# Dynamics of Immune Responses are Inconsistent when Trauma Patients are Grouped by Injury Severity Score and Clinical Outcomes

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**Research**

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# Dynamics of immune responses are inconsistent when trauma patients are grouped by injury severity score and clinical outcomes

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Short title: Immune dynamics in trauma patients

**Abstract**

**Background:** The injury severity score (ISS) is used in daily practice to evaluate the severity of trauma patients, however the score is not always consistent with the prognosis. After injury, systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS) are related to the prognosis of trauma patients.

**Methods:** Patients who admitted to the Trauma Intensive Care Unit (ICU) were eligible. Whole blood samples were collected at admission, and then 6, 12, 24, 48 and 72 hours after admission. Natural killer (NK) cells, lymphocyte subset population and cytokines release were identified using flow cytometry. We grouped patients by their ISS ($\leq 25$ and $> 25$ as very severe injury) and ICU stay ($\leq 10$ days as a short ICU stay and $>10$ days as a long ICU stay) for evaluation.

**Results:** Fifty-three patients were enrolled. ICU stay but not ISS was close correlated with activity daily living (ADL) at discharge. Patients with a long ICU stay had an immediate increase in NK cells followed by lymphopenia which persisted for 48 hours. Immediate activation of $CD8^+$ T cells and then exhaustion with a higher programmed cell death-1 (PD-1) expression and suppression of $CD4^+$ T cells with a shift to an anti-inflammatory Th2 phenotype were also observed in the patients with a long ICU stay. When the patients were grouped by ISS, the dynamics of immune responses were inconsistent to those when the patients were grouped by ICU stay.

**Conclusions:** Immune responses are associated with the prognosis of trauma patients, however the currently used clinical parameters may not accurately reflect immune responses. Further investigations are needed to identify accurate predictors of prognosis in trauma patients.

**Keywords:** Trauma, injury severity score, lymphopenia; Natural killer

## Background

Trauma is a leading cause of mortality and morbidity worldwide, and it is also the leading cause of a loss of years of productive life. It is very heterogeneous in terms of the underlying causes, and it is characterized by considerable prognostic uncertainty. Although traditional vital signs such as blood pressure, heart rate, respiratory rate and body temperature are routinely monitored in trauma patients, their prognostic ability in these patients is unclear. The injury severity score (ISS) is widely used to evaluate trauma patients [1]. The ISS is calculated as the sum of the square of the three most severe injuries ranges from 3 (least) to 75 (most) injured. However it only considers one injury per body region. An ISS of 1–8 is considered minor, 9–15 moderate, 16–24 severe, and 25 and higher very severe. ISS is widely used to predict mortality but is not linear [2, 3].

Following trauma, exaggerated innate immunity results in systemic inflammatory response syndrome (SIRS), and almost simultaneously, an appropriate and extremely important counter-inflammatory response is initiated known as compensatory anti-inflammatory response syndrome (CARS). The large amount of tissue injury caused by trauma releases various antigens and mediators. The response is sterile, and these endogenous factors interact with immune cells to initiate inflammatory responses [4]. SIRS commonly follows traumatic injury in humans and is the result of innate immune system activation [5-7]. The purpose of the inflammatory response is to limit further damage, clear debris and promote healing, and the degree of immune system activation is related to the concentration of cytokines produced [8]. CARS usually reflects all autoimmunosuppression caused by a major insult such as sepsis, burns, or tissue injury and is mediated primarily by the adaptive immune system and, in particular, by T cells. It is well-known that trauma alters the immune system, and that this process is complex and dynamic. However, the relationship between the dynamics of immunity and prognosis in trauma patients is uncertain.

Several studies [9-13] have shown that short- and intermediate-term mortality outcomes were worse for patients who had prolonged stays in ICU. The definition of prolonged ICU stay is different by choosing different break point which results in the effect of a prolonged stay in ICU on prognosis in critically ill patients remains controversial and uncertain. Some study showed an apparent cut-off point appeared to occur on day 10 for hospital mortality and long-term survival because of the physiological insults of a critical illness occur within the first 10 days of the onset of a critical illness [14].

Activity of daily living (ADL) is used as an indicator of a person's functional status [15] and the inability to perform ADLs results in the dependence of other individuals and/or mechanical devices. ADL is an important predictor of admission to nursing homes, need for alternative living arrangements, and use of paid home care which is related to the prognosis in trauma patients [16-19]. In general, the scores of 0-20 indicate "total" dependency, 21-60 indicate "severe" dependency, 61-90 indicate "moderate" dependency, and 91-99 indicates "slight" dependency [20]. Most studies apply the 60/61 cutting point.

The logistical challenges of conducting trauma research mean that few studies have specifically examined consecutive immune cell populations after injury, and few have focused on the first 2 hours. Therefore, the aim of this prospective observational cohort study was to investigate associations between the immune response and prognosis in trauma patients.

## Methods

### Study design

This is a prospective study conducted from March 2018 to January 2021, patients older than 20 years admitted to the Trauma Intensive Care Unit (ICU) at the Department of Traumatology of National Taiwan University Hospital, the level I trauma center in Taipei, Taiwan, were screened. Those without systemic diseases (severe liver disease, known bleeding abnormality, etc.), substance abuse, concomitant use of drugs (anticoagulant medication, etc.), or smoking habit were enrolled. The exclusion criteria included: age < 20 years, transfer from another hospital, arrival > 120 minutes from injury, and those refused informed consent. Data including demographics, co-morbidities, trauma mechanism, severity of ISS, ICU stay and hospital length of stay (LOS), ICU and hospital mortality were collected. Activity of daily living (ADL) at discharge of survivors were also recorded for prognostic assessment. Laboratory data were measured daily as part of routine patient care. Blood samples for further evaluations were collected from ICU admission. Eight healthy volunteers (4 were males and 4 were females) were also recruited in the period for control group. This study was approved by the Institutional Ethical Committee of National Taiwan University Hospital (NTUH IRB No. 201702060 RINA). Informed consent was obtained in writing from each patient before inclusion in the study.

**Sampling and peripheral blood mononuclear cell (PBMC) isolation**

Whole blood samples were collected into heparin–containing tubes from the trauma patients at admission, and then 6, 12, 24, 48 and 72 hours after admission. PBMCs were isolated from the whole blood samples using the standard gradient centrifugation method.

**Flow cytometry**

Cell subset analysis was carried out using flow cytometry with the following antibodies: FITC conjugated anti-CD8a, FITC conjugated anti-IL-4, PE conjugated anti-*IFN-γ*, PerCP/Cy5.5 conjugated anti-CD4, PE/Cy7 conjugated anti-CD3, PE/Cy7 conjugated anti-PD-1, APC conjugated anti-CD8a, APC/Cy7 conjugated anti-CD56 and APC/Cy7 conjugated anti-CD3. The staining protocol was performed following the instructions provided with each antibody kit. Fresh isolated PBMCs ($1x10^6$ cells/ml supplemented with RPMI 1640) were incubated with phorbol-12-myristate 13-acetate (81 nM), ionomycin (1338.6 nM), and brefeldin A (5 μg/ml) for 3 hours in a humidified incubator at 37°C and 5% carbon dioxide to induce intracellular cytokine production. The cell surface/intracellular markers were stained and analyzed via flow cytometry. These markers could be used to separate T, $CD4^+$ T, $CD8^+$ T and natural killer (NK) cells. We used *IFN-γ* and IL-4 expression on $CD4^+$ T cells to identify $CD4^+$ Th1 and $CD4^+$ Th2 T cells, respecitvely.

**Statistical analysis**

Continuous variables were expressed as either the mean ± standard deviation (SD) or the median and range, while categorical variables were presented as frequency and percentage. All clinical and flow cytometry data were analyzed using SPSS STATISTICS version 20 (IBM, Armonk, NY, USA). The Kruskal-Wallis H test and Mann-Whitney nonparametric U test were used for comparisons between groups. Categorical variables were examined using either the chi-square test or Fisher's exact test. All tests were two-sided, and a 2-sided $p$ value $< 0.05$ was considered to indicate a statistically significant difference.

## Results

**Patients' characteristics**

Fifty-three polytrauma patients (mean age ± SD, 52.6 ± 19.5 years) treated consecutively at our ICU were enrolled. The mean ISS was 26.5 ± 9.6 (range from 16 to 75). Thirty-seven patients (69.8%) were males. We first grouped patients according to their ISS. By definition an ISS of 25 and higher is very severe injury, there were 31 patients had an ISS ≤ 25 and 22 had an ISS > 25. Five of the patients (9.4%) died during admission, one of whom had an ISS of 45 and the other four had an ISS of 25. The mortality rate was 12.9% in patients with an ISS ≤ 25 and 4.5% in patients with an ISS > 25. 96.2% of patients sustained blunt mechanism of trauma, with the top 2 leading causes of trauma were motor vehicle collision (69.8%) and falls (26.4%).

The median ICU LOS was 10.7 ± 8 days. There was strong correlation between ICU stay and ADL at discharge of survivors (Fig. 1A). On the other hand, there was no significant correlation between ISS and ADL at discharge (Fig. 1B). ICU stay was 9.1 days when we used the equation to find the best fit value of ADL 60 ($Y = -2.262*X + 80.53$). We then grouped trauma patients as ICU stay longer than 10 days or not. Thirty-two patients had an ICU stay ≤ 10 days (short ICU stay) and the other 21 patients had an ICU > 10 days (long ICU stay). The patients who died were included in the long ICU stay group.

There were no significant differences in age and gender when the patients were grouped by either ISS or ICU stay. There was no significant difference in ICU stay when the patients were grouped by ISS, and there was no significant difference in ISS when the patients were grouped by ICU stay (Table 1).

**NK cells analysis**

When the patients were grouped by ISS, those with an ISS ≤ 25 had significantly higher percentages (% of total lymphocytes) of CD3-CD56+ NK cells 12 hours after ICU admission compared to those with an ISS > 25 (Fig. 2A and 2C). When the patients were grouped by ICU stay, the percentages and absolute numbers of NK cells were immediately increased after injury in the patients with a long ICU stay compared to those with a short ICU stay and the control group, and then returned to a normal level 24 hours after injury (Fig. 2B and 2D).

**Lymphocytes analysis**

Trauma patients had lymphopenia when compared to the control group. When the patients were grouped by ISS, the percentage of lymphocytes (% of PBMCs) in the patients with ISS > 25 was lower compared to those with an ISS ≤ 25 at admission, but then became similar (Fig. 3A). However, when the patients were grouped by ICU stay, the percentage of lymphocytes in the patients with a long ICU stay was significantly lower from 12 to 48 hours after ICU admission compared to those with a short ICU stay (Fig. 3B). There were no significant differences in the absolute numbers of lymphocytes between the patients grouped by ISS or ICU stay (Fig. 3C and 3D).

**T cells analysis**

The trauma patients had a lower $CD3^+$ T cells compared to the control group. When the patients were grouped by ISS, those with an ISS ≤ 25 had a lower percentage of T cells (% of lymphocytes) 12 hours after ICU admission compared to those with an ISS > 25 (Fig. 4A). When the patients were grouped by ICU stay, those with a long ICU stay had a lower percentage of T cells (% of

lymphocytes) from 6 hours to 48 hours after ICU admission compared to those with a short ICU stay (Fig. 4B).

**Analysis of PD-1 expression on T cells**

The expression of PD-1 on $CD3^+$ T cells was higher in the trauma patients compared to the control group. When the patients were grouped by ISS, those with an ISS $\leq$ 25 had a higher PD-1 expression on $CD3^+$ T cells compared to those with an ISS > 25, however there was no significant difference between the groups (Fig. 4C). When the patients were grouped by ICU stay, the PD-1 expression on $CD3^+$ T cells was significantly higher in the patients with long ICU stay compared to those with a short ICU stay (Fig. 4D).

**$CD4^+$ T cells analysis**

When the patients were grouped by ISS, those with an ISS $\leq$ 25 had reduced $CD4^+$ T cells (% of T lymphocytes) compared to those with an ISS > 25, however there was no significant difference between the groups (Fig. 5A). When the patients were grouped by ICU stay, those with a long ICU stay had reduced $CD4^+$ T cells (% of T lymphocytes) compared to that with a short ICU stay at 12 hours after ICU admission (Fig. 5B).

**Analysis of interferon gamma (*IFN*-γ) production by $CD4^+$ Th1 cells**

The trauma patients had lower *IFN-γ* production by $CD4^+$ Th1 cells compared to the control group. When the patients were grouped by ISS or ICU stay, there were no significant differences in *IFN-γ* production by $CD4^+$ Th1 cells between the groups (Additional file 1: figure S1).

**Analysis of IL-4 production by $CD4^+$ Th2 cells**

There was no significant difference in IL-4 production by $CD4^+$ Th2 cells between the ISS groups. However, when the patients were grouped by ICU stay, those with a long ICU stay had significantly higher IL-4 production by $CD4^+$ Th2 cells compared to those with a short ICU stay and the control group (Additional file 1: figure S1).

**$CD8^+$ T cells analysis**

When the patients were grouped by ISS, those with an ISS ≤ 25 had an increased $CD8^+$ T cells (% of T lymphocytes) compared to those with an ISS > 25 without significant differences (Fig. 5C). When the patients were grouped by ICU stay, those with a long ICU stay had a significantly increased $CD8^+$ T cells (% of T lymphocytes) compared to those with a short ICU stay at 48 hours after admission (Fig. 5D).

**Analysis of PD-1 expression on $CD8^+$ T cells**

The trauma patients had a higher expression of PD-1 on $CD8^+$ T cells compared to the control group. When the patients were grouped by ISS, those with an ISS ≤ 25 had a higher expression of PD-1 on $CD8^+$ T cells compared to those with an ISS > 25. When the patients were grouped by ICU stay, those with a long ICU stay had a higher expression of PD1 on $CD8^+$ T cells compared to those with a short ICU stay (Additional file 1: figure S2). There were no significant differences between groups when we grouped patients by ISS or ICU stay.

**Analysis of *IFN-* $\gamma$ production by $CD8^+$ cells**

The trauma patients had reduced *IFN-γ* production by $CD8^+$ T cells compared to the control group. When the patients were grouped by ISS or ICU stay, there were no significant differences in *IFN-γ* production by $CD8^+$ T cells between the groups (Additional file 1: figure S2).

## Discussion

The ISS is used to evaluate the severity of trauma patients clinically. A prolonged ICU stay may be considered a risk factor for a poor prognosis because of a slow or absent response to ICU therapy. Trauma induces SIRS and CARS due to disorders of the innate and acquired immune response [21]. In this prospective study, we consecutively evaluated dynamics of immunity in trauma patients from ICU admission. Our findings showed that changes in immunity were inconsistent when the patients were grouped by ISS and ICU stay, and that dynamics of immunity could be useful predictors or potential therapeutic targets in trauma patients.

SIRS is the manifestation of immunoinflammatory activation that occurs in response to injury and released factors from disrupted tissue which induces cytokines, proinflammatory lipids, and related proteins. The presence of a systemic inflammatory response at admission has been associated with a poor outcome after trauma [22-24]. NK cells are lymphocytes in the same family as T and B cells and come from a common progenitor, however they play an important role in innate immune responses [25]. As cells of the innate immune system, NK cells are classified as group I innate lymphocytes and respond quickly to a wide variety of pathological challenges. Some studies have showed that number of NK cells increases immediately after trauma (2 hours), possibly in response to neurotransmitters such as catecholamine [26]. This may reflect rapid mobilization from bone marrow and other secondary lymphoid tissues [27]. In our study, those

with an ISS ≤ 25 had significantly higher percentages (% of total lymphocytes) of NK cells 12 hours after ICU admission compared to those with an ISS > 25. However, the patients with a long ICU stay had a significantly higher NK cell expression compared to the patients with a short ICU stay from admission to 12 hours and 72 hours after admission. Cytokine concentrations have been shown to increase within 30 minutes of injury and can remain elevated for several days [28-30]. Persistence of this inflammatory response has been associated with multi-organ dysfunction syndrome (MODS) and a high possibility of death [31-34]. For trauma patients, rapid and persistent NK cell elevation may reflect exaggerated innate immunity leading to excessive SIRS and longer ICU stay.

CARS is a systemic deactivation of the immune system tasked with restoring homeostasis from an inflammatory state. However, uncontrolled CARS can further result in immunoparalysis, impaired wound healing, recurrent nosocomial infections, and late MODS, all of which are associated with a poor prognosis. Lymphocytes can interact with innate and adaptive immune responses, and their anergy or decreased responsiveness to stimuli has been demonstrated following major surgery, blunt trauma, and thermal injury. Lymphopenia itself is known to occur after severe injury, and a lack of lymphocyte recovery has been shown to impact survival [35]. Recent evidence suggests that lymphopenia develops within 24 hours of injury, and that lymphopenia at 48 hours is an early indicator of a poor outcome [36]. More of our trauma patients had lymphopenia than the healthy control, and significantly more of the patients with an ISS > 25 had lymphopenia compared to those with an ISS ≤ 25 on admission to the ICU, but the proportion of patients with lymphopenia became similar after that. When the patients were grouped by ICU stay, significantly more of those with a long ICU stay had lymphopenia compared to those with a short ICU stay from 12 hours to 48 hours after admission. However, there were differences of

percentages but not cell numbers of lymphocytes. It might be secondary impact of an expansion or decrease in a different cell type. Persistent lymphopenia reflected the critical condition of the patients and was related to a poor prognosis. SIRS and CARS are complex immunologic responses to injury. After injury, the patients with a long ICU stay had an immediate increase in NK cells followed by lymphopenia which persisted for 48 hours (Fig. 6). This indicates that rapid and persistent SIRS and subsequently severe CARS may be related to a prolonged ICU stay.

As an essential component of adaptive immunity, T cells are involved in both cellular and humoral immunity. T-cell function is an important mechanism of immunosuppression observed after injury which may contribute to the development of complications [37]. The period of immunoparalysis after trauma is characterized by increased expressions of inhibitory coreceptors (PD-1, CD47, CTLA4) on T lymphocytes [38]. A high expression of PD-1 on T lymphocytes has been correlated with the severity of illness after major trauma [39]. In our study, the trauma patients had a lower T cells and higher PD-1 expression on T cells compared to the control group. When the patients were grouped by ISS, those with an ISS $\leq$ 25 had a greater reduction in T cells and increased PD-1 expression on T cells compared to those with an ISS > 25. When the patients were grouped by ICU stay, those with a long ICU stay had a significant reduction in percentages of T cells from 6 hours to 48 hours after ICU admission and higher PD-1 expression on T cells compared to those with a short ICU stay (Additional file 1: figure S3). T cells may display limited function after injury as a result of exhaustion, which has been associated with the expression of some immune-inhibitory factors including PD-1 on T cell surface, and this has been related to a prolonged ICU stay and poor prognosis.

Injured cells release endogenous damage-associated molecular patterns into the circulation, which create a sepsis-like state [40]. Some studies have shown that trauma patients exhibit $CD4^+$

T-cell loss with a relative increase in regulatory T-cell count, both of which are associated with unfavorable outcomes after septic shock [41]. When the patients were grouped by ISS, a decrease in $CD4^+$ T cells was observed in those with an ISS $\leq$ 25 compared to those with an ISS > 25 during the first 24 hours after ICU admission without significant differences. When the patients were grouped by ICU stay, those with a long ICU stay had a significantly reduced $CD4^+$ T cells compared to that with a short ICU stay at 12 hours after ICU admission.

Beyond changes in numbers, circulating effector T lymphocytes also change from a pro-inflammatory Th1 phenotype to an anti-inflammatory Th2 phenotype after injury [42]. The impairment of effector helper T lymphocytes after trauma has also been shown to result in a reduction in *IFN-γ* production by Th1 polarized cells [43]. In our study, the trauma patients had suppressed *IFN-γ* production by Th1 cells compared to the control group. When the patients were grouped by ISS and ICU stay, there were no significant differences in *IFN-γ* production by $CD4^+$ Th1 cells between the groups. IL-4 is produced primarily by mast cells, $CD4^+$ Th2 cells, eosinophils and basophils, and further induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. When the patients were grouped by ICU stay, those with a long ICU stay had significantly higher IL-4 production by $CD4^+$ Th2 cells compared to the control group and compared to those with a short ICU stay. For trauma patients, $CD4^+$ T suppression and shift to an anti-inflammatory Th2 phenotype with increased IL-4 secretion reflect immunoparalysis and are associated with a prolonged ICU stay.

$CD8^+$ T cells are important mediators of cytotoxic adaptive immunity. Some studies have reported no significant differences in $CD8^+$ and CD4/CD8 ratio among healthy controls and patients with mild and severe trauma [44]. Another study reported that trauma plasma could induce an increase in $CD8^+$ T cell and NK cell abundance, and the plasma from 1 day but not 3 days after

trauma increased $CD8^+$ T cells in PBMC cultures by manual gating [45]. When our patients were grouped by ICU stay, those with a long ICU stay had an increased $CD8^+$ T cells compared to those with a short ICU stay at 48 hours after admission. The T cell subpopulation analysis showed the patients with a long ICU stay had an increased $CD8^+$ T cells and reduced $CD4^+$ T cells 12 and 48 hours after admission (Additional file 1: figure S4).

The inhibitory receptor, PD-1, plays a major role in $CD8^+$ T cell exhaustion during chronic infections and cancer [46-48], and is also expressed during the early phase of T cell activation when naive $CD8^+$ T cells differentiate into effector cells [49]. In our study, the trauma patients had an increased expression of PD-1 on $CD8^+$ T cells and reduced *IFN-γ* production by $CD8^+$ T cells compared to the control group. The PD-1 expression on $CD8^+$ T cells was higher in the patients with a long ICU stay compared to those with a short ICU stay. There were no significant differences in *IFN-γ* production by $CD8^+$ T cells the patients were grouped by ISS or ICU stay. Immediate activation of $CD8^+$ T cells after injury and then exhaustion was related to a prolonged ICU stay in the trauma patients.

There are several limitations of this study. First, because of the great heterogeneity in terms of etiology, mechanism, pathology, severity and treatment with widely varying outcomes, we could just enrolled poly-trauma patients and most of them were suffered from blunt injury for evaluation. Second, because our hospital is a Level I trauma center, our patients had higher ISS than average. Third, some patients were excluded because they died before 72 hours and didn't have the data for all time points.

## Conclusions

Immune responses are closely correlated with prognosis. However, the dynamics of immune responses were inconsistent when the patients were grouped by ISS and ICU stay, indicating that the currently used clinical parameters may not accurately reflect immune responses. Understanding the dynamics of immunity could allow clinicians to stratify trauma patients and identify effective treatments in an acute setting to improve their management and prognosis [50]. Beyond the currently used scoring system, further evaluations should be conducted to identify an accurate predictor of prognosis in trauma patients.

**Abbreviations**

ISS: injury severity score; SIRS: systemic inflammatory response syndrome; CARS: compensatory anti-inflammatory response syndrome; ICU: intensive care unit; LOS: length of stay; PBMC: peripheral blood mononuclear cell; MODS: multi-organ dysfunction syndrome; IFN-γ: interferon gamma; PD-1: programmed cell death-1

## Supplementary Information

**Additional file 1: figure S1:** $CD4^+$ T were shift to an anti-inflammatory Th2 phenotype. (A) (B) Trauma patients had suppressed *IFN-γ* production by $CD4^+$ Th1 cells compared to the control group. (C) IL-4 production by $CD4^+$ Th2 T cell in trauma patients grouped by ISS. (D) The patients with a long ICU stay had increased IL-4 production by $CD4^+$ Th2 cells compared to those with a short ICU stay, $*p < 0.05$.

**Additional file 2: figure S2:** Trauma patients had increased PD1 expression on $CD8^+$ T cells and reduced *IFN- γ* production by CD8+ T cells as compared to control group (A) The expression of

PD1 on $CD8^+$ T cells in trauma patients grouped by ISS. (B) The patients with a long ICU stay had a higher expression of PD1 on $CD8^+$ T cells compared to those with a short ICU stay. (C) (D) *IFN- γ* production by $CD8^+$ T cells in trauma patients grouped by ISS and ICU stay.

**Additional file 3: figure S3:** Trauma patients with long ICU stay had suppressed T cells and increased PD-1 expression on T cells compared to that with short ICU stay. (A) (C) T cells and PD-1 expression on T cells in patients with short ICU stay. (B) (D) T cells and PD-1 expression on T cells in patients with long ICU stay.

**Additional file 4: figure S4:** Trauma patients with long ICU stay had suppressed $CD4^+$ T cells and increased $CD8^+$T cells compared to that with short ICU stay. (A) $CD4^+$ and $CD8^+$ T cells in patients with short ICU stay. (B) $CD4^+$ and $CD8^+$ T cells in patients with long ICU stay.

**Additional file 5: Supplements Table 1:** Dynamics of immune responses in patients grouped by ISS.

**Additional file 6: Supplements Table 2.** Dynamics of immune responses in patients grouped by ICU stay.

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**Authors' contributions**

Ray-Heng Hu and Ya-Wen Yang participated in the conception and method design of the study. Ya-Wen Yang, Huei-Ting Tsai, Ying-Ru Chen, Yu-Ping Chang, and Tiffany E. Wu performed the data acquisition. Ya-Wen Yang and Che-Hsiung Wu performed the statistical analyses and data interpretation. Ya-Wen Yang drafted the initial manuscript. Yin- Yi Han and Ray-Heng Hu critically revised and finalized the manuscript.


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### Availability of data and materials

The data set used and analyzed are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Institutional Ethical Committee of National Taiwan University Hospital (NTUH IRB No. 201702060 RINA). Informed consent was obtained in writing from each patient before inclusion in the study.

### Consent for publication

All authors consented for publication of the study.

## Competing interests

The authors declare that they have no competing interests.

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**Figure legends:**

**Fig. 1.** The correlation of ALD and ICU stay (A) and ISS (B) in trauma patients (survivors).

**Fig. 2.** NK cells in trauma patients. (A) Patients with an ISS ≤ 25 had higher percentages of NK cells compared to those with an ISS > 25. (B) and (D) Patients with a long ICU stay had increased NK cells compared to that with a short ICU stay. (C) The NK cells in trauma patients grouped by ISS, $*p < 0.05$.

**Fig. 3.** Trauma patients had lymphopenia compared to control ones. (A) (C) Lymphocytes in trauma patients grouped by ISS. (B) The percentage of lymphocytes was significantly reduced in the patients with a long ICU stay compared to those with a short ICU stay. (D) The absolute numbers of lymphocytes between patients grouped ICU stay, $*p < 0.05$.

**Fig. 4.** Trauma patients had suppressed T cells and increased expression of PD-1 on T cells compared to control group. (A) T cells in trauma patients grouped by ISS. (B) T cells were significantly suppressed in the patients with a long ICU stay compared to those with a short ICU stay. (C) PD-1 expression on T cells in trauma patients grouped by ISS. (D) PD-1 expression on T cells was significantly increased in the patients with a long ICU stay compared to those with a short ICU stay, $*p < 0.05$.

**Fig. 5.** $CD4^+$ T and $CD8^+$ T cells in trauma patients. (A) $CD4^+$ T cells in trauma patients grouped by ISS. (B) $CD4^+$ T cells were more suppressed in the patient with a long ICU stay compared to those with a short ICU stay. (C) $CD8^+$ T cells in trauma patients grouped by ISS. (D) The patients with a long ICU stay had an increased $CD8^+$ T cells compared to those with a short ICU stay.

**Fig. 6.** Trauma patients with long ICU stay had increased NK cells and lymphopenia compared to that with short ICU stay. (A) (C) NK cells and lymphocytes in patient with short ICU stay. (B)(D) NK cells and lymphocytes in patient with long ICU stay.

**Table 1**. Baseline Characteristics of trauma patients grouped by ISS (A) and ICU stay (B).

(A)

| | ISS ≤ 25 (*n* = 31) | ISS > 25 (*n* = 22) | *P* value |
|---|---|---|---|
| Age | 53.65 ± 20.07 | 51.06 ± 19.04 | 0.64 |
| ICU stay | 10.67 ± 6.46 | 12.48 ± 9.73 | 0.44 |
| Gender (male, %) | 22 (71%) | 15 (68.2%) | 0.53 |

(B)

| | ICU ≤ 10 days (*n* = 27) | ICU > 10 days (*n* = 26) | *P* value |
|---|---|---|---|
| Age | 47.98 ± 19.91 | 57.35 ± 18.24 | 0.08 |
| ISS | 25.33 ± 6.25 | 27.69 ± 12.18 | 0.38 |
| Gender (male, %) | 16 (59.3%) | 21 (80.8%) | 0.14 |

# Figures

Fig. 1

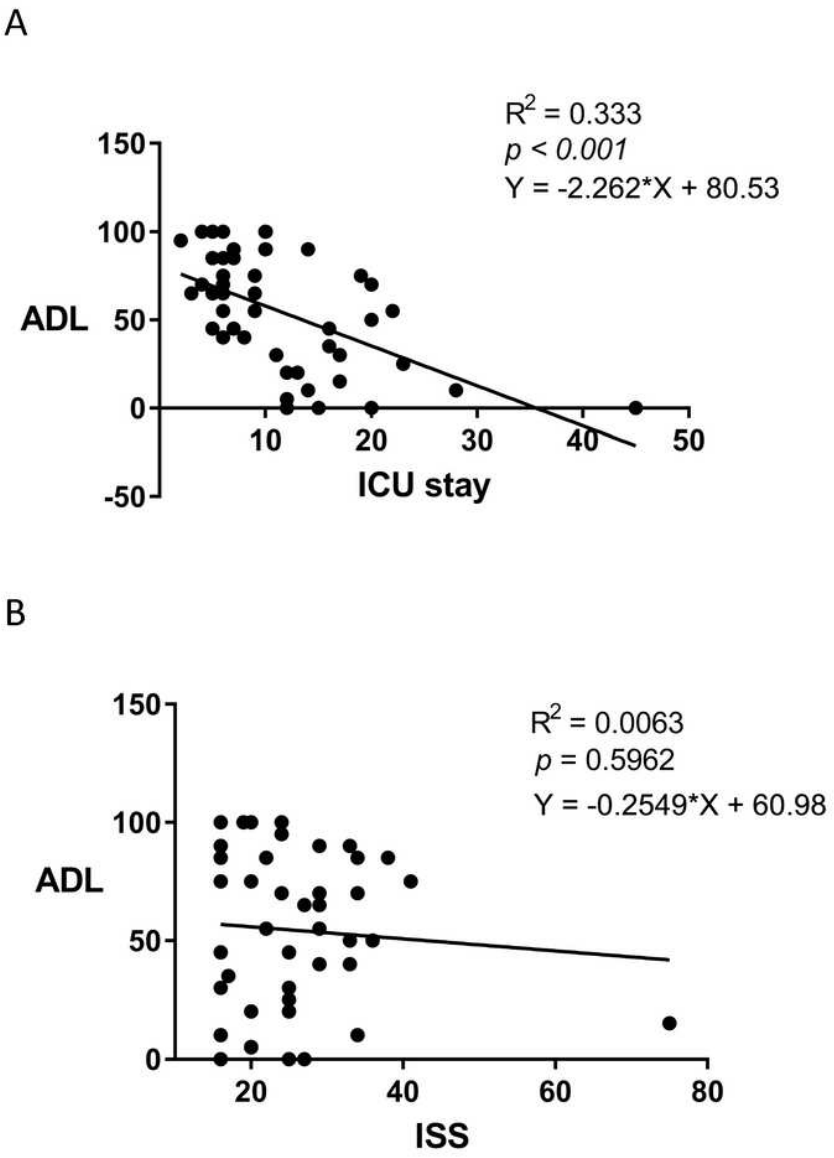
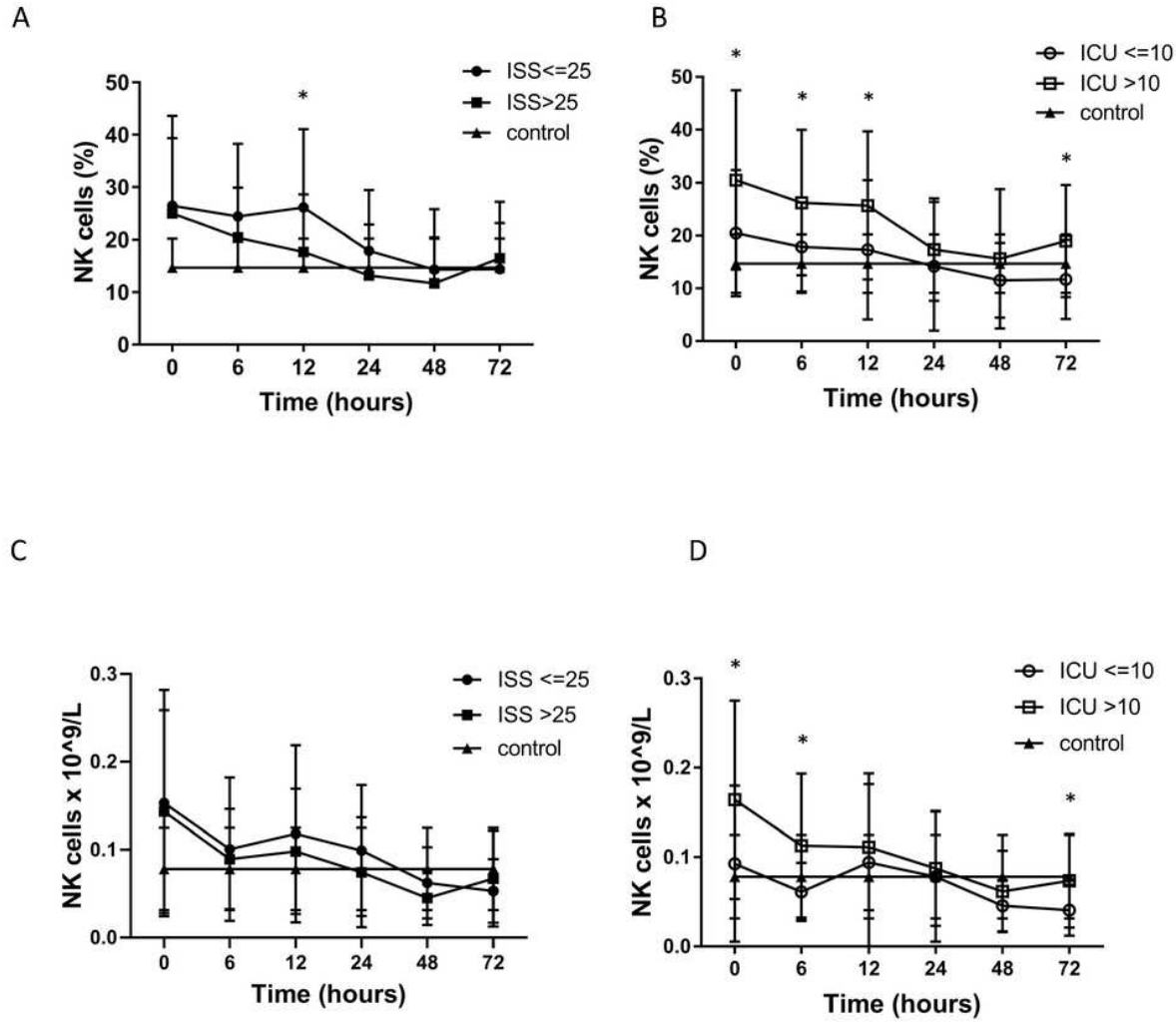



**Figure 1**

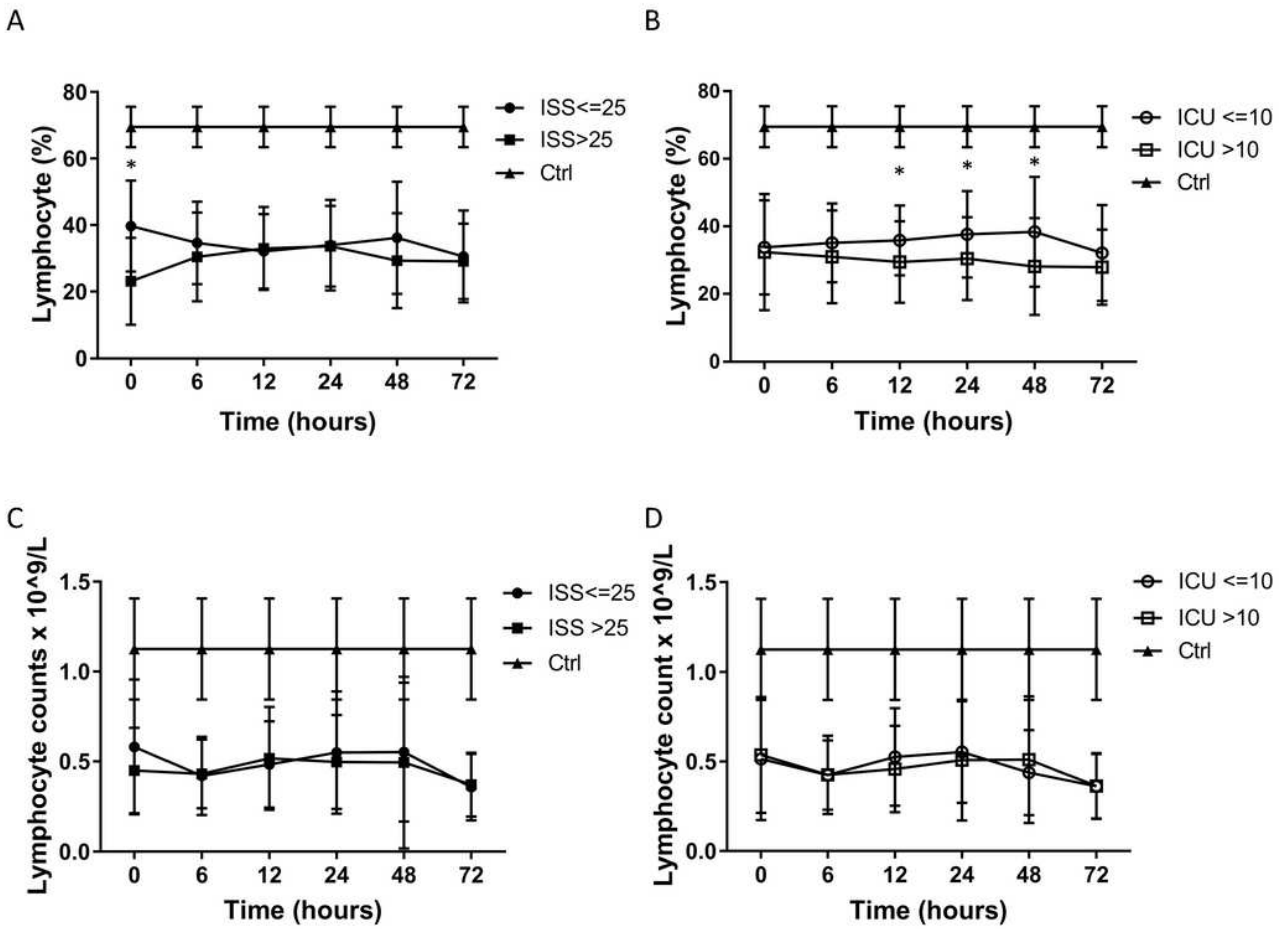
The correlation of ALD and ICU stay (A) and ISS (B) in trauma patients (survivors).

Fig. 2




## Figure 2

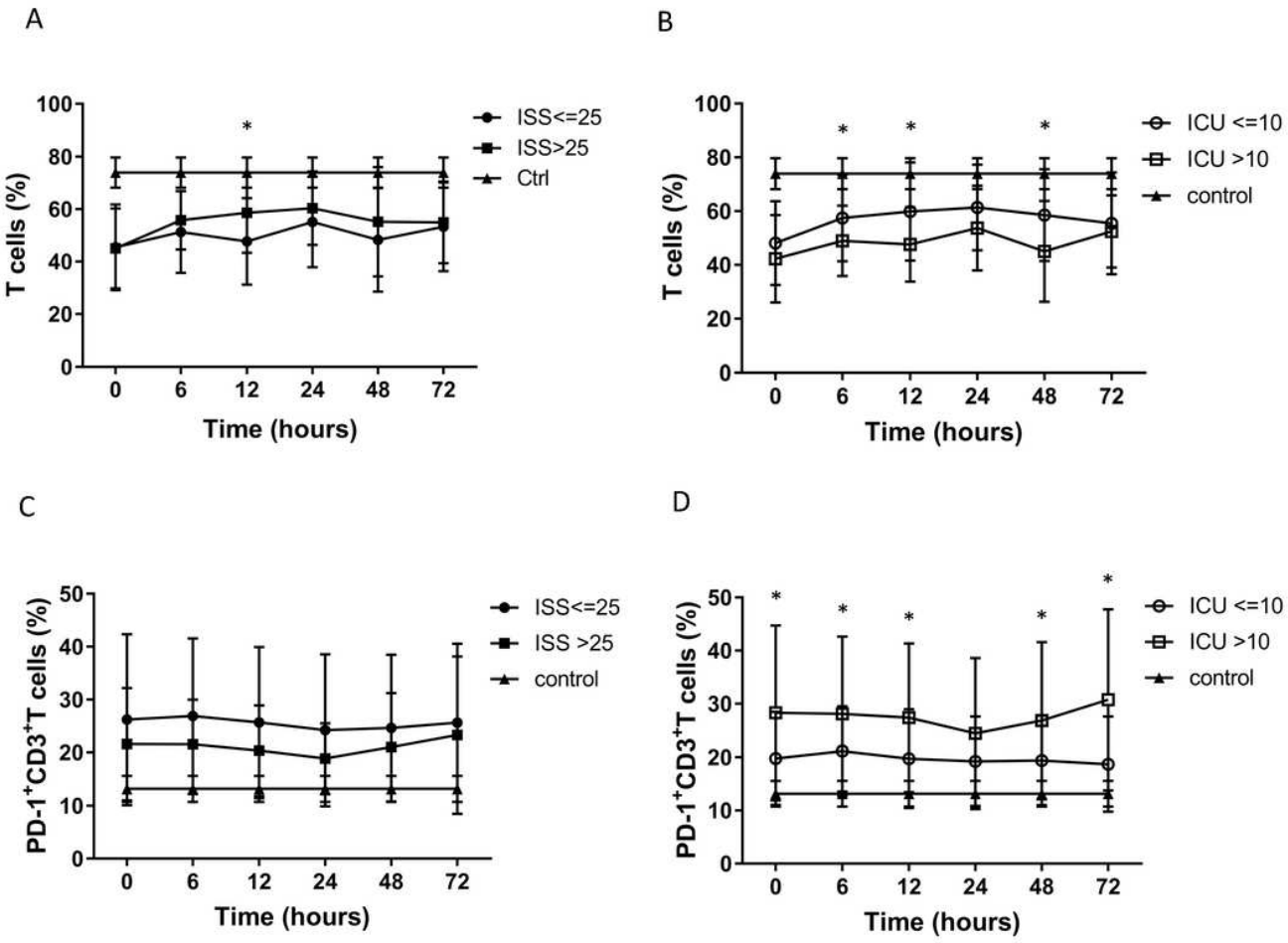
NK cells in trauma patients. (A) Patients with an ISS ≤ 25 had higher percentages of NK cells compared to those with an ISS > 25. (B) and (D) Patients with a long ICU stay had increased NK cells compared to that with a short ICU stay. (C) The NK cells in trauma patients grouped by ISS, *$p < 0.05$.




## Figure 3

Trauma patients had lymphopenia compared to control ones. (A) (C) Lymphocytes in trauma patients grouped by ISS. (B) The percentage of lymphocytes was significantly reduced in the patients with a long ICU stay compared to those with a short ICU stay. (D) The absolute numbers of lymphocytes between patients grouped ICU stay, *p < 0.05.

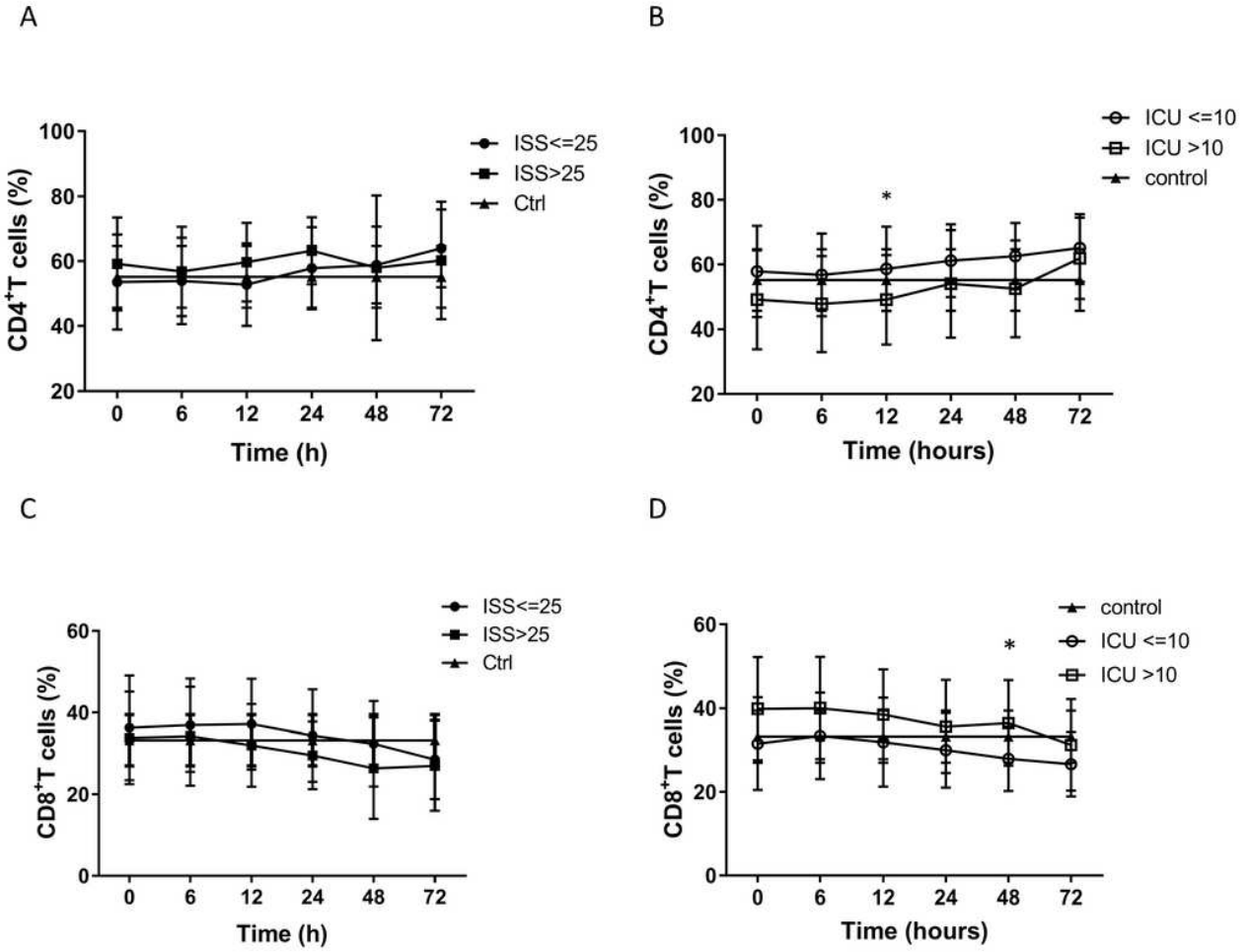
Fig. 4




## Figure 4

Trauma patients had suppressed T cells and increased expression of PD-1 on T cells compared to control group. (A) T cells in trauma patients grouped by ISS. (B) T cells were significantly suppressed in the patients with a long ICU stay compared to those with a short ICU stay. (C) PD-1 expression on T cells in trauma patients grouped by ISS. (D) PD-1 expression on T cells was significantly increased in the patients with a long ICU stay compared to those with a short ICU stay, *$p < 0.05$.

Fig. 5




## Figure 5

CD4⁺T and CD8⁺T cells in trauma patients. (A) CD4+ T cells in trauma patients grouped by ISS. (B) CD4⁺T cells were more suppressed in the patient with a long ICU stay compared to those with a short ICU stay. (C) CD8+ T cells in trauma patients grouped by ISS. (D) The patients with a long ICU stay had an increased CD8+ T cells compared to those with a short ICU stay.

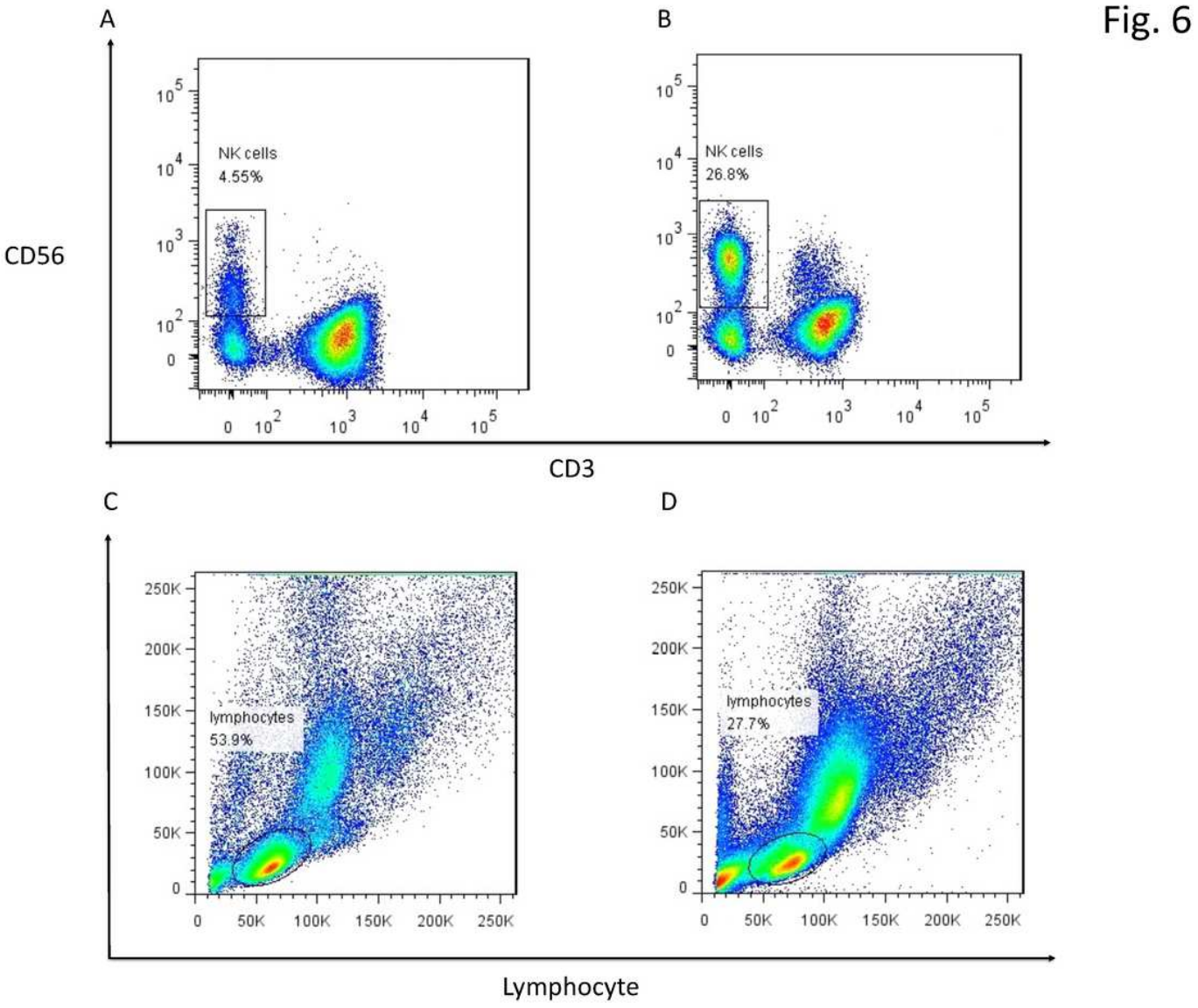



**Figure 6**

Trauma patients with long ICU stay had increased NK cells and lymphopenia compared to that with short ICU stay. (A) (C) NK cells and lymphocytes in patient with short ICU stay. (B)(D) NK cells and lymphocytes in patient with long ICU stay.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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