Correspondence

High-fluorescent lymphocytes are increased in patients with COVID-19

Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, the coronavirus disease 2019 (COVID-19) has spread to most of the world. The understanding of the pathomechanism of SARS-CoV-2 is extremely limited.

SARS-CoV-2 is a member of β-coronavirus family, which includes SARS-CoV and Middle East respiratory syndrome (MERS)-CoV. Studies have found lymphocytes play a significant role in the anti-viral reaction against coronavirus. Lymphocytes, especially T cells, are also destroyed by the virus. The interaction between lymphocytes and SARS-CoV-2 in vivo is largely unknown. Epidemiological investigation has found blood lymphocytes are decreased in patients with COVID-19. As to lymphocyte subgroups, CD8+ T cells are decreased during the course of COVID-19, while the percentage of exhausted CD8+ T cells (NKG2A+ T cells) is increased. The mechanism underlying the lymphopenia in patients with COVID-19 is that lymphocytes, especially T cells, are attracted into the infection sites or killed by the coronavirus; described in detail in a recent review. B cells are the source of antibodies to SARS-CoV-2 and are a subtype of lymphocyte, but the levels of circulating B cells, especially activated B cells or plasma cells, are unclear. One study found that B cells were decreased substantially in patients with severe COVID-19, while another study suggested the level was not changed.

High-fluorescent lymphocytes (HFLs) in the blood are cells related with activated B cells or plasma cells. HFLs can be easily counted by an automated haematology analyser as one of the parameters of a full blood count. In the present study, we conducted retrospective analyses of the HFLs counts of 111 patients with COVID-19. The HFLs levels of patients with COVID-19 were compared with those of healthy individuals. To our knowledge, this is the first study to count and compare the numbers of HFLs in patients with differing severity of COVID-19. The present study may provide insight into understanding the interaction of B cells with SARS-CoV-2 and a clue to monitoring disease severity in patients with COVID-19.

We retrospectively analysed the full blood count results of patients with COVID-19 admitted to Wuhan Union Hospital, China, from 29 January to 8 March 2020. The diagnosis of COVID-19 was based on the Guideline provided by the National Health Commission of China. Patients with COVID-19 were classified into ‘mild’ or ‘severe’ subgroups according to the Guideline. Severe cases were defined as having any of the following features: (i) respiratory rate ≥30 breaths/min, (ii) oxygen saturation at rest of ≤93%; (iii) ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2) ≤300; (iv) pulmonary imaging showing that patients’ lesions had increased >50% within 24–48 h. Cases with other than a severe condition were classified as ‘mild’. Full blood count results including HFLs count were performed using Sysmex XE-5000 automated cytometry (Sysmex, Kobe, Japan). The healthy controls were enrolled before the SARS-CoV-2 outbreak in late November 2019. Healthy controls were from regular physical examination groups. Subjects with a fever, cough, or diarrhoea symptoms were excluded. Subjects with any lung, heart, liver, kidney and other infectious diseases were also excluded. All the results for the full blood count, including HFLs counts, were retrospectively retrieved from the information-processing unit (IPU) of Sysmex XE-5000 automated haematology analyser. The study was approved by the Ethics Committee of Wuhan Union Hospital. Non-normal data were described as medians with interquartile ranges. The Kruskal–Wallis test and Dunn’s multiple comparisons test were used to compare variables among groups. All statistical analyses were performed by GraphPad Prism version 8.0 (Graphpad Software Inc., La Jolla, CA, USA). A P < 0.05 was considered as statistically significant.

There were 111 patients with COVID-19 enrolled in the present study (Table 1). All were from the epicentre of Wuhan city at the beginning of the COVID-19 pandemic and 46 (41.4%) were male. The median (range) overall age of the patients with COVID-19 was 48.6 (24–89) years. In all, 19 patients (17.1%) were classified into the ‘severe’ group and five (4.5%) of them died. Patients in the severe group were older than patients in...

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the mild group (median age: 66 vs. 42 years, \( P < 0.001 \)). There was no significant difference in gender and age between the patients with COVID-19 and the healthy controls.

All 111 patients had lymphocyte count results for analyses; 24 patients had no HFLs count results, thus 87 were included for HFLs analyses. Lymphocyte counts were significantly lower in patients with mild and severe COVID-19 compared with the healthy controls (1400 ± 9 10^6/l and 820 ± 9 10^6/l vs. 2100 ± 9 10^6/l, \( P < 0.0001 \)). Lymphocyte counts in the severe group demonstrated a lower trend as compared to that in the mild group (\( P = 0.080 \), Fig 1).

The HFLs counts were significantly higher in patients with mild and severe COVID-19 compared with healthy controls (11-8 ± 10^6/l and 20-4 ± 10^6/l vs. 0-0 ± 10^6/l, \( P < 0.0001 \)). The HFLs counts tended to be higher in patients with severe COVID-19 than in those with mild COVID-19 (Fig 1). To explore the relative change between HFLs and lymphocytes in individuals, the ratio of HFLs/lymphocytes was calculated. The changing trends of HFLs/lymphocytes were similar to HFLs. Two of five patients with the highest HFLs/lymphocytes ratio died.

Since the beginning of the SARS-CoV-2 outbreak, the epidemiological and clinical features of COVID-19 have been reported.\(^2\,9\) Consistent with these reports, the present study showed that middle-aged people were more susceptible to SARS-CoV-2 and senior citizens more likely to develop a more severe condition. The fatality rate in the present study was 4-5%, which fell between 1-4%\(^2\) and 15%\(^9\) reported by two other studies. One characteristic that differed from other reports was that females represented the majority of the patients with COVID-19.

In the present study, we found that circulating lymphocytes were lower in patients with COVID-19, and those in the severe group tended to have fewer circulating lymphocytes than those in the mild group. Our present data further demonstrated the decreasing trend of lymphocytes in patients with COVID-19\(^2\,9\). Contrary to the overall decreasing trend of lymphocytes, we found that HFLs were increased in patients with COVID-19. Our present result concurred with a recent case report that found plasmacytoid lymphocytes in the blood films of patients with COVID-19.\(^10\) A study also reported that the levels of antibodies specific to SARS-CoV-2 were higher in patients with severe COVID-19.\(^11\) We found that HFLs tended to increase as the condition of the patients with COVID-19 got worse. This is reasonable, as HFLs are related to the level of circulating plasma cells.\(^7\)

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**Table I.** Demographics, baseline characteristics of the 111 patients with COVID-19.

| Characteristics                          | All patients | Mild patients | Severe patients |
|------------------------------------------|--------------|---------------|----------------|
| Males, n (%)                             | 46 (41-4)    | 37 (40-2)     | 9 (47-1)       |
| Age, years, median (IQR)                 | 47-0 (33-0-63-0) | 42-0 (32-0-57-0) | 66-0 (48-0-78-0) |
| Any comorbidity, n (%)                   |              |               |                |
| Hypertension                             | 22 (19-8)    | 14 (15-2)     | 8 (42-1)       |
| Diabetes                                 | 11 (9-9)     | 6 (6-5)       | 5 (26-3)       |
| Coronary heart disease                   | 8 (7-2)      | 5 (5-4)       | 3 (15-8)       |
| Signs and symptoms, n (%)                |              |               |                |
| Fever                                    | 75 (67-6)    | 60 (65-2)     | 15 (78-9)      |
| Cough                                    | 49 (44-1)    | 40 (43-5)     | 10 (52-6)      |
| Fatigue                                  | 41 (36-9)    | 35 (38-0)     | 7 (36-8)       |
| Myalgia                                  | 17 (15-3)    | 14 (15-2)     | 3 (15-8)       |
| Pharyngalia                              | 13 (11-7)    | 13 (14-1)     | 0 (0)          |
| Diarrhoea                                | 19 (17-1)    | 18 (19-6)     | 1 (5-3)        |

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**Fig 1.** Blood cells counts in patients with COVID-19 and healthy controls. Lymphocytes count (A), high-fluorescent lymphocytes (HFLs) count (B) and HFLs/lymphocytes (C) in patients with COVID-19 and healthy controls. \( ***P < 0.001 \), \( ****P < 0.0001 \).
In conclusion, the SARS-CoV-2 infection induced lymphopenia but increased HFLs. The HFLs level might be correlated with disease severity in patients with COVID-19. Given that HFLs can be conveniently counted by a haematology analyser, it might be a useful parameter for clinical monitoring and mechanism studies of COVID-19. Studies based on larger samples are warranted to confirm our present finding.

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Conflicts of interest

The authors do not have any conflict of interest to declare.

Author contributions

Zhao Wang and Zhaoming Tang conceived the study and wrote the paper. Yu He and Huaqing Shu analysed the data and wrote the paper. Ping Wang, Hui Xing and Xiaqian Zeng collected the data. All authors contributed to critical revision and final approval of the manuscript to be published.

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Molecular mechanisms for thrombosis risk in Black people: a role in excess mortality from COVID-19

We read with interest your recent article by Fogarty et al., in particular their conclusion that differences in thrombotic risk may contribute to ethnic disparities in mortality from coronavirus disease 2019 (COVID-19). This is especially important in the UK, where age-sex adjusted hospital death rates for COVID-19 are 2.17-times higher for people with ethnicity recorded as Black compared to those recorded as White, and 1.95 higher for those recorded as Asian. This excess mortality persists after adjustment for deprivation, body mass index (BMI), smoking and comorbidities, and