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IL-6 drives T cell death to participate in lymphopenia in COVID-19

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ABSTRACT

Lymphopenia is a common observation in patients with COVID-19. To explore the cause of T cell lymphopenia in the disease, laboratory results of 64 hospitalized COVID-19 patients were retrospectively analyzed and six patients were randomly selected to trace their changes of T lymphocytes and plasma concentration of IL-6 for the course of disease. Results confirmed that the *T*-cell lymphopenia, especially $CD4^+$ T cell reduction in COVID-19 patients, was a reliable indicator of severity and hospitalization in infected patients. And $CD4^+$ T cell count below 200 cells/μL predicts critical illness in COVID-19 patients. *In vitro* assay supported that exposure to key contributors (IL-1β, IL-6, TNF-α and IFN-γ) of COVID-19 cytokine storm caused substantial death of activated T cells. Among these contributors, IL-6 level was found to probably reversely correlate with T cell counts in patients. And IL-6 alone was potent to induce T cell reduction by gasderminE-mediated pyroptosis, inferring IL-6 took a part in affecting the function and status of T cells in COVID-19 patients. Intervention of IL-6 mediated T cell pryprotosis may effectively delay disease progression, maintain normal immune status at an early stage of infection.

1. Introduction

Multiple drugs to treat COVID-19 are developed or have entered clinical trials based on preliminary understanding of the pathogenesis of COVID-19 [1]. In severely affected patients, the sudden clinical deterioration following the initial symptom onset is driven by a unique pattern of immune dysfunction [2], with immune cells being excessively activated at first, and becoming exhausted thereafter [3,4]. T cell-mediated immunity is the central element of the adaptive immune system [5]. $CD8^+$ T cells are important for directly attacking and killing virus-infected cells, whereas $CD4^+$ T cells are crucial to prime both $CD8^+$ T cells and B cells [6]. The insufficient number and function of T lymphocytes is an important factor for exacerbation and mortality of COVID-19 patients [7]. Lymphopenia, especially T cell depletion, is considered to be an indicator associated with disease severity [8,9]. For instance, Diao *et al.* described that the number of total T cells, as well as $CD4^+$ and $CD8^+$ T cell subsets, were strongly reduced in COVID-19 patients, especially in those requiring ICU care. Counts of total T cells, $CD8^+$ or $CD4^+$ T cell subsets below 800, 300 or 400 cells/μL, respectively, exhibited negative correlation with patient survival [10]. Lessons from HIV infection indicated that a low $CD4^+$ T cell counts increases the risk of opportunistic infections and lower antiviral immune surveillance [11]. Thus, more attention should to be given to patients in critical condition, and non-ICU patients with total T cell counts lower than 800 cells/μL may still require urgent intervention, even in the absence of more severe symptoms, due to a high risk for further deterioration in condition [10].

The reasons for T cell reduction in severe COVID-19 patients were widely concerned. Complex immune dysfunction in severe COVID-19 may contribution to lymphopenia [12,13] via increasing expression of caspase-1 in T cells [14], elevating plasma level of sFasL in patients [15], and especially, cytokine storm [16]. Several pro-inflammatory or anti-inflammatory cytokines can accelerate the depletion and exhaustion of T cells with their respective functions [17,18], among them, IL-6 was the

Abbreviations: COVID-19, coronavirus disease 2019; ICU, intensive care unit; OR, odds ratio; CI, confidence interval. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CKs, cytokine mixture.

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unique pattern of immune dysregulation, associated with sustained cytokine production and hyper-inflammation [19]. The dynamic change in IL-6 also have been reported as a marker for disease monitoring and closely correlated with severity of COVID-19 [20], predicting poor prognosis. However, it has not been elucidated yet whether IL-6 could directly affect the number and function of T cell.

In this study, to explore the cause of T cell lymphopenia in the disease, laboratory results of 64 hospitalized COVID-19 patients were retrospectively analyzed and six patients were randomly selected to trace their changes of T lymphocytes and plasma concentration of IL-6 for the course of disease. Our results confirmed the clinical severity-dependent reduction and amelioration-dependent restoration in T cells, especially in $CD4^+$ T cell numbers, and revealed an association between the dynamic change of $CD4^+$ T cell counts and the clinical course and outcome of COVID-19. And we also found that, among key contributors of COVID-19 cytokine storm, IL-6 alone was potent to induce T cell reduction by gasderminE-mediated pyroptosis, suggesting intervention of IL-6 mediated T cell pryprotosis may effectively delay disease progression, maintain normal immune status at an early stage of infection.

2. Materials and methods

2.1. Data collection

In the part of retrospective study, all COVID-19 patients included were admitted between 7 February and 13 March 2020 to Zhongnan Hospital of Wuhan University, Wuhan, China. The electronic medical records of the 64 patients, including demographic information, clinical course, laboratory data, and outcome of the disease were analyzed.

2.2. Detection of lymphocyte subsets and interleukin-6 in COVID-19 patients

Peripheral venous blood was collected from patients with COVID-19, and lymphocyte subsets and plasma IL-6 were measured at indicated time points between admission and discharge/transfer. Cells were stained using the BD Multitest™ IMK kit (BD ltd., San Jose, CA, USA) according to the manufacturer' s instruction and were analyzed by flow cytometry (BD FACSCanto™ II Flow Cytometer). Interleukin-6 (IL-6) was detected using the automatic electrochemiluminescence immunoassay system (Cobas e601, Roche, Basel, Switzerland), elecsys IL-6 (Cobas 07027532501V1.0, Roche, Switzerland) immunoassay was used for the quantitative determination of IL-6 in plasma according to the manufacturer's instructions.

2.3. Cell culture

PBMCs from healthy donors were isolated by density gradient using Lymphoprep (STEMCELL, 07851). Cells were resuspended in RPMI 1640 with 10 % FBS and penicillin and streptomycin (Biosharp, BL505A) or were resuspended in cryopreservation medium and then stored at liquid nitrogen until needed.

T cell were isolated from PBMCs using EasySep Human T cell Isolation Kit (STEMCELL, 17951) according to manufacturer's protocol. Cells were cultured in RPMI 1640 (Gibco, C11875500BT) supplemented with 1 % non-essential amino acids (Gibco, 11140-050), 1 % sodium pyrubate (Gibco, 11360070) and 10 % FBS (Gibco), and 1 % penicillin and streptomycin. Activated T cells were obtained by 2–5 h of incubation in plates pre-coated with anti-CD3 (Bioxcell, BE0001-2) and anti-CD28 (Bioxcell, BE0248).

PBMCs or T cells were stimulated with following cytokines at concentrations where indicated unless otherwise noted: 1 ng/ml of IL-1β (PeproTech, 200-01B), 20 ng/ml of IL-6 (PeproTech, 200-06), 5 ng/ml of TNF-α (PeproTech, 200-01A) and 1 ng/ml IFN-γ (PeproTech, 300-02).

The release of LDH was measured using LDH Cytoxicity Assay Kit (Beyotime, C0016, China) according to manufacturer's instruction.

2.4. Flow cytometry analysis

PBMCs or T cells were stained with PE-Cy7 anti-CD3 (BD, 560910), APC-Cy7 anti-CD4 (BD, 557871), BV510 anti-CD8 (BD, 344732) for T cell subset analysis. Propidium iodide (PI, 556547, BD Bioscience) were used for cell death analysis according to manufacturer's instruction. Data were acquired using the flow cytometer (BD verse) and analyzed with the Flowjo software.

2.5. Immunoblot analysis

T cells were lysated in RIPA lysis buffer (Fudebio-tech, FD009). Equal amounts of protein were resolved by 10 % SDS-PAGE. After electrophoresis, separated proteins were transferred onto PVDF membranes (Millipore, IPVH00010). The membrane was blocked in 5 % skim milk, followed by overnight incubation with GSDME-N terminal antibody (Abcam, ab215191, 1:1000). After incubation with HRP-conjugated secondary antibody, the positive immune reactive signal was detected using FDbio-Dura ECL Kit (Fdbio-tech, FD8020). β-actin was used as an endogenous control.

2.6. Statistical analysis

Statistical significance was determined by *t* test (two-tailed) for two groups or by one-way ANOVA for three or more groups. Categorical variables were expressed as number (%) and compared by Fisher's exact test between the ICU and non-ICU group in Table. Statistical analyses were performed using the GraphPad Prism software Version 8.3.0 and SPSS Version 22.0.

3. Results

3.1. $CD4^+$ T cell count closely relates to the severity of COVID-19

Of the 64 patients with confirmed COVID-19 diagnosis who were enrolled in this study, 13 were admitted to the ICU. In line with previous reports [21], our investigation observed the elevated neutrophil-to-lymphocyte ratios in these cases (Fig. S1) and confirmed that T lymphocyte counts in peripheral blood ($CD3^+$ cells) did decrease after SARS-CoV-2 infection, as well as $CD4^+$ ($CD3^+CD4^+$) and cytotoxic $CD8^+$ ($CD3^+CD8^+$) T subsets (data not shown). To check the relationship between *T*-cell lymphopenia and the severity of COVID-19, T cell counts in ICU and non-ICU groups were compared. Fig. 1A showed that T cell counts fell to a lower level in ICU patients, as well as $CD4^+$ T subset counts. Although $CD8^+$ T subset counts showed the same tendency, the difference between two groups was not statistically significant. Further analysis suggested that patients with a $CD4^+$ T cell count of less than 200 cells/μL were more likely to be admitted to the ICU ($P = 0.004$, $OR = 9.26$, 95 %*CI*: (2.10, 43.94)), which was not observed for $CD8^+$ T cell counts ($P = 0.22$, $OR = 2.53$, 95 %*CI*: (0.70, 9.25)) (Fig. 1B, Table 1).

To better evaluate the predictive character of T cell counts on the clinical course and outcome of the disease, dynamic changes of T cells were monitored in six cases (Fig. 1C, Patient 1–6, P1-P6) from admission until death or discharge. At the time of enrollment, three patients (P1-P3) were treated on a general ward, and three patients (P4-P6) on the ICU. For P1 and P4, the counts of total T cells and T subsets were slightly lower than normal range (805–4459 cells/μl), but for the others (P2, P3, P5, P6), these values were nearly-one third of the lower reference limit. As the disease progressed, all three non-ICU patients were admitted to the ICU after their T cell counts decreased to an extremely low level. When T cell counts in the ICU patients, especially $CD4^+$ counts, increased and reached to the normal range within the following three weeks, their condition improved, and they were transferred to the general ward and finally discharged. However, the clinical condition of

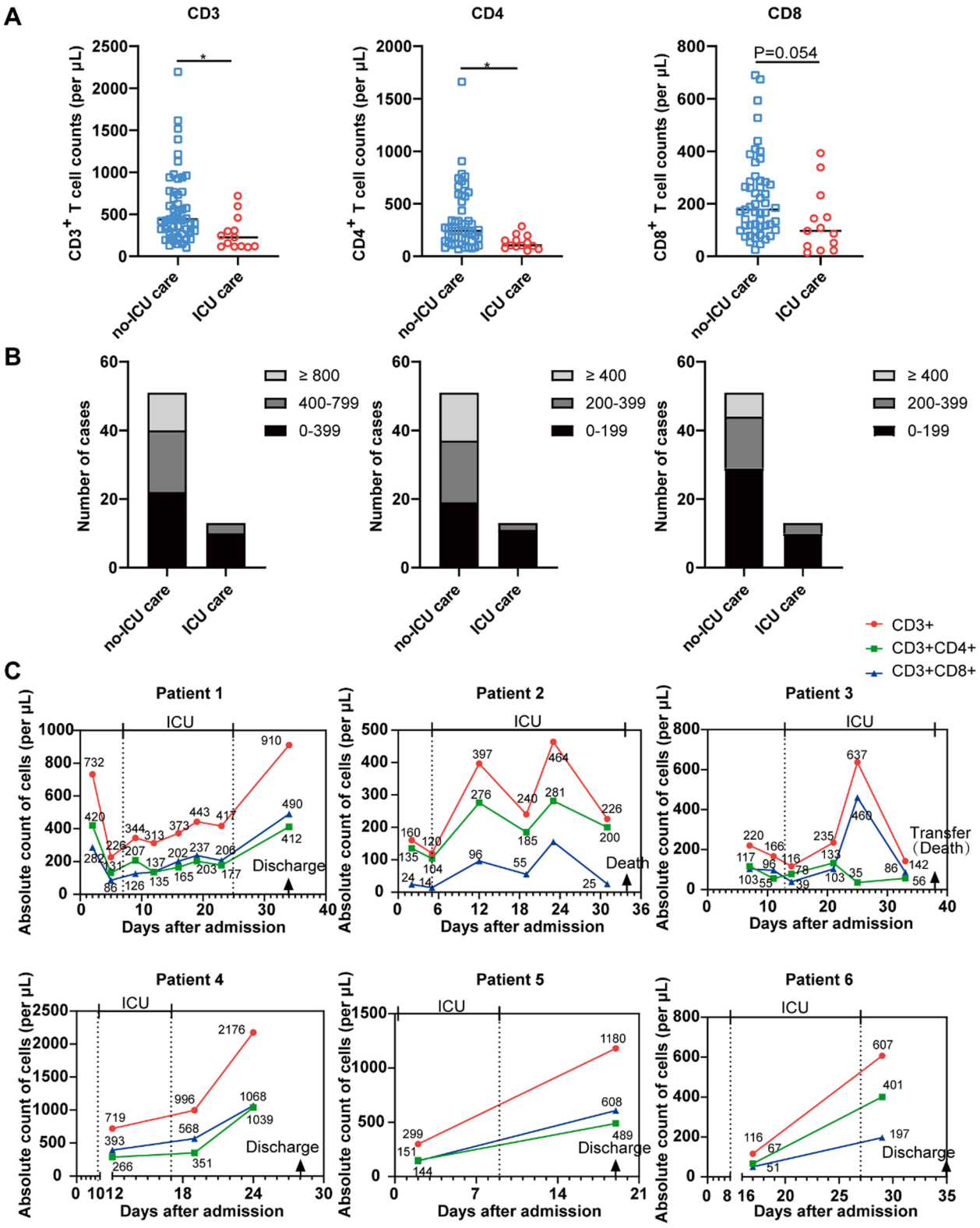

Fig. 1. Counts of T cell subsets in ICU and no-ICU COVID-19 patients. (A) Counts of T cell subsets in COVID-19 patients admitted to ICU or not. Each dot represents a count for T cell subsets. Data were analyzed using unpaired *t* test. $^{*}P < 0.05$. (B) Different T cell subset counts and ICU care distribution of COVID-19 patients. Number of hospital admissions grouped by ICU admission or not. Light grey bars represent T cell counts greater than 800 cells/μl ($CD3^{+}$) or 400 cells/μl ($CD4^{+}$ and $CD8^{+}$), median grey bars represent counts from 400 to 799 cells/μl ($CD3^{+}$) or 200 to 399 cells/μl ($CD4^{+}$ and $CD8^{+}$), black bars represent counts of less than 400 cells/μl ($CD3^{+}$) or 200 cells/μl ($CD4^{+}$ and $CD8^{+}$), n = 64. (C)Timeline charts illustrate T cells ($CD3^{+}$, $CD3^{+}CD4^{+}$, $CD3^{+}CD8^{+}$) on the day after admission to hospital in 6 representative COVID-19 patients.

Table 1
$CD4^+$ and $CD8^+$ T cell count thresholds in ICU care and non-ICU care patients.

$CD4^+$ T cell count cells/μL	ICU care		Total	*P value*	*OR (95 %CI)*
	ICU care	no-ICU care			
< 200	11	19	30	**0.002**	**9.26(1.85, 46.34)**
≥200	2	32	34		
Total	13	51	64		
$CD8^+$ T cell count cells/μL	ICU care		Total	*P value*	*OR (95%CI)*
	ICU care	no-ICU care			
<200	10	29	39	**0.19**	**2.53(0.62, 10.30)**
≥200	3	22	25		
Total	13	51	64		

Note: Data are given as number of patients in different groups. *P* values comparing ICU care and no ICU care from χ^2. $P < 0.05$ was considered statistically significant. *OR* values refer to the ratio of the number of T cells ($CD4^+$, $CD8^+$) less or greater than 200 cells/μL in the case group divided by the ratio of the number of T cell ($CD4^+$, $CD8^+$) less or greater than 200 cells/μL in the control group. Abbreviation: ICU, intensive care unit; OR, odds ratio; CI, confidence interval.

P2 and P3 deteriorated and their T cell counts decreased further, resulting in a lethal outcome. These results suggest that the count of T cells, especially of $CD4^+$ T cells, is closely related to the severity of COVID-19.

3.2. Activated T cells are sensitive to cytokine-induced death

After confirming the predictive impact of T cell counts on the clinical course and outcome of the disease, next, we need to explore the underlying mechanism of *T*-cell lymphopenia during the COVID-19 infection. The occurrence of cytokine storm is closely associated with the rapid deterioration and high mortality of severe cases in COVID-19. And cytokine storm has been reported to affect the number and function of T cells [22–24]. To mimic the cytokinaemia, IL-1β, IL-6, TNF-α and IFN-γ, which are the key contributors to cytokine storm in COVID-19 patients [25–28], were applied to detect their impact on T cell death. The gating strategy and analysis method were depicted in Fig. 2A. Results suggested that T cells in healthy donor-derived PBMCs, along with $CD4^+$ T subset, underwent significant cell death when exposed to these cytokine mixtures at low concentrations (Fig. 2B). And the cytokines induced T cell death in a dose dependent manner (Fig. 2C). To resemble the priming and activation of naïve T cells by virus antigens, the isolated T subsets were pre-stimulated with CD3/CD28 antibodies and then conditioned by cytokine mixtures. Results showed that the activated T cells were more sensitive to cytokines induced death (Fig. 2D) than resting subsets. Multiple healthy samples verified that the activated T cells were vulnerable to cytokine mixtures-induced death (Fig. 2E).

3.3. IL-6 level negatively correlates with T cell counts

IL-6 is the cytokine with more significant changes in COVID-19 patients [29,30]. Its increment in patients with other critical illnesses was reported to associate with hypercytokinemia and unexplained clinical severity-related lymphopenia [31]. To deepen pathobiological understanding of *T*-cell lymphopenia and cytokine storm in COVID-19 patients, next, we analyze whether the lymphopenia is mediated at least in part by elevated IL-6. In our six COVID-19 cases as specified above, IL-6 detection was available for 3 patients (P1, P2 and P4). In these cases, the plasma IL-6 level fluctuated reversely with the leukocyte count (Fig. 3A), as well as with percentage of lymphocyte (Fig. 3C), but with no correlation with neutrophil percent (Fig. 3B). Besides, counts of T cell and subsets might negatively associated with IL-6 level. The counts declined with increase of IL-6, and when counts reached the minimum, IL-6 level reached their maximum. And when conditions were improved in patient 1 and 4, T cell counts restored step by step while with a sharp decline of IL-6. In patient 2, with the deteriorating condition, T cell counts fluctuated while IL-6 level still maintained at low levels for nearly 3 weeks, followed by a sharp increase of IL-6 before a lethal outcome (Fig. 3D).

3.4. IL-6 induces the GSDME-mediated T cell pyroptosis

To detect thereaction to IL-6, activated healthy T cells were conditioned with IL-6 at different concentrations. Results manifested that IL-6 significantly induced death of $CD4^+$ and $CD8^+$ T subsets in a dose dependent manner. And $CD4^+$ subset was more sensitive to IL-6 condition, which was induced to death with statistical significance at concentration of 2 ng/ml, while $CD8^+$ subset at 20 ng/ml (Fig. 4A). Multiple healthy samples confirmed the IL-6-induced T cell death (Fig. 4B). Pyroptosis represents the pathways of genetically encoded necrotic cell death which can protect the host against microbial pathogens. However its dyregulation allows the release of inflammatory cytokines, to trigger inflammation [32]. It has been reported cytokine storm syndromes in COVID-19 triggers inflammatory cell death which related with pyroptosis [14]. To test the cell death modalities of IL-6-conditioned T cells, their expression of GSDME were monitored. Data showed that IL-6 stimulation facilitated cells to release more GSDME-*N*-terminal to the plasma membrane, causing pyroptosis (Fig. 4C), while with no effect on release of cleaved Caspase-3 (Fig. 4D). Meanwhile, IL-6-conditioned cells lose membrane integrity and lyse, releasing more lactate dehydrogenase (LDH) than control (Fig. 4E), suggesting the participation of IL-6 in *T*-cell lymphopenia in COVID-19 patients through the GSDME-mediated pyroptosis rather than Caspase-3-mediated apoptosis.

4. Discussion

In this study, we confirm that COVID-19 patients exhibit *T*-cell lymphopenia and $CD4^+$ T cell count is helpful in evaluating the severity of SARS-CoV-2 infection. If $CD4^+$ T cell counts progressively decrease below 200 cells/μL, COVID-19 patients should be admitted to ICU. Restoration of $CD4^+$ T cell count to normal levels indicate an improvement of the clinical condition and predict a favorable clinical course for critical ill patients, while fluctuation of $CD4^+$ T cell counts at low levels point at deterioration and a lethal outcome.

In agreement with previous reports [33–35], our findings confirm that lymphopenia is a common feature in most of COVID-19 patients, and predicts COVID-19 severity. Lymphocytes play an important role in maintaining immune homeostasis and regulation of the inflammatory response throughout the body [36,37]. Lymphocyte exhaustion may partially explain the immune dysregulation that occurs in COVID-19 patients [2]. Among lymphocytes, T cells, particularly $CD4^+$ and $CD8^+$ T cell subsets, play a crucial role in promoting effective immunity and viral clearance during viral infection [5]. Previous reports have demonstrated that the counts of peripheral $CD4^+$ and $CD8^+$ T cells were substantially reduced and hyperactivated [38], and the decrease of $CD4^+$ T cells was more pronounced in severe COVID-19 cases [39]. Our study verifies that counts of total T cells, $CD4^+$ and $CD8^+$ T subsets decrease in a large fraction of patients to a relatively low level, and the decrease of $CD4^+$ T cells was more evident in severely affected patients. $CD4^+$ T cell counts of less than 200 cells/μL have been identified to be a risk factor for the patients' ICU admission.

In order to better understand the potential of T cells and T subset counts for predicting the clinical course and outcome of COVID-19, dynamic changes of T lymphocyte count and their association to disease severity were further analyzed. With an OR of 9.26, the $CD4^+$ T cell count was a strong indicator for the requirement of ICU treatment. Diao *et al.* reported 400 cells/μL of $CD4^+$ T subsets would be indicator for patients requiring urgent intervention [10]. Here, we reported a decrease of $CD4^+$ T cells below 200 cells/μl could serve as an early

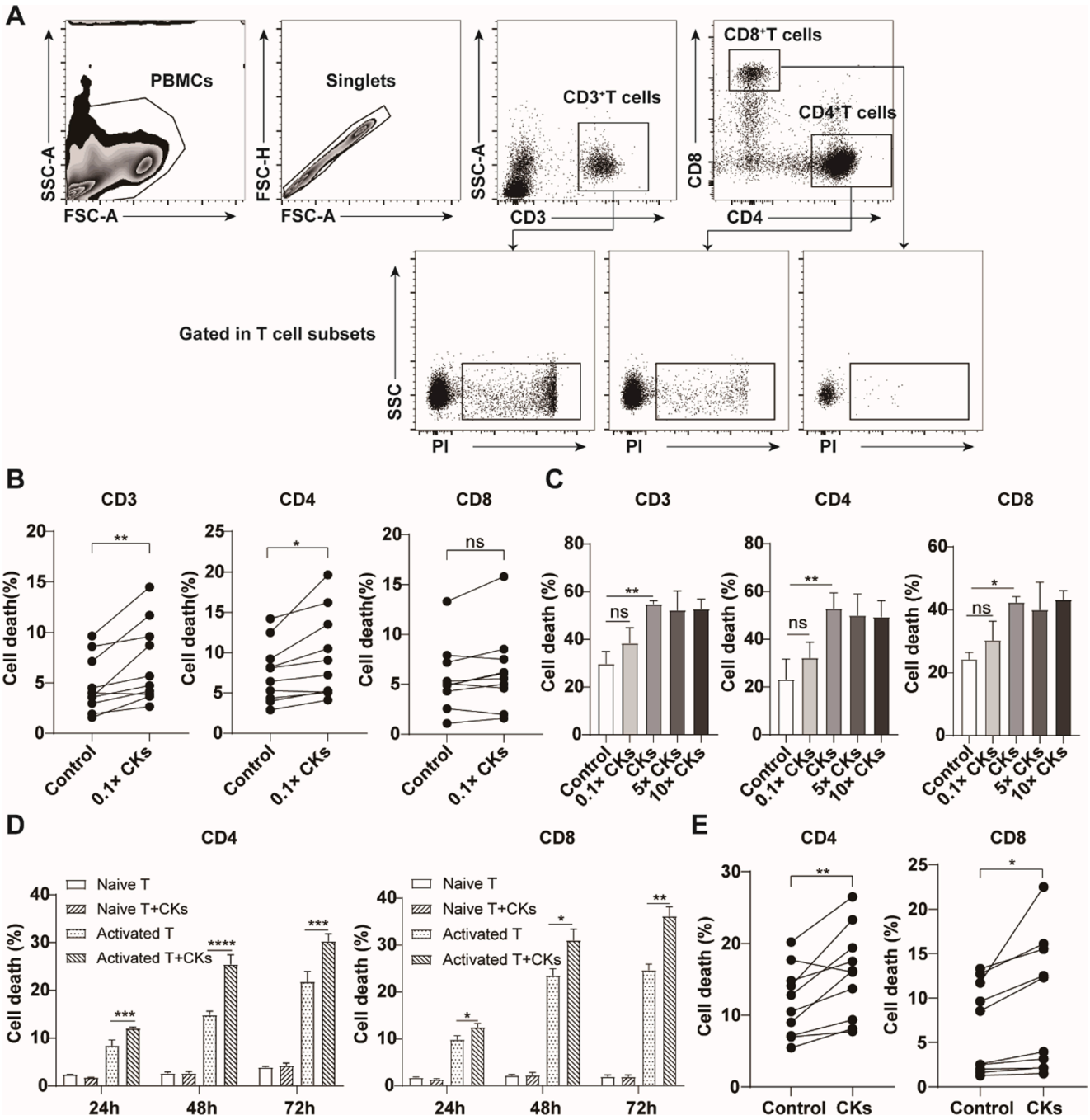

Fig. 2. Cytokine mixtures promote T cell death. (A) The representative plots for analyzing the death of T cell subsets by FCM. (B) Freshly isolated or (C) Cryopreserved PBMCs from healthy donors were stimulated with cytokine mixtures (1 ng/ml IL-1β, 20 ng/ml IL-6, 5 ng/ml TNF-α and 1 ng/ml IFN-γ) at indicated folds for 48 h. Cells were stained with PI for cell death assay. (D) T cells isolated from healthy donors were pre-activated with anti-CD3 and anti-CD28 for 2–5 h. Then cells were treated with cytokine mixtures, followed by death detection at indicated timepoints. Data were representative of 3–5 independent experiments. (E) Death rates of T cells stimulated with or without cytokine mixtures (48 h), n = 10. Data were analyzed using one-way ANOVA or paired *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

marker for clinical deterioration, when other aspects of COVID-19 severity like impaired pulmonary gas exchange are not yet apparent.

In a detailed follow-up of six cases, we observed the clinical severity-dependent reduction and amelioration-dependent restoration in T cells, especially in $CD4^+$ T cell numbers. The duration of low $CD4^+$ T cell counts was an indicator for the length of the ICU stay. $CD8^+$ T cells directly attack and kill virus-infected cells, whereas $CD4^+$ T subsets play a major role in initiating and shaping adaptive immune responses [40]. Hence, the depletion and restoration of the $CD4^+$ T cell subset reflects the immune dysregulation which occurs in COVID-19 patients, and thus could serve as a reliable indicator for disease severity, therapeutic response and outcome.

The mechanisms causing T cell reduction in COVID-19 patients have been discussed extensively. It is concluded that lymphocyte sequestration to specific target organs [41], induction of an immune-mediated destruction of infected lymphocytes [42], direct inhibition of bone marrow or inhibition of lymphocytes by metabolic molecules [43] and hyperactive T cells [44], are all factors which contribute to lymphocyte deficiency. Cytokine storm is a condition of uncontrolled systemic hyper-inflammation caused by cytokine excess, leading to multi-organ

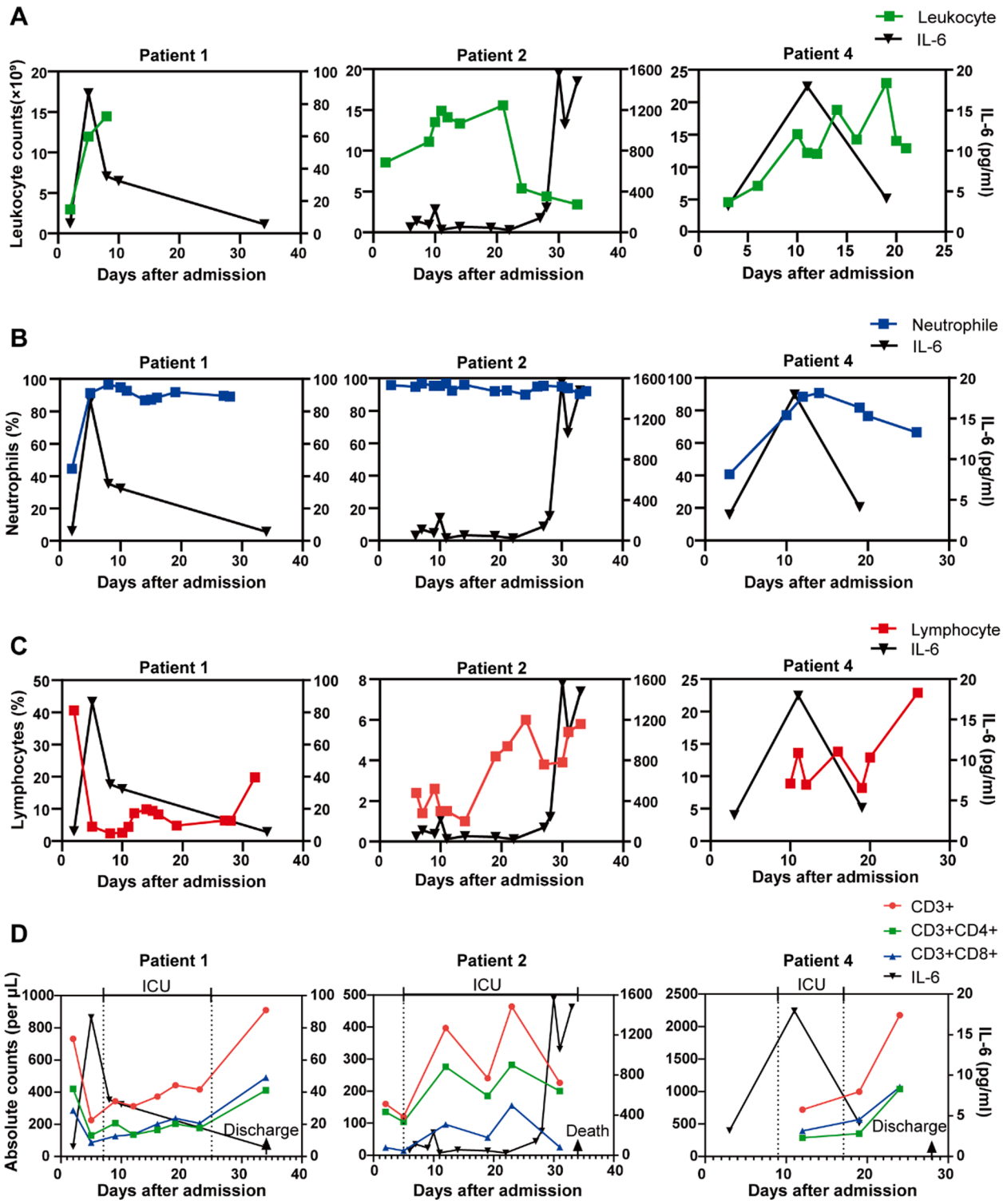

Fig. 3. Dynamic changes of T cell count and IL-6 in COVID-19 patients. Timeline charts illustrate IL-6 levels and leukocyte counts (A), percentage of neutrophils (B) and lymphocytes (C), T subset counts (D) on the day after admission to hospital in COVID-19 patients.

failure and even death [45]. Studies have indicated that rapid clinical deterioration and high mortality risk in COVID-19 could be related to cytokine storm [16]. Variety of cytokines, especially the IL-1 family, IL-6, TNF-α and interferon (IFN)-γ are involved [25,27,46–49]. In this study, we confirmed that activated T cells are sensitive to death induced by these cytokines.

Among these cytokines, IL-6, although its production in COVID-19 is not comparable to in other critical illnesses associated with elevated cytokine concentrations [50], as one of most important cytokines in COVID-19 related cytokines storm, has been widely concerned [20,30]. A study showed IL-6 was more elevated in no-survivors than survivors from COVID-19 [29]. Physiological concentrations of IL-6 in human serum are normally low (1–5 pg/ml), but during disease, IL-6 is rapidly induced and in extreme circumstances reaches μg/ml quantities [51]. Similar circumstances could be found in severe COVID-19 patients, the concentration of IL-6 could reached to 1600 pg/ml in P2.

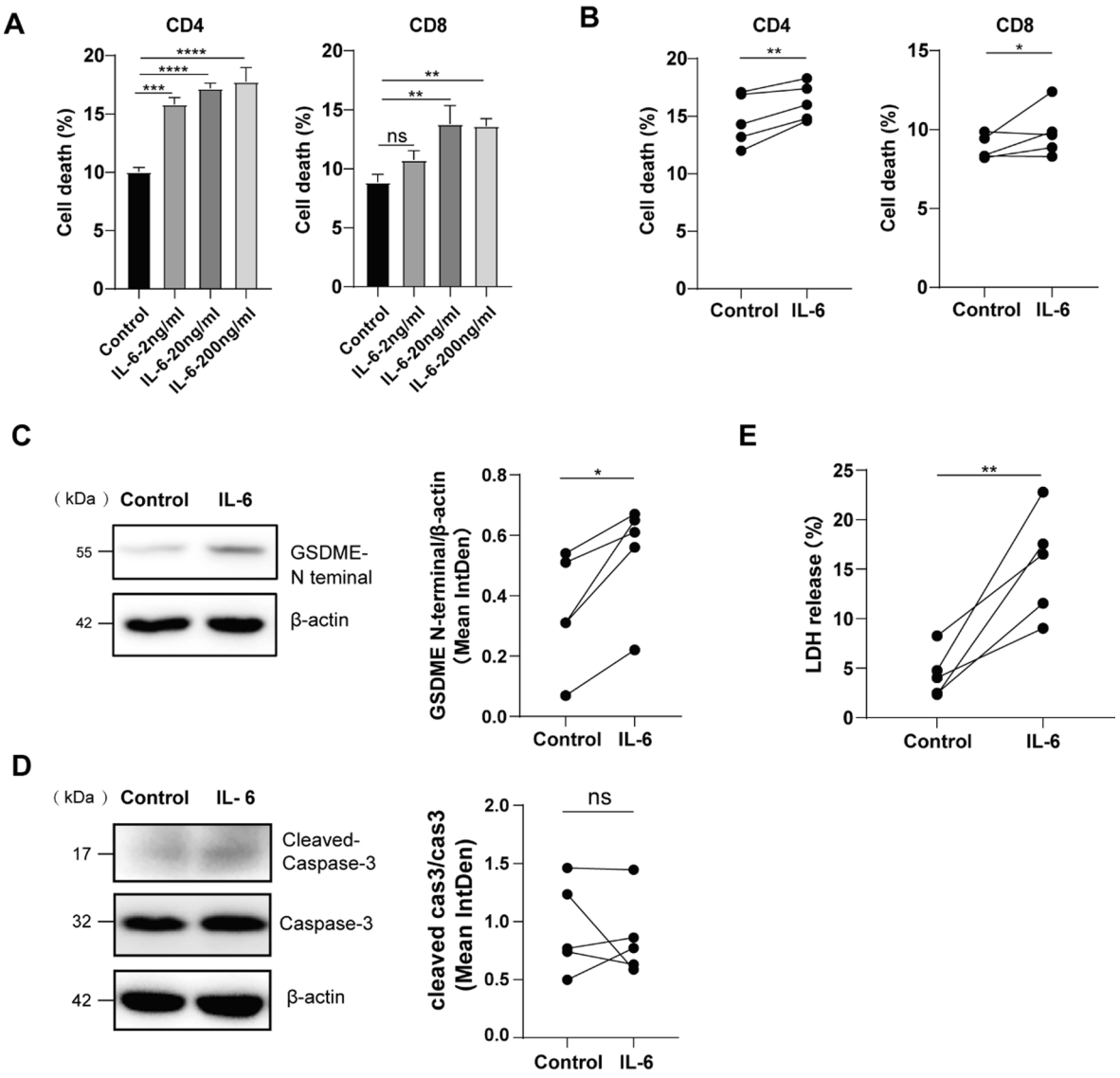

Fig. 4. IL-6 induces T cell death through a GSDME-mediated pyroptosis way.(A) T cells isolated from healthy donors were pre-activated with anti-CD3 and anti-CD28 for 2–5 h. Then cells were treated with IL-6 at indicated concentrations for 48 h, Death of $CD4^+$ and $CD8^+$ T subsets were determined by FCM. Data were representative of three independent experiments. (B) Death rates of T cells stimulated with or without IL-6 (20 ng/ml, 48 h). WB analysis for GSDME (C) and Caspase-3 (D) in T cells stimulated with or without IL-6. Representative image (right) and integrated density ratio (GSDME-N/β-actin or cleaved Caspase-3/Caspase-3) were shown. (E) LDH release analysis. T cell were stimulated with 20 ng/ml IL-6 for 48 h. LDH release was calculated as LDH release (%) = 100 × (experimental release - spontaneous release) / (maximum release - spontaneous release). Data were representative of three independent experiments and analyzed using one-way ANOVA or paired *t* test. n = 5. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

IL-6 is important for *T*-cell mediated adaptive immunity and plays an important role in the pathogenesis of proinflammatory diseases [52]. In our study, we assumed that T cell counts negatively correlate with IL-6 levels and IL-6 could induce T cell death through GSDME-mediated pyroptosis rather than caspase 3-mediated apoptosis. This mode of T cell death would contribute to more cytokine release [53] and further exacerbate the disease. Therefore, dysregulated activation of T cells can be considered as a major pathological mechanism in COVID 19-associated cytokine storm wherein IL-6 is the key cytokines [16]. Combined with reports that IL-6 blocks lymphopoiesis by elevating the expansion of uncommitted progenitors and suppressing the lymphoid option [31], it could be speculated that IL-6 is a significant inflammatory mediator affecting the function and status of T cells in COVID-19 patients.

Some clinical trial targeting IL-6 and IL-6R have been carried out, however randomised trials (RCTs) of IL-6 blockade for severe COVID-19 have proven essentially null, and cytokine blockade in severe COVID-19 also has yet to be proven effective [54]. However, fully understanding the mechanism of T cell pyroptosis and intervening and blocking the course may solve the dramatic decrease in the number of lymphocytes caused by cytokine storm, the collapse of the immune system, and ultimately lead to death to a certain extent.

Apart from lymphocytes, we observed that neutrophil counts also changed, with an exact opposite trend to lymphocyte counts. This observation is consistent with the report that the increase of neutrophil-to-lymphocyte ratio indicates higher disease severity and poor clinical outcome in COVID-19 patients [21]. One plausible explanation is that neutrophils with suppressive properties such as granulocytic myeloid-derived suppressor cells (G-MDSCs) suppress T lymphocytes expansion and give rise to the lymphopenia in severe COVID-19 patients [55]. However, the function of this kinds of subset G-MDSCs have not been fully investigated, the underlying mechanism is worthy of further investigation.

In conclusion, $CD4^+$ T cell count below 200 cells/μL predicts critical illness in COVID-19 patients, and dynamic changes of $CD4^+$ T cell counts

can indicate aggravation or recovery from COVID-19. IL-6 has a role in mediating T cell pyroptosis by GSDME-mediated pathway. Intervention of IL-6 mediated T cell pryprotosis may effectively delay disease progression, maintain normal immune status at an early stage.

Ethics approval

This study was approved by the institutional ethics board of Tongji Medical College, Huazhong University of Science and Technology; Written informed consent was not obtained because the data were analyzed retrospectively and anonymously.

Author contributions

Y.GM and L.YR performed flow cytometry analyses. H.Y was responsible for the collection and summary of clinical cases. Z.XQ, H.Y and L.P analyzed the data and wrote the manuscript. S.GX, P.X, H.Y and L.P supervised the study.

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CRediT authorship contribution statement

Xiaoqi Zhou: Investigation, Visualization, Writing – original draft. **Guangming Ye:** Investigation, Visualization, Resources. **Yibing Lv:** Validation. **Yanyan Guo:** Investigation. **Xingfei Pan:** Supervision. **Yirong Li:** Investigation, Resources. **Guanxin Shen:** Supervision. **Yong He:** Conceptualization, Visualization, Supervision. **Ping Lei:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2022.109132.

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