Clinical characteristics and plasma antibody titer of patients with COVID-19 in Zhejiang, China *#
reverse transcription-polymerase chain reaction (RT-PCR) via throat swab or sputum. The time of CP collection was approximately 7, 14, 21, 28, 35, and 49 d after symptom onset. A commercial COVID-19 IgG/IgM Rapid Test Cassette produced by Zhejiang Orient Gene Biotech Co., Ltd. (Huzhou, China) was applied to detect the samples’ stock solution and their dilutions. Each IgG/IgM positive CP sample was diluted in saline at volume ratios of 1:80, 1:160, 1:320, 1:640, and 1:1280. We also retrospectively retrieved data on the epidemiological characteristics and clinical information of patients diagnosed with COVID-19 in Wenzhou Central Hospital as of February 24, 2020 from the electronic medical record system.

Of the 139 CP samples from Wenzhou Central Hospital, 55 were collected within 4 weeks after symptom onset, 50 were collected within 4–6 weeks after symptom onset, and 34 were collected more than 6 weeks after symptom onset. These were mostly discontinuous samples, and we were fortunate to find four patients with continuous samples. We found that in the first month, the titer level of IgG was mainly between 80 and 160, rising to 160–320 in the next 2 weeks and then to 320 or higher after 6 weeks (Table 1). There were four patients for whom we had enough consecutive blood samples from the first week (symptom onset) to the seventh week. We learned that IgG antibody titer levels of 320–640 or higher always appeared within 4–6 weeks after symptom onset (Fig. 1). This result was consistent with other studies showing that IgG reached higher levels after four weeks from the onset of COVID-19 symptoms (Li et al., 2020; Long et al., 2020), which can thus be considered the best time to donate plasma. However, many samples still also showed positive IgM titer results in this period, which should be taken into consideration in screening plasma donor. IgM is a serologic marker which usually represents a recent or current infection, and low IgM levels may not be required for CP donor selection if available donors are limited (Singh et al., 2015; Samanta and Willis, 2016; Zhang et al., 2020).

Table 1  Antibody titer levels from COVID-19 patients’ plasma (discontinuous) in different stages of disease progress in Wenzhou Central Hospital of Zhejiang Province (n=139)

| Titer level | IgG-positive case (within 4 weeks after symptom onset) | IgG-positive case (within 4–6 weeks after symptom onset) | IgG-positive case (more than 6 weeks after symptom onset) |
|------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| 80         | 20                                                   | 8                                                    | 3                                                    |
| 160        | 21                                                   | 22                                                  | 10                                                   |
| 320        | 13                                                   | 16                                                  | 17                                                   |
| 640        | 1                                                    | 4                                                   | 4                                                    |

Of the 139 CP samples from Wenzhou Central Hospital, 55 were collected within 4 weeks after symptom onset, 50 were collected within 4–6 weeks after symptom onset, and 34 were collected more than 6 weeks after symptom onset. These were mostly discontinuous samples, and we were fortunate to find four patients with continuous samples. We found that in the first month, the titer level of IgG was mainly between 80 and 160, rising to 160–320 in the next 2 weeks and then to 320 or higher after 6 weeks (Table 1). There were four patients for whom we had enough consecutive blood samples from the first week (symptom onset) to the seventh week. We learned that IgG antibody titer levels of 320–640 or higher always appeared within 4–6 weeks after symptom onset (Fig. 1). This result was consistent with other studies showing that IgG reached higher levels after four weeks from the onset of COVID-19 symptoms (Li et al., 2020; Long et al., 2020), which can thus be considered the best time to donate plasma. However, many samples still also showed positive IgM titer results in this period, which should be taken into consideration in screening plasma donor. IgM is a serologic marker which usually represents a recent or current infection, and low IgM levels may not be required for CP donor selection if available donors are limited (Singh et al., 2015; Samanta and Willis, 2016; Zhang et al., 2020). Positive IgM samples were obtained from a total of 260 COVID-19 convalescent patients from three hospitals, including Wenzhou Central Hospital (139 total cases, 86 men and 53 women), the First People’s Hospital of Jiaxing City (13 total cases, 10 men and 3 women), and Ningbo Huamei Hospital (108 total cases, 55 men and 53 women). The IgM positivity rate was higher among men and the difference by sex was statistically significant (P<0.05). This result may be due to pre-existing fitness and health levels or other factors like differences in immunoregulation between male and female, which needs further research. We discovered correlations between IgG/IgM and different laboratory indices such as C-reactive protein (CRP), prothrombin time (PT), absolute value of lymphocyte count (LYM), albumin (ALB), lactic dehydrogenase (LDH), and hemoglobin (HGB), which are shown in Fig. S1. The IgM titer in CP had a positive correlation with CRP and PT, but a negative correlation with LYM and ALB. Meanwhile, the IgG titer in CP had a positive correlation with CRP and LDH, but a negative correlation with LYM and HGB (P<0.05). These indices are related to the degree of inflammation, immunologic or coagulation function. In addition, the IgM titer in CP shared the same growth trend with CRP and PT, which supports the idea that patients with persistent IgM may have a shorter duration of positive RT-PCR, resulting in clinical conditions that worsened less compared to patients without the presence of anti-SARS-CoV-2 IgM antibodies (Lee et al., 2020).

In our study, 13 patients were severely or critically ill patients reported in Wenzhou Central Hospital, aged 37–77 years, with a mean age of 56.4 years. Eleven of these 13 patients were male (84.6%) and two were female (15.4%), which may indicate that
middle-aged men tend to experience worse symptoms from the virus. The reported routes of transmission for these severely and critically ill patients included living in the epidemic area (69.2%), a history of contact with a COVID-19 patient in public (15.4%), or close contact with a COVID-19-positive family member (15.4%), supporting the importance of quarantine measures such as wearing masks, washing hands, and disinfecting frequently to reduce the risk of transmission. Seven of the 13 patients (53.8%), including two (50.0%) of the critically ill and five (55.6%) of the severely ill patients, presented with gastrointestinal symptoms such as diarrhea, nausea, and vomiting. On admission, other frequent symptoms included fever in 12 (92.3%), cough in 11 (84.6%), dyspnea in eight (61.5%), and fatigue in eight (61.5%) patients. What calls for special attention is that presented symptoms represent multiple systems such as the digestive tract and the respiratory tract, which makes it difficult to distinguish COVID-19 from other diseases with overlapping symptoms.

As Table 2 shows, among the 13 severe and critically ill patients, eight (61.5%) patients were found to have increased serum creatine kinase levels, including six severely ill and two critically ill patients. Eleven patients (84.6%), including four of the critically ill patients (100.0%) and seven of the severely ill patients (77.8%), showed elevated serum lactate dehydrogenase levels. Of the 13 patients, eight patients (61.5%) showed decreased serum potassium levels, including three of the critically ill patients (75.0%), and five of the severely ill patients (55.6%). Also, eight (61.5%) patients presented reduced blood calcium concentrations, including two of the critically ill patients (50.0%) and six of the severely ill patients (66.7%), suggesting that electrolyte disturbances occur during the process of the disease. It is necessary to address any water and electrolyte metabolism disorders in time to avoid heart failure and prevent or mitigate liver and kidney dysfunctions.

Meanwhile, we must also pay attention to patients with complicated courses of the diseases. The absolute value of lymphocytes was decreased in 12 (92.3%) patients, including four (100.0%) of the critically ill and eight (88.9%) of the severely ill patients, although the degree of decline was higher among the former. CRP levels were increased for all patients. CRP is a very sensitive indicator of an acute-phase reaction and is commonly used in clinic to identify the existence of co-infection. When the body is resistant to infection, or following infection, the function of the systemic immune system is weak and shows a poor ability to resist bacteria. This highlights the fact that it is important to actively work to prevent hospital-related infections, such as the occurrence of ventilator-associated pneumonia or tube infections. To supply oxygen to severely ill patients, nasal catheter oxygenation and mask oxygenation were used, while none were given noninvasive/invasive ventilator oxygenation. Either mask oxygen or nasal catheter oxygen can meet the body’s oxygen demands in severely ill patients. All four critically ill patients required noninvasive ventilator oxygenation (100.0%). The latest research has found that the mortality rate of COVID-19 patients with acute respiratory distress syndrome (ARDS) is 50%–70% (Liu et al., 2020). Early noninvasive positive-pressure ventilation should be deployed as conditions allow; this can increase the body’s oxygen supply and improve prognosis by facilitating the recovery of lung function during rehabilitation.

There are several limitations in this study. The sample size was small, limited to Zhejiang Province, and lacked a sufficient quantity of consecutive samples. Because of this, we were not able to carry out a large-scale, well-designed clinical trial and the result may not be representative of the large target populations. More samples and subjects should be included in similar studies in the future to explore the correlation of antibody levels with disease severity.
In conclusion, IgG antibody titer levels of 320–640 or higher always appeared within 4–6 weeks after symptom onset, which indicates that this is the best time to collect CP. At that point, some patients still showed positive IgM titer results in the plasma. The positive rate of IgM in CP tended to be higher among males than among females. Critically ill patients needed to rely on noninvasive ventilation for survival. Early noninvasive positive-pressure ventilation should be deployed as conditions allow in order to reduce the mortality rate. Almost all severe and critical patients had water and electrolyte metabolism disorders, which indicates that attending physicians need to pay more attention to this issue and take intervention measures as soon as possible. The incidence of coagulation function disorder and inflammation in the early stages of the disease can influence the antibody titer of IgG and IgM in CP, which may help to screen appropriate

| Table 2 Epidemiology and clinical characteristics of severely and critically ill COVID-19 patients at Wenzhou Central Hospital of Zhejiang Province |
|---------------------------------------------------|-------------------|-------------------|
| Characteristics                                    | Total (n=13)       | Severe disease (n=9) | Critical illness (n=4) |
| Epidemiological data                               |                   |                   |                   |
| Male patient                                       | 11 (84.6%)        | 9 (100.0%)         | 2 (50.0%)          |
| Female patient                                     | 2 (15.4%)         | 0 (0%)             | 2 (50.0%)          |
| Age (year)                                         | 56.4 (37.0–77.0)  | 57.7 (46.0–77.0)   | 54.5 (37.0–71.0)   |
| History of exposure to the epidemic area           | 9 (69.2%)         | 6 (66.7%)          | 3 (75.0%)          |
| History of contact with an infected patient        | 2 (15.4%)         | 2 (22.2%)          | 0 (0%)             |
| No obvious history of contact with an infected patient | 2 (15.4%)         | 1 (11.1%)          | 1 (25.0%)          |
| Days to diagnosis (d)                              | 6.5 (3.0–17.0)    | 9.4 (3.0–17.0)     | 5.3 (3.0–7.0)      |
| Days to diagnosis (d)                              |                   |                   |                   |
| Days to diagnosis (d)                              | 6.5 (3.0–17.0)    | 9.4 (3.0–17.0)     | 5.3 (3.0–7.0)      |
| Fever                                              | 12 (92.3%)        | 8 (88.9%)          | 4 (100.0%)         |
| Fatigue                                            | 8 (61.5%)         | 7 (77.8%)          | 1 (25.0%)          |
| Cough                                              | 11 (84.6%)        | 7 (77.8%)          | 4 (100.0%)         |
| Chest distress                                     | 1 (7.6%)          | 0 (0%)             | 1 (25.0%)          |
| Dyspnea                                            | 8 (61.5%)         | 4 (44.4%)          | 4 (100.0%)         |
| Diarrhea                                           | 7 (53.8%)         | 5 (55.6%)          | 2 (50.0%)          |
| Nausea and vomiting                                | 7 (53.8%)         | 5 (55.6%)          | 2 (50.0%)          |
| Laboratory test (reference value)                  |                   |                   |                   |
| White blood cell (×10^9 L^-1) (4–10)               | 4.5 (2.4–8.8)     | 4.3 (2.4–8.8)      | 5.0 (3.8–7.6)      |
| Decreased white blood cell                         | 8 (61.5%)         | 6 (66.7%)          | 2 (50.0%)          |
| Leukomonocyte (×10^9 L^-1) (1.1–3.2)               | 0.7 (0.1–1.0)     | 0.6 (0.3–1.0)      | 0.5 (0.1–0.8)      |
| Decreased leukomonocyte                            | 12 (92.3%)        | 8 (88.9%)          | 4 (100.0%)         |
| Creatine kinase (U/L) (55–170)                     | 359.4 (31.0–1933.0) | 229.4 (31.0–273.7) | 603.3 (80.0–1933.0) |
| Increased creatine kinase                          | 8 (61.5%)         | 6 (66.7%)          | 2 (50.0%)          |
| C-reactive protein (mg/L) (<8)                     | 47.2 (22.8–101.9) | 43.4 (22.8–96.2)   | 55.87 (36.6–101.9) |
| Increased C-reactive protein                       | 13 (100%)         | 9 (100.0%)         | 4 (100.0%)         |
| Lactic dehydrogenase (U/L) (114–240)               | 298.1 (196.0–457.0) | 265.0 (196.0–384.0) | 372.8 (295.0–457.0) |
| Increased lactic dehydrogenase                     | 11 (84.6%)        | 7 (77.8%)          | 4 (100.0%)         |
| Potassium (mol/L) (3.5–5.5)                        | 3.42 (2.76–4.39)  | 3.58 (3.40–4.39)   | 3.06 (2.76–3.76)   |
| Decreased potassium                                | 8 (61.5%)         | 5 (55.6%)          | 3 (75.0%)          |
| Serum calcium (mol/L) (2.05–2.60)                  | 1.80 (0.93–2.29)  | 1.70 (0.93–2.29)   | 2.03 (1.98–2.08)   |
| Decreased serum calcium                            | 8 (61.5%)         | 6 (66.7%)          | 2 (50.0%)          |
| Oxygen/respiratory support                         |                   |                   |                   |
| Nasal catheter for oxygen                          | 11 (84.6%)        | 8 (88.9%)          | 3 (75.0%)          |
| Mask oxygen                                        | 4 (30.7%)         | 0 (0%)             | 4 (100.0%)         |
| Noninvasive ventilator support                     | 4 (30.7%)         | 0 (0%)             | 4 (100.0%)         |
| Invasive ventilator support                        | 0 (0%)            | 0 (0%)             | 0 (0%)             |

Data are expressed as number (percentage) or average (range)
CP donors in advance. COVID-19 patients were at risk of concurrent infections due to their compromised immune systems. The antibody profile has clear links to some clinical lab indexes (lymphopenia, CRP, and creatine), which may lay a foundation for future therapy.

Contributors
Wei-ling Xiang and Jing-jing Cheng conducted the testing. Lian-peng Wu, Fei-hang GE, Dong Chen, and Wei Zhang collected the clinical data. Bing-yu Chen, Wen-xin Li, and Dan-ying Qiu performed the statistics and analyses of the data. Wei-ling Xiang wrote the first draft of the manuscript. Dong Chen and Zhen Wang designed the study. All authors have read and approved the final manuscript and, therefore, have full access to all of the data in the study and take responsibility for the integrity and accuracy of the data.

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Compliance with ethics guidelines
Wei-ling Xiang, Jing-jing Cheng, Lian-peng Wu, Bing-yu Chen, Wen-xin Li, Dan-ying Qiu, Wei Zhang, Fei-hang GE, Dong Chen, and Zhen Wang declare that they have no conflict of interest.

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中文概要

题目：浙江省新冠病毒肺炎患者的临床特征和血浆抗体滴度的调查与分析

目的：利用胶体金快速检测试剂盒测定恢复期患者血浆中IgG/IgM的抗体滴度，回顾性分析新型冠状病毒肺炎（COVID-19）患者临床特征、抗体效价变化特点，以及两者间的相关性。

创新点：利用胶体金检测试剂盒检测恢复期血浆抗体滴度，结合重症及危重症患者病例特点及实验室检测指标分析血浆抗体效价变化规律，及其临床特征的关系，为临床重症及危重症患者诊治以及筛选恢复期血浆供者提供参考依据。

方法：收集293份来自COVID-19康复者随访过程中的血液标本，取血浆原液，用生理盐水按不同比例（1:80、1:160、1:320和1:640）稀释后进行胶体金试纸法IgG/IgM抗体检测，并记录相应患者检验指标水平。通过电子病历系统检索回顾性分析温州市第六人民医院截至2020年2月24日确诊的COVID-19患者的流行病学特征和临床信息。通过线性回归分析两变量之间的相关性，P<0.05时具有统计学意义。

结论：症状出现4～6周时，COVID-19患者恢复期血浆IgG滴度水平可达到1:320～1:640或者更高水平。此时部分患者的血浆IgM仍为阳性。COVID-19患者恢复期血浆IgM抗体滴度检测结果显示男性较女性高。本研究中危重症患者均需要无创通气来维持生命，且重症和极危重症患者均易发生水电解质紊乱。

关键词：新型冠状病毒肺炎（COVID-19）；恢复期血浆；抗体滴度