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**Diagnostic value and dynamic variance of serum antibody in
coronavirus disease 2019**

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Highlights

- Viral serological test is an effective diagnostic means for SARS-CoV-2 infection.
- The positive rate and titer variance of serum IgG were higher than that of IgM in COVID-19 course.
- The median titer of IgG after viral negative was twice than that before.

Abstract

Objective: To investigate the diagnostic value of serological test and dynamic variance of serum antibody in coronavirus disease 2019 (COVID-19).

Methods: We retrospectively included 43 patients with a laboratory-confirmed infection and 33 patients with suspected infection who were finally excluded. The IgM/IgG titer of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was measured by chemiluminescence immunoassay analysis.

Results: Compared to molecular detection, the sensitivity of serum IgM and IgG antibodies to diagnose COVID-19 were 48.1% and 88.9%, and the specificity were 100% and 90.9%, respectively. In COVID-19 group, IgM positive rate almost increased first then decreased over time, however, IgG positive rate increased till 100% and was higher than IgM all the time. IgM positive rate and titer were not

significantly different before and after viral negative. IgG positive rate was up to 90% and not significantly different before and after viral negative. However, the median titer of IgG after viral negative was twice than that before with significant difference.

Conclusion: Viral serological test is an effective diagnostic means for SARS-CoV-2 infection. The positive rate and titer variance of IgG are higher than that of IgM in COVID-19.

Keywords: COVID-19; SARS-CoV-2; diagnosis; serological test; Immunoglobulin M; Immunoglobulin G

Introduction

Since December 2019, a group of pneumonia patients of unknown cause have been identified in Wuhan, Hubei province, China [1]. The pathogen has been identified as a novel coronavirus and named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as it has a phylogenetic similarity to SARS-CoV [2]. SARS-CoV-2 spread rapidly and the resulting coronavirus disease 2019 (COVID-19) has been

declared a public health emergency of international concern by the world Health Organization [3]. As of 4 March 2020, 93091 laboratory-confirmed cases and 3198 deaths have been documented globally [4].

Coronaviruses, enveloped non-segmented positive-sense RNA viruses, belong to the Orthocoronavirinae subfamily. Although most human coronavirus infection are mild, SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV), betacoronaviruses zoonotic in origin, have been associated with potentially fatal disease during the outbreaks in 2003 and 2012, respectively [5,6]. Currently, the mortality rate of SARS-CoV-2, a novel betacoronavirus, is about 3.4%, which is lower than 10% for SARS-CoV and 34% for MERS-CoV [4,7,8]. However, SARS-CoV-2 has potentially higher transmissibility than both SARS-CoV and MERS-CoV [9].

The rapid and accurate diagnostics of COVID-19 contributes to disease and outbreak management by enabling prompt and accurate public health surveillance, prevention and control measures. Real time reverse transcriptase polymerase chain reaction (RT-PCR) is the primary means for diagnosing SARS-CoV-2 [1]. However,

molecular detection may have risk of false-negatives because of low viral loads in specimens [10]. Serological testing, another common laboratory diagnostic, can diagnose illness by detecting antibodies. The serological study regarding SARS-CoV-2, to our knowledge, is scarce. Here, we aimed to investigate the diagnostic value of serological detection to COVID-19 and the dynamic variance of viral antibodies in SARS-CoV-2 infection.

Methods

Data sources

We conducted a retrospective study at Xixi hospital of Hangzhou, a designated hospital for emerging infectious disease, in Zhejiang province, China, from January 2020 to 4 March 2020. Case definitions of confirmed COVID-19 are based on the WHO interim guidance [11]. 43 Patients with a laboratory confirmed infection and at least one viral serological test performed in hospital were enrolled in this study. 33 patients with suspected SARS-CoV-2 infection, who were finally excluded in our hospital and quarantined at home, were included as control group. The definition of

suspected SARS-CoV-2 infection presents a fever or any respiratory symptoms, and especially in those with a history of travel to Wuhan or exposure to infected cases within two weeks before the onset of disease since January 2020 [12]. Suspected infected patients were discharged from hospital once the results of two times of molecular tests in a 24h interval were negative. The demographic and clinical data of these patients were extracted from their medical records.

24 patients were laboratory-confirmed in other hospital and transferred to our hospital. Oral swab or sputum specimens collected from the remaining 19 cases at admission were sent to the Center for Disease Control of Hangzhou and tested by real time RT-PCR for SARS-CoV-2 RNA. Laboratory confirmation of the virus was based on the result of real time RT-PCR [1]. Virus detection was repeated twice every 24 hours. Fitness for discharge of COVID-19 patients was based on normal body temperature for at least 3 days, with improvement of chest radiographic evidence and viral clearance in respiratory samples from upper respiratory tract twice. The study was approved by the Ethics Committee of Xixi Hospital of Hangzhou and written informed consent was obtained from each participant.

Serological test

Serum was separated by centrifugation at 2500 g for 5 min within 12h of collection.

SARS-CoV-2 IgM and IgG chemiluminescence immunoassay (CLIA) kits were from Shenzhen YHLO Biotech Co., Ltd (China), with two antigens of SARS-CoV-2 coated on the magnetic beads of these CLIA assays (Nucleocapsid protein or N protein, Spike Protein or S proteins). All serum antibody tests were performed by iFlash3000 fully automatic chemiluminescence immunoassay analyzer from Shenzhen YHLO Biotech Co., Ltd (China). SARS-CoV-2 IgM/IgG titer (AU/ml) was calculated automatically by the immunoassay analyzer on the basis of relative light unit (RLU) because viral antibody titer was positively associated with RLU. The cutoff value of SARS-CoV-2 IgM/IgG positive was 10 AU/ml according to the manufacturer's instructions.

Statistical analysis

Continuous variables were expressed as mean (SD) if normally distributed or median (IQR) if not, and categorical variables were described as count (%). Serum antibody titers before and after viral negative were compared using Wilcoxon

matched-pairs signed-ranks test. Proportions for categorical were compared using Fisher exact test. All analyses were done with SPSS software (version 23.0). A 2-sided of less than 0.05 was considered statistically significant.

Results

43 patients with laboratory-confirmed SARS-CoV-2 infection were included in the study. Among them, 24 patients were transferred to our hospital after laboratory-confirmed and the remaining 19 patients were confirmed in our hospital. Meanwhile, 33 suspected COVID-19 patients, who were finally excluded, were chosen as control group. The demographic and clinical characteristics of COVID-19 and control group are shown in Table 1. The median age of COVID-19 patients was 47.0 years (IQR 34.0-59.0), ranging from 7 years to 74 years. A total of 39.5% were men in Covid-19 group. Among both groups, less patients had chronic disease, including hypertension, diabetes and liver disease. Fever was present in 62.8% of COVID-19 patients before or on admission. The secondly most common symptom was cough (60.5%). Similarly, Fever and cough were also most common symptom of control group. The

durations from first symptoms to hospital admission, laboratory confirmation, and first serological test in COVID-19 group were 3 days (IQR 2-7), 3 days (IQR 2-7) and 18 days (IQR 11-23), respectively. Among them, 2 patients having history of exposure to infected cases had represented without any symptom until first serological test.

IgM and IgG positive rate were 0 (0/33) and 9.1% (3/33) in control group respectively. IgG titers of 3 positive patients were all less than 15 AU/ml. Whereas, in COVID-19 group, 27 patients were tested for viral antibody before viral negative (including oral swabs, anal swabs, or sputum). The median durations from first symptoms to serological test in these 27 patients were 16 days ((IQR 9-20). Among these people, we found 13 IgM positive (48.1%) and 24 IgG positive (88.9%). 3 IgG negative patients were also IgM negative. According to molecular detection as gold standard, the sensitivity of serum IgM and IgG antibodies to diagnose COVID-19 were 48.1% (13/27) and 88.9% (24/27) respectively, and the specificity were 100% (33/33) and 90.9% (30/33) respectively. Moreover, the positive predictive values of

IgM and IgG antibodies were 100% (13/13) and 88.9% (24/27) respectively, and the negative predictive values were 70.2% (33/47) and 90.9% (30/33).

After 43 cases were laboratory-confirmed in COVID-19 group, 98 serological tests were performed. Figure 1A displays that IgM positive rate almost increased first then decreased as days from laboratory-confirmed to serological detection increased, however, IgG positive rate increased till 100% and was higher than IgM all the time. Meanwhile, viral positive rate almost decreased overtime. Before laboratory-confirmed, 2 serological tests were performed in one case and viral IgM and IgG were both positive in 2 results. Figure 1B shows the similar trend as durations from symptom onset to serological test increased. We also found IgM and IgG were both not high in the first 5 days from symptom onset.

In COVID-19 group, 34 patients were tested for viral antibody after two times of oral swabs negative in a 24h interval without other molecular detection positive. IgM and IgG positive rate were 55.9% (19/34) and 94.1% (32/34), respectively. 2 IgG negative patients were also IgM negative. The median titers of IgM and IgG among these 34 people were 12.1 AU/ml (IQR 5.2-46.6) and 132.2 AU/ml (IQR 65.5-179.1),

respectively. All patients have been discharged and the median time from this serological test to discharge was 2 days (IQR 1-3).

Among these 34 people, serological test was also performed in 20 patients before viral negative. Table 2 shows that IgM positive rate and titer were not significantly different before and after viral negative. IgG positive rate was up to 90% and also not significantly different before and after viral negative. However, the median titer of IgG after viral negative was twice than that before with significant difference. The median interval between these two serological tests was 6 days (IQR 4-9).

Discussion

As the diagnosis of COVID-19 was complicated by the diversity in symptoms and imaging findings, molecular and serological detection tools have been rapidly developed. The laboratory-confirmation of this virus was based on a positive result on real time RT-PCR. The molecular and serological study regarding this virus, to our knowledge, was less. In the study, we included 43 patients with laboratory-confirmed COVID-19 infection and 33 suspected patients who were finally excluded by nucleic

acid test twice and discharged soon, to investigate the diagnostic value of serological detection to COVID-19. Meanwhile, we also surveyed the dynamic variance of viral antibodies during SARS-CoV-2 infection.

Most of the infected cases in our study were female patients, but the age range of individuals is large because children and those older than 65 years were also infected. The proportion of any coexisting illness in infected individuals was low in our study, consistent with that of non-severe patients in a study of Guan W et al [13]. Through media and national advocacy, those having contact with infected cases, or patients with suspected infection were asked to go to hospital at an early stage as soon as possible. Furthermore, our hospital was a designed tertiary hospital and mainly admitted patients with mild to moderate symptoms in Hangzhou. Fever and cough were the dominant symptoms, in concert with recent studies [1,12,13]. In control group, patients with lower age, more male sex, and less common presence of any coexisting illness than those in infected group.

We found the specificity of serum IgM and IgG to diagnose COVID-19 were both more than 90% compared to molecular detection. Moreover, IgG titers of 3 positive

patients in control group presented weakly positive. In COVID-19 group, we also found IgM and IgG both positive in one case before laboratory-confirmed for the first time. Therefore, COVID-19 should be considered when serum IgM or IgG was positive. In COVID-19 patients, we found IgG positive rate (88.9%) was higher than IgM (48.1%) before viral molecular detection negative. Interestingly, 3 IgG negative patients were also IgM negative in our study. The durations from symptom onset to this serological test in these 3 patients were 0 day, 5 days and 8 days, respectively. Furthermore, we observed that serum viral antibodies almost increased at the early stage of disease. Hence, it may be explained that serum viral antibodies have not been produced yet and could be undetectable. Zhang W et al also found IgM and IgG positive rate were 50% and 81% in day0 (the day of first sampling) and increased to 81% and 100% on day 5, respectively [14]. Thus, Covid-19 cannot be excluded at an early stage when viral serological test negative.

We also observed the trend of IgM positive rate upward first then downward, however, that of IgG positive rate upward and stable as time went on. Meanwhile, IgG positive rate was always higher than IgM, and this phenomenon was also

observed in a study of Zhang W et al [14]. There were 2 patients with both IgM and IgG negative after oral swabs negative twice. The intervals between laboratory-confirmed and serological test were 10 and 14 days respectively. A lag period was found as antibodies specifically targeting MERS-CoV would normally appear between 14 and 28 days after the illness onset [15]. Therefore, we cannot infer whether viral antibodies have not been produced yet or turned to negative because of the only one serological test. Besides high positive rate, we found IgG titer after viral negative was twice than before. In our study, these COVID-19 patients were discharged soon after two times of oral swabs negative. Whether higher antibodies titers in SARS-CoV-2 infection were associated with better outcome need to be further studied.

Our study has several limitations. Firstly, only 43 laboratory-confirmed patients and 33 controls were included, the study results should be interpreted with caution. Secondly, the time of viral molecular detection and serological test was irregular and depending on clinician's judgement because of a retrospective study. Thirdly, since infected patients included in the study were non-severe, the value of serological test in

severe patients need to be assessed. Fourthly, the median duration from symptom onset to serological test was long as viral serological detection kits were available late. Finally, follow-up data regarding these individuals who have discharged is scarce.

In conclusion, viral serological test is an effective diagnostic means for SARS-CoV-2 infection. The positive rate and titer variance of IgG are higher than that of IgM in COVID-19 course.

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Ethical approval

The study was approved by the Ethics Committee of Xixi Hospital of Hangzhou and written informed consent was obtained from each participant.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020; 395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017.
3. WHO. Coronavirus disease (COVID-19) outbreak. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>, 2020(accessed 04 March, 2020).
4. WHO. Coronavirus disease 2019 (COVID-19) Situation Report-44. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200304-sitrep-44-covid-19.pdf?sfvrsn=93937f92_6/, 2020(accessed 04 March, 2020).

5. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA.
Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. doi:10.1056/NEJMoa1211721.
6. Zhong NS, Zheng BJ, Li YM, Poon, Xie ZH, Chan KH, Li PH, Tan SY, Chang Q, Xie JP, Liu XQ, Xu J, Li DX, Yuen KY, Peiris, Guan Y. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet. 2003;362(9393):1353-8. doi:10.1016/s0140-6736(03)14630-2.
7. WHO. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. https://www.who.int/csr/sars/country/table2003_09_23/en/, 2020(accessed 04 March, 2020).
8. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). <https://www.who.int/emergencies/mers-cov/en/>, 2020(accessed 04 March, 2020).
9. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect. 2020; S1286-4579(20)30026-5. doi: 10.1016/j.micinf.2020.01.004.

10. Pang J, Wang MX, Ang IYH, Tan SHX, Lewis RF, Chen JI, Gutierrez RA, Gwee SXW, Chua PEY, Yang Q, Ng XY, Yap RK, Tan HY, Teo YY, Tan CC, Cook AR, Yap JC, Hsu LY. Potential Rapid Diagnostics, Vaccine and Therapeutics for 2019 Novel Coronavirus (2019-nCoV): A Systematic Review. *J Clin Med*. 2020;9(3): E623. doi:10.3390/jcm9030623.
11. WHO. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. Jan 11, 2020. [https://www.who.int/internal-publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/internal-publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected).
12. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ*. 2020;368:m606. doi:10.1136/bmj.m606.
13. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY,

Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P,

Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY,

Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical

Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020. doi:

10.1056/NEJMoa2002032.

14. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, Wang YY, Xiao GF, Yan B,

Shi ZL, Zhou P. Molecular and serological investigation of 2019-nCoV infected

patients: implication of multiple shedding routes. *Emerg Microbes Infect*. 2020;

9(1):386-389. doi: 10.1080/22221751.2020.1729071.

15. Al Johani S, Hajeer AH. MERS-CoV diagnosis: An update. *J Infect Public Health*.

2016;9(3):216-9. doi: 10.1016/j.jiph.2016.04.005.

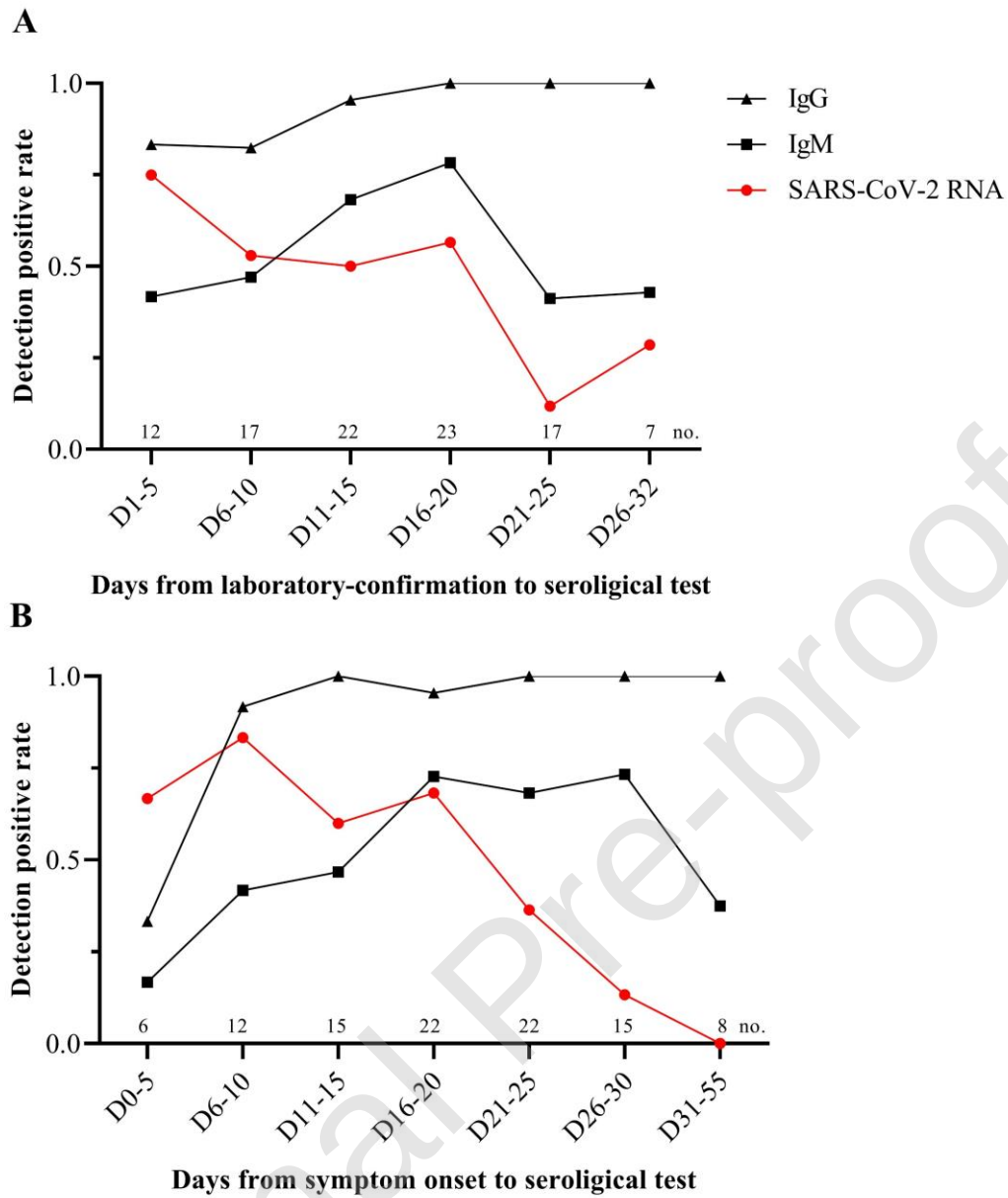


Figure1. Serological test positive rate and SARS-CoV-2 viral load in different intervals

Table1. Demographic and clinical characteristics of COVID-19 and control group

Characteristic	COVID-19 group (n=43)	Control group (n=33)
Age, year	47.0 (34.0-59.0)	31.0 (25.5-37.5)
Male sex, n (%)	17 (39.5)	22 (66.7)
Smoking history, n (%)	6 (14.0)	6 (18.2)
Diabetes, n (%)	3 (7.0)	1 (3.0)
Hypertension, n (%)	10 (23.3)	4 (12.1)
Liver disease, n (%)	2 (4.7)	0
Fever, n (%)	27 (62.8)	24 (72.7)
Cough, n (%)	26 (60.5)	15 (45.5)
Fatigue or Myalgia, n (%)	9 (20.9)	3 (9.1)
Sputum production, n (%)	8 (18.6)	6 (18.2)
Onset of Symptom to, days		
Hospital admission	3.0 (2.0-7.0)	1.0 (1.0-7.0)
Laboratory confirmation	3.0 (2.0-7.0)	-
First serological test	18.0 (11.0-23.0)	3.0 (2.0-8.0)

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Table2. The comparison of SARS-CoV-2 antibodies before and after negative

	Before negative (n=20)	After negative (n=20)	P
IgM positive, n (%)	10 (50.0)	10 (50.0)	1
IgM titer, AU/ml	8.8 (4.2-14.7)	7.8 (4.3-27.8)	0.198
IgG positive, n (%)	18 (90.0)	19 (95.0)	1
IgG titer, AU/ml	78.6 (56.5-107.9)	161.2 (102.6-184.3)	<0.001