1] named severe acute respiratory syndrome coronavirus
60 2 (SARS-CoV-2) causes a recent cluster cases of respiratory illness named corona
61 virus disease 2019 (COVID-19) in multiple regions of the world, and it leads to a
62 serious public health problem especially in Wuhan city, Hubei province, China since
63 December 2019. [2] By March 1, 2020, more than 80,000 confirmed cases have been
64 identified globally both in China and other 58 countries spanning Asia, Europe,
65 Oceania, North and South America, and Northeast Africa. It is evidenced that
66 SARS-CoV-2 can transmit rapidly from person to person, which is evidently found in
67 hospital and family settings. [3-5]

68 The SARS-CoV-2 is the seventh member of enveloped RNA coronaviruses
69 (CoVs). [6-8] Sequence and phylogenetic tree of CoVs analysis indicates that
70 SARS-CoV-2 is genetically distinct from SARS-CoV and is more closely related to
71 bat-SL-CoV ZC45 and bat-SL-CoV ZXC21. [1] SARS-CoV-2 owns a similar
72 receptor-binding domain structure to that of SARS-CoV. [1] A typical CoV contains four
73 main structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N)
74 proteins. The S protein homotrimers are required for attachment to host receptors, [9]
75 and both the M protein and the E protein play important roles in virus assembly. [10,11]
76 The N protein is responsible for packaging the encapsidated genome into virions, [12,13]
77 and acts as a viral RNA silencing suppressor that is beneficial for the viral replication.
78 [14] Furthermore, the N protein has high immunogenic activity and is profusely
79 overexpressed during infection, [15] indicating that N protein should be a potential

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80 source of a diagnostic antigen for detecting SARS-CoV-2 infection. Many diagnostic
81 methods based on the N protein have been developed for SARS-CoV detection. [16-18]
82 In addition, different CoVs possess special structural and accessory proteins, such as
83 HE protein, 3a/b protein, and 4a/b protein. [19]

84 Both nucleic acid test and serological assay are commonly used for infectious
85 disease screening and diagnosis. In the present case of SARS-CoV-2, nucleic acid test
86 has been being routinely used to detect causative viruses from respiratory secretions
87 by real-time RT-PCR in China. However, the nucleic acid tests appeared to have a
88 high false negative rate because of several unavoidable reasons, including the
89 sensitivity of the detection kits that have not been well assessed, sampling location
90 and technique, etc. A large number of clinically-suspected patients, whose nucleic
91 acid tests were negative, are unable to get timely confirmed-diagnosis and hospital
92 treatment, which potentially promotes the spread of SARS-CoV-2 and leads to a rapid
93 disease progress of the suspected patients.

94 In this study, a newly-developed IgM and IgG antibody detecting Enzyme-linked
95 immunosorbent assays (ELISA) based on a recombinant fragment of SARS-CoV-2 N
96 protein were used to detect IgM and IgG against SARS-CoV-2 in serum of 238
97 admitted hospital patients with confirmed or suspected SARS-CoV-2 infection. The
98 results strongly indicated that the suspected patients were infected. We also analyze
99 the diagnostic value of the IgM and IgG testing in COVID-19, even in the early stage
100 of disease.

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102 **Methods**

103 ***Patients and samples***

104 All consecutive patients (n=238) with confirmed or suspected SARS-CoV-2
105 infection who have been tested by real-time RT-PCR for viral infection and were
106 being treated in General Hospital of Central Theater Command of PLA from February
107 6 to February 14, 2020, were enrolled. The general information (age, sex, vital signs,
108 coexisting disorders), clinical, laboratory, and radiological characteristics data of the
109 patients on admission were extracted from electronic medical records. Among the 238
110 recruited patients, 153 patients were laboratory-confirmed cases, who were tested
111 positive for viral RNA by real time RT-PCR assay on pharyngeal swab specimens, and
112 the remaining 85 patients having negative results for real time RT-PCR assay were
113 clinically diagnosed as highly-suspected cases according to the notice on the issuance
114 of strategic guidelines for diagnosis and treatment of COVID-19. [20] The serum
115 samples were collected once from each recruited patient. Meanwhile, the serum
116 samples from 70 ordinary patients and 50 healthy blood donors were randomly
117 selected as the controls. The study was approved by the Hospital Ethics Committee
118 and written informed consent was waived for emerging infectious diseases.

119 ***Real time Reverse Transcription Polymerase Chain Reaction (RT-PCR) Assay***

120 Pharyngeal swab specimens were collected from patients and placed into a
121 collection tube with 200 μL of virus preservation solution. Total RNA was extracted
122 using the respiratory sample RNA isolation kit (Shuoshi, Shanghai, China). After
123 vortex, 50 μL of cell lysates were transferred into another collection tube. The

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124 collection tube was centrifugated at 1000 rpm/min for 5 min after standing at room
125 temperature for 10 minutes. 5 μL RNA was prepared and used for real time RT-PCR.
126 Real time RT-PCR was performed using the nucleic acid testing kit (Daan,
127 Guangzhou, China) for SARS-CoV-2 detection. The open reading frame 1ab
128 (*ORF1ab*) and nucleocapsid protein (N) were simultaneously selected as the two
129 target genes. The human *GAPDH* gene was used as an internal control. The specific
130 primers and probes set for *ORF1ab* and N were as follows: *ORF1ab*-forward primer
131 5'-ACCTTCTCTTGCCACTGTAGC-3'; *ORF1ab*-reverse primer
132 5'-AGTATCAACCATATCCAACCATGTC-3'; and the probe
133 5'-FAM-ACGCATCACCCAACTAGCAGGCATAT-BHQ1-3'; N-forward primer
134 5'-TTCAAGAAATTCAACTCCAG-3'; N-reverse primer
135 5'-AGCAGCAAAGCAAGAGCAGCATC-3'; and the probe
136 5'-VIC-TCCTGCTAGAATGGCTGGCAATGGCG-BHQ1-3'. The real time RT-PCR
137 experiment was thoroughly performed according to kit's instructions. The reaction
138 mixture contains 17 μL of reaction buffer A, 3 μL of reaction buffer B, and 5 μL RNA
139 template. The real time RT-PCR assay was performed under the following conditions:
140 incubation at 50 °C for 15 min and 95 °C for 15 min, 45 cycles of denaturation at
141 94 °C for 15 s, and extending and collecting fluorescence signal at 55 °C for 45 s. A
142 cycle threshold value (Ct-value) ≤ 40 was defined as a positive test result, and a
143 Ct-value > 40 was defined as a negative test.

144 ***Enzyme-Linked Immunosorbent Assay (ELISA)***

145 Serological assay was performed using Enzyme-Linked Immunosorbent Assays

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146 kit (Lizhu, Zhuhai, China), which was established for detecting IgM or IgG antibody
147 against N protein of SARS-CoV-2. For IgM detection, ELISA plates were previously
148 coated with mouse anti-human IgM (μ chain) monoclonal antibody. 100 μL diluted
149 (1:100) serum sample was added into the pre-coated plates with three replicating
150 wells for each sample and incubated at 37 °C for 1 h. The heat-inactivated positive
151 and negative serum were included on each plate. After washing, 100 μL horse radish
152 peroxidase (HRP) conjugated recombinant (rN) protein of SARS-CoV-2 was added.
153 Then the plate was incubated at 37 °C for 30 min followed by washing. 50 μL of
154 TMB substrate solution and 50 μL of the corresponding buffer were added and
155 incubated at 37 °C for 15 min. The reaction was terminated by adding 50 μL of 2 M
156 sulfuric acid, and the absorbance value at 450 nm (A_{450}) was determined. The cut off
157 value was calculated by sum of 0.100 and average A_{450} of negative control replicates.
158 A_{450} less than cut off value was defined as a negative test, and A_{450} greater than or
159 equal to cut off value was defined as a positive test.

160 For IgG detection，ELISA plates were previously coated with rN protein. 5 μL
161 serum sample diluted with 100 μL dilution buffer were added into the plates. After
162 incubation and washing, HRP-conjugated mouse anti-human IgG monoclonal
163 antibody was added into the plates for detection. The other operation steps were
164 performed as described in the above IgM detection. The cut off value was calculated
165 by the sum of 0.130 and average A_{450} of negative control replicates. A_{450} less than cut
166 off value was defined as a negative test, and A_{450} greater than or equal to cut off value
167 was defined as a positive test.

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168 ***Statistical analysis***

169 Continuous variables were described as the means and standard deviations or
170 medians and interquartile ranges (IQR) values. Categorical variables were expressed
171 as the counts and percentages. Independent group t tests were applied to continuous
172 variables that were normally distributed; otherwise, the Mann-Whitney test was used.
173 Categorical variables were compared using the chi-square tests, while the Fisher exact
174 test was used when the data were limited. Statistical analyses were performed using
175 Statistical Package for the Social Sciences (SPSS) version 22.0 software. A two-sided
176 α of less than 0.05 was considered statistically significant.

177

178 **Results**

179 ***Demographics and patient characteristics***

180 The serum samples were collected from 238 admitted hospital patients with
181 confirmed or suspected SARS-CoV-2 infection in General Hospital of Central Theater
182 Command of PLA from February 6 to February 14, 2020. The clinical characteristics
183 of the patients were shown in Table 1. The median age was 55 years (IQR, 38.3-65),
184 and 138 (58.0%) of the patients were men. Hypertension (63 [26.5%]), diabetes (25
185 [10.5%]), and cardiovascular disease (24 [10.1%]) were the most common coexisting
186 disorders. Of these patients, the most common symptoms at illness onset were fever
187 (206 [86.6%]), dry cough (128 [53.8%]), and fatigue (78 [32.8%]). A small number of
188 patients possessed the symptoms of abdominal pain (1 [0.4%]), vomiting (3 [1.3%]),
189 and dizziness (4 [1.7%]). On admission, leucocytes were below the normal range in

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190 41 (17.2%) patients and above the normal range in 21 (8.8%) patients. 125 (52.5%)
191 patients had lymphocytes below the normal range and no patients were found to have
192 lymphocytes above the normal range. Neutrophils were below the normal range in 28
193 (11.8%) patients and above the normal range in 32 (13.4%) patients. According to CT,
194 235 (98.7%) patients showed ground-glass opacity and/or patchy shadowing.

195 According to the positive or negative results of real time RT-PCR assay for
196 pharyngeal swab specimens, the enrolled patients were divided into two groups: the
197 confirmed group and the suspected group. There were no statistical differences of
198 baseline characteristics of the two groups patients.

199 ***Performance and validation of ELISA assays for viral specific IgM and IgG***
200 ***antibodies***

201 Each serum sample of 238 patients were respectively tested for IgM and IgG
202 antibodies against SARS-CoV-2 by using newly-developed ELISA kits based on N
203 protein of SARS-CoV-2. The IgM and/or IgG could be detected in 194 serum samples,
204 and the positive rate (81.5%) was significantly higher than that of SARS-CoV-2
205 RNA detected by real time RT-PCR, which was 64.3% (153/238) (Fig.1A).
206 Importantly, there were no difference in positive rates of IgM and/or IgG between the
207 confirmed patients (83.0%, 127/153) and the suspected patients (78.8%, 67/85)
208 (Fig.1B), suggesting that the clinically suspected patients, who were viral RNA
209 negative detected by RT-PCR, were mostly infected.

210 To verify the specificity of the ELISA assays, the serum samples from 70
211 randomly-selected ordinary patients and 50 healthy blood donors were simultaneously

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212 detected. Four samples from the ordinary patients were identified as antibody positive
213 (including one dual positive sample, two IgM-positive samples, and one IgG-positive
214 sample) and no positive was found in the samples of healthy blood donors (Fig.1A).
215 These results confirmed the specificity of the IgG and IgM ELISA assays.

216 ***Dynamic analysis of ELISA and RT-PCR assays***

217 To study the diagnostic value of ELISA assay for virus-specific antibodies,
218 especially in the early stage of the disease, we tried to analyze the positive rates of
219 ELISA and RT-PCR assays in the different stages of disease. To this end, each patient
220 was assigned to different days after initial onset of symptoms based on the time when
221 the pharyngeal swab specimen was detected to be positive or the last recoded
222 detection was still negative, or the blood was collected. The positive rates of viral
223 RNA, IgM and/or IgG were compared in every day after initial onset of symptoms
224 (Table 2). Due to a small number of samples in each individual day, we pooled the
225 samples in which the positive rates were similar in consecutive days. Thus, the disease
226 process were divided into five phases of 0-5, 6-10,11-12, 13-15 days and more than
227 16 days after initial onset of symptoms (Table 3 & Fig.2). The data showed that
228 positive rates of IgM and/or IgG were very low in the first five days after initial onset
229 of symptoms because there was no antibody produced in most of patients in this early
230 stage, and then rapidly increased as the disease progressed. Day 11 after initial onset
231 of symptoms is a key time point because the positive rates of IgM and/or IgG jumped
232 to above 80% from less than 50% at this time point. The dynamic pattern is consistent
233 with SARS-CoV infection. [21] On the contrary, the real-time RT-PCR was more

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234 effective for detecting SARS-CoV-2 infection than ELISA in the early stage of disease.
235 The positive rate of viral RNA detected by RT-PCR was maintained above 60% in the
236 first 11 days after initial onset of symptoms, and then rapidly decreased with the rapid
237 increase of positive rate of antibodies. These results demonstrated that ELISA-based
238 IgM and/or IgG detection should be used as a major viral diagnostic test for the
239 patients with symptoms for more than 10 days. Because about 50%
240 clinically-suspected patients with symptoms for 6-10 days were detected to be
241 positive by ELISA-based IgM and/or IgG detection (Table 4), the combination of
242 ELISA and RT-PCR assays will greatly improve the detection efficacy, even in the
243 early stage of COVID-19 infection.
244

245 **Discussion**

246 The outbreak of the recently emerged novel coronavirus (SARS-CoV-2) poses a
247 challenge for public health laboratories, especially for clinical laboratories of the
248 hospitals in Wuhan, China. Although serological assay is a frequently used method for
249 viral infection screening and diagnosis, there are few reports about serological assay
250 in detection of SARS-CoV-2 up to now. In this study, we report the application of the
251 SARS-CoV-2 N protein-based ELISA for detection of IgM and IgG antibodies in the
252 admitted hospital patients with confirmed or suspected SARS-CoV-2 infection. The
253 results showed that the positive rates of IgM and IgG were significantly higher than
254 that of viral RNA detected by real-time RT-PCR on the pharyngeal swab specimens of
255 all enrolled patients. This result is further supported by the fact that the suspected

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256 patients had the same positive rate of antibody as the confirmed patients. These data
257 strongly demonstrated that the clinically suspected patients were mostly infected by
258 SARS-CoV-2.

259 In this study, the serum samples were collected in a time period of 9 days from
260 the patients in different stages of disease. The positive rates of nucleic acid test and
261 serological assay in total populations cannot reflect their diagnostic value in
262 surveillance and control of the disease, because the production of antiviral antibodies
263 will decrease the positive rates of the nucleic acid test as the disease progresses. In
264 order to objectively determine the disease stage of the patients, we used the initial
265 onset of symptoms of the patients as the start time point. Based on the day when the
266 test sample was collected, all patients were defined to the different stages of disease.
267 The resulted dynamics patterns of positive rates of viral RNA and antiviral antibodies
268 proved the rationality of the disease stage definition. Our analysis identified the 11th
269 day after initial onset of symptoms as a key time point in the disease process when
270 most infected patients produce antiviral antibodies. After this time point, the diagnosis
271 for viral infection should majorly depend on serological assay. Before this time point,
272 nucleic acid test is important for confirmation of viral infection. The combination of
273 serological assay can greatly improve the diagnostic efficacy.

274 According to the rapid advice guideline for the diagnosis and treatment of
275 SARS-CoV-2 currently implemented in China, [20] a confirmed case of COVID-19
276 patients exclusively depends on the positive result of nucleic acid test or virus gene
277 sequencing. Although this is a preliminary ELISA assay for SARS-CoV-2, our study

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278 strongly demonstrate that serological assay is very important for surveillance and
279 control of the current COVID-19, especially in Wuhan of China, where a lot of
280 patients are waiting to be confirmed at present. Moreover, most of patients had
281 symptoms for more than 11 days.

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289 **Conflict of interest**

290 The authors declare that no conflict of interest exists.

291 **Authors' contributions**

292 S.Z., S.W., and L.L. conceived the study and designed experimental procedures;
293 W.L., Y.Z., W.N.,Y.D., W.W., S.T., X.J., J.D., Q.H., Z.H., W.X., Y.Z., B.Z., Z.T.,
294 X.Z., H.L., Z.R., H.J., and X.R. collected patients' samples. Q.W. and L.T. performed
295 viral RNA tests. G.K.,W.L. W.N., and Y.Z established ELISA and performed
296 serological assays. S.W., L.L., S.Z., and W.L. wrote the paper. All authors contributed
297 to data acquisition, data analysis, or data interpretation, and reviewed and approved
298 the final version.

299

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Table 1. Demographic and baseline characteristics of 238 enrolled patients

Characteristics	All patients (N=238)	Confirmed (N=153)	Suspected (N=85)	P value
Age, Median (IQR) – y	55.0 (38.3-65.0)	54.0 (39.0-64.0)	55.0 (38.0-65.0)	0.656
Sex				
Male	138 (58.0%)	93 (60.8%)	45 (52.3%)	0.240
Female	100 (42.0%)	60 (39.2%)	40 (47.6%)	
Vital signs				
Heart rate	89 (80-100)	89 (80-99)	90 (80-104)	0.496
Respiratory rate	18 (18-20)	19 (18-20)	18 (18-20)	0.059
Oxygen saturation	96% (94%-98%)	96% (94%-98%)	97% (95%-98%)	0.292
CT findings of ground-glass opacity and/or patchy shadowing	235/238 (98.7)	151/153 (98.7)	84/85 (98.8)	0.931
Leukocytes (×10^9 per L; normal range 3.5-9.5)				
Decreased	41/238 (17.2)	25/153 (16.3)	16/85 (18.8)	0.627
Increased	21/238 (8.8)	16/153 (10.5)	5/85 (5.9)	0.233
Lymphocytes (×10^9 per L; normal range 1.1-3.2)				
Decreased	125/238 (52.5)	84/153 (54.9)	41/85 (48.2)	0.324
Neutrophils (×10^9 per L; normal range 1.8-6.3)				
Decreased	28/238 (11.8)	17/153 (11.1)	11/85 (12.9)	0.675
Increased	32/238 (13.4)	22/153 (14.4)	10/85 (11.8)	0.571
Signs and symptoms				
Fever	206/238 (86.6)	134/153 (87.6)	72/85 (84.7)	0.533
Dry cough	128/238 (53.8)	86/153 (56.2)	42/85 (49.4)	0.314
Fatigue	78/238 (32.8)	47/153 (30.7)	31/85 (36.5)	0.365
Myalgia	46/238 (19.3)	29/153 (19.0)	17/85 (20.0)	0.845
Dyspnea	44/238 (18.5)	31/153 (20.3)	13/85 (15.3)	0.344
Chill	31/238 (13.0)	20/153 (13.1)	11/85 (12.9)	0.977
Anorexia	29/238 (12.2)	14/153 (9.2)	15/85 (17.6)	0.055
Diarrhea	24/238 (10.1)	14/153 (9.2)	10/85 (11.8)	0.521
Expectoration	21/238 (8.8)	14/153 (9.2)	7/85 (8.2)	0.812
Headache	15/238 (6.3)	12/153 (7.8)	3/85 (3.5)	0.189
Pharyngalgia	14/238 (5.9)	9/153 (5.9)	6/85 (7.1)	0.720
Palpitation	9/238 (3.8)	8/153 (5.2)	1/85 (1.2)	0.120
Chest pain	6/238 (2.5)	4/153 (2.6)	2/85 (2.4)	0.902
Nausea	4/238 (1.7)	2/153 (1.3)	2/85 (2.4)	0.548
Dizziness	4/238 (1.7)	4/153 (2.6)	0/85 (0.0)	0.133
Vomiting	3/238 (1.3)	3/153 (2.0)	0/85 (0.0)	0.194
Abdominal pain	1/238 (0.4)	1/153 (0.7)	0/85 (0.0)	0.455
Coexisting disorders				
Hypertension	63/238 (26.5)	42/153 (27.5)	21/85 (24.7)	0.646

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Diabetes	25/238 (10.5)	15/153 (9.8)	10/85 (11.8)	0.636
Cardiovascular disease	24/238 (10.1)	16/153 (10.5)	8/85 (9.4)	0.797
Malignancy	12/238 (5.0)	6/153 (3.9)	6/85 (7.1)	0.289
Cerebrovascular disease	8/238 (3.4)	5/153 (3.3)	3/85 (3.5)	0.915
COPD	3/238 (1.3)	1/153 (0.7)	2/85 (2.4)	0.260

357 Data are median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. P values comparing the
358 confirmed patients and the suspected patients are from χ^2 test, Fisher's exact test, or Mann-Whitney U test. Confirmed=confirmed
359 patients. Suspected=suspected patients.

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363 **Table 2. Viral RNA and antibody positive rates of the patients detected each day**
364 **from initial onset of symptoms**

Day	Viral RNA$^+$	IgM$^+$and/or IgG$^+$	IgM$^+$	IgG$^+$	IgM$^+$and IgG$^+$
0	100.0 (2/2)	50.0 (1/2)	0.0 (0/2)	50.0 (1/2)	0.0 (0/2)
1	83.3 (5/6)	33.3 (1/3)	0.0 (0/3)	33.3 (1/3)	0.0 (0/3)
2	71.4 (5/7)	0.0 (0/3)	0.0 (0/3)	0.0 (0/3)	0.0 (0/3)
3	61.5 (8/13)	25.0 (1/4)	25.0 (1/4)	0.0 (0/4)	0.0 (0/4)
4	69.2 (9/13)	50.0 (2/4)	0.0 (0/4)	0.0 (0/4)	50.0 (2/4)
5	92.3 (12/13)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)	0.0 (0/1)
6	62.5 (5/8)	60.0 (3/5)	0.0 (0/5)	0.0 (0/5)	60.0 (3/5)
7	88.9 (8/9)	50.0 (2/4)	25.0 (1/4)	0.0 (0/4)	25.0 (1/4)
8	81.8 (18/22)	54.5 (6/11)	18.2 (2/11)	0.0 (0/11)	36.4 (4/11)
9	85.0 (17/20)	42.9 (6/14)	7.1 (1/14)	7.1 (1/14)	28.6 (4/14)
10	75.0 (9/12)	42.9 (3/7)	14.3 (1/7)	0.0 (0/7)	28.6 (2/7)
11	72.2 (13/18)	81.8 (9/11)	18.2 (2/11)	27.3 (3/11)	36.4 (4/11)
12	50.0 (5/10)	80.0 (8/10)	30.0 (3/10)	20.0 (2/10)	30.0 (3/10)
13	44.4 (4/9)	90.9 (10/11)	27.3 (3/11)	9.1 (1/11)	54.5 (6/11)
14	69.2 (9/13)	100.0 (17/17)	29.4 (5/17)	0.0 (0/17)	70.6 (12/17)
15	30.8 (4/13)	90.0 (18/20)	10.0 (2/20)	15.0 (3/20)	65.0 (13/20)
16	50.0 (4/8)	100.0 (16/16)	12.5 (2/16)	25.0 (4/16)	62.5 (10/16)
17	57.1 (4/7)	88.9 (16/18)	0.0 (0/18)	5.6 (1/18)	83.3 (15/18)
18	33.3 (2/6)	100.0 (16/16)	0.0 (0/16)	6.3 (1/16)	93.8 (15/16)
19	33.3 (3/9)	100.0 (19/19)	10.5 (2/19)	21.1 (4/19)	68.4 (13/19)
20	0.0 (0/1)	83.3 (5/6)	0.0 (0/6)	33.3 (2/6)	50.0 (3/6)
>20	36.8 (7/19)	97.2 (35/36)	2.8 (1/36)	8.3 (3/36)	86.1 (31/36)

365 Data are % (n/N). Day= the day after initial onset of symptoms. Viral RNA$^+$=a positive result detected by real time RT-PCR.
366 IgM$^+$=a positive result detected by IgM ELISA and simultaneously a negative result detected by IgG ELISA. IgG$^+$=a positive
367 result detected by IgG ELISA and simultaneously a negative result detected by IgM ELISA. IgM$^+$ and IgG$^+$=a dual positive result
368 detected by IgM and IgG ELISA. IgM$^+$and/or IgG$^+$=at least a positive result detected by IgM and IgG ELISA.

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375 **Table 3. Viral RNA and antibody positive rates of the patients detected in**
376 **different stages of disease**

Days	Viral RNA$^+$	IgM$^+$and/or IgG$^+$	IgM$^+$	IgG$^+$	IgM$^+$and IgG$^+$
0-5	75.9 (41/54)	29.4 (5/17)	5.9 (1/17)	11.8 (2/17)	11.8 (2/17)
6-10	80.3 (57/71)	48.8 (20/41)	12.2 (5/41)	2.4 (1/41)	34.1 (14/41)
11-12	64.3 (18/28)	81.0 (17/21)	23.8 (5/21)	23.8 (5/21)	33.3 (7/21)
13-15	48.6 (17/35)	93.8 (45/48)	20.8 (10/48)	8.3 (4/48)	64.6 (31/48)
≥16	40.0 (20/50)	96.4 (107/111)	4.5 (5/111)	13.5 (15/111)	78.4 (87/111)
Total	64.3 (153/238)	81.5 (194/238)	10.9 (26/238)	11.3 (27/238)	59.2 (141/238)

377 Data are % (n/N). Days= the day after initial onset of symptoms. Viral RNA$^+$=a positive result detected by real time RT-PCR.
378 IgM$^+$=a positive result detected by IgM ELISA and simultaneously a negative result detected by IgG ELISA. IgG$^+$=a positive
379 result detected by IgG ELISA and simultaneously a negative result detected by IgM ELISA. IgM$^+$ and IgG$^+$=a dual positive result
380 detected by IgM and IgG ELISA. IgM$^+$and/or IgG$^+$=at least a positive result detected by IgM and IgG ELISA.

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386 **Table 4. Comparison of the antibody positive rates between the confirmed and**
387 **suspected patients**

	0-5 days	6-10 days	≥11 days
Confirmed	55.6 (5/9)	44.0 (11/25)	93.3 (111/119)
Suspected	0.0 (0/8)	56.3 (9/16)	95.1 (58/61)

388 Data are % (n/N). Confirmed=confirmed patients. Suspected=suspected patients.

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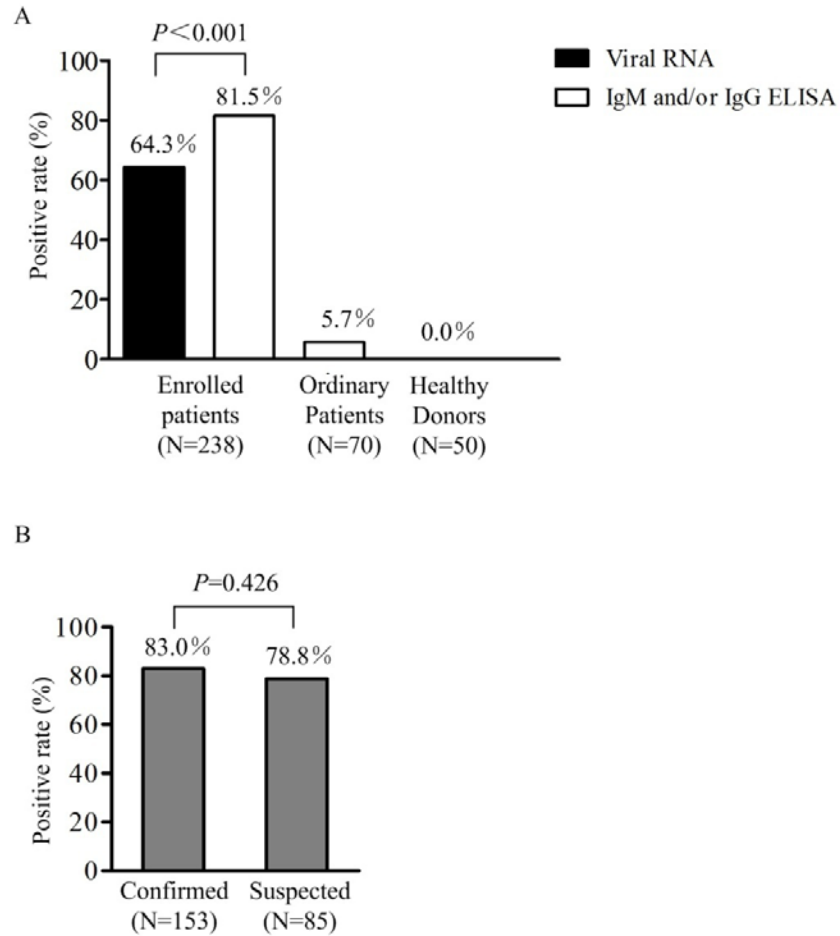

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396 **Figure 1. Positive rate of viral RNA and antibody in different samples.** A) The
397 positive rate of viral RNA (black column) and antibody (white column) in 238
398 enrolled patients (two columns on the left), as well as the positive rate of antibody in
399 ordinary patients and healthy donors (two columns on the right). B) Comparison of
400 positive rate of antibody between the laboratory-confirmed (left) and highly-suspected
401 patients (right). Results were compared by chi-square tests.

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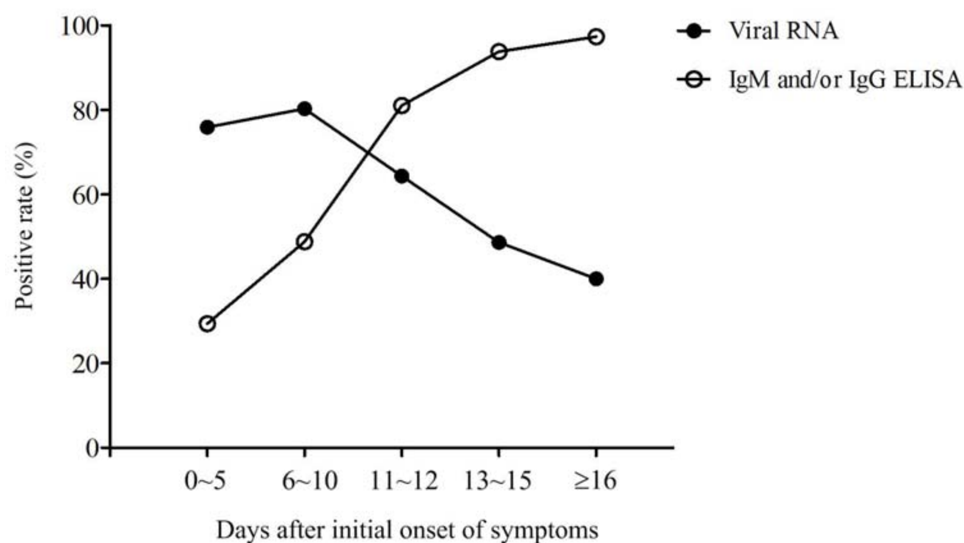

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411 **Figure 2. Dynamics of the positive rate of viral RNA and antibody of the patients**
412 **at the different stages of disease.** The disease courses were divided into five phases
413 of 0-5, 6-10,11-12, 13-15 days and more than 16 days after initial onset of symptoms.
414 The positive rate of viral RNA (solid circle) and antibody (hollow circle) of the
415 patients at the different phase of disease was shown.

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