Early persistent lymphopenia and risk of death in critically ill patients with and without sepsis.

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Prognostic value of persistent lymphopenia in Critical Illness: PIVOTAL Study

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Drafting the manuscript: DA, SF.

Reviewing manuscript for important intellectual content and approving final version: All authors.
ABSTRACT

Purpose
To determine the proportion of critically ill patients with and without sepsis who exhibit persistent lymphopenia and examine its relationship with hospital survival.

Methods
Database analysis of adult intensive care unit (ICU) patients at two hospitals in Queensland, Australia and the MIMIC III database from Boston, USA.

Results
We defined persistent lymphopenia at two thresholds (absolute lymphocyte count [ALC] <1.0 and <0.75 x 10^9/L) based on two qualifying values recorded during the first four days in ICU. In the USA cohort 27,646/32,528 (85.0%) patients did not have two ALCs recorded with evidence that data were not missing at random; consequently, we report the analysis of the Australian cohort. In the Australian cohort 7605/8507 (89.4%) patients had two ALCs recorded, of these 1482 (19.5%) had sepsis. Persistent lymphopenia (ALC<1.0) was present in 728/1482 (49.1%) and 2302/6123 (37.6%) of patients with and without sepsis, respectively. For ALC <0.75 the results were 487/1482 (32.9%) and 1125/6123 (18.4%), respectively. 562/3030 (18.5%) patients with persistent lymphopenia (ALC<1.0) died in hospital compared with 439/4575 (9.6%) patients without persistent lymphopenia. Persistent lymphopenia was an independent risk factor for in hospital death in all patients. The hazard ratio for death at ALC<1.0 was 1.89 (95%CI 1.31 – 2.85) and 1.17 (1.02 – 1.36) in patients with and without sepsis respectively.

Conclusions
Persistent lymphopenia is common in critically ill patients and associated with increased risk of death. The association is stronger in patients with sepsis. Trials testing the hypothesis that reversing lymphopenia reduces mortality should initially target patients with sepsis.

Keywords
Sepsis, lymphopenia, critical illness
Take Home Message

Early persistent lymphopenia occurs frequently in critically ill patients with and without sepsis. In both these cohorts it is independently associated with an increased risk of in-hospital death although the association is stronger in patients with sepsis. Trials testing the hypothesis that reversing lymphopenia reduces mortality should initially target patients with sepsis.
INTRODUCTION

Persistent lymphopenia is known to be associated with increased mortality in critically ill patients with sepsis [1] and trauma [2, 3]. Patients who survive critically illness suffer long term morbidity affecting physical function, cognition, and mental health [4, 5]. These long-term health effects are similar in patients with and without sepsis [6]. The terms “post-intensive care syndrome” and “posts-sepsis syndrome” describe these persisting health effects. Additionally, readmission rates to hospital for sepsis-survivors are high. In high income countries around 40% of sepsis survivors are readmitted to hospital within 90 days with recurrent sepsis being a common reason for readmission, supporting the hypothesis that survivors suffer persistent immunosuppression [7]. In patients with sepsis multiple factors influence the immune system, including the microbiome,[8] impacting the post-sepsis metabolic[9] and epigenetic[8] immune composition. This results in complex alterations to cellular immunity with consequent immunodeficiency. This immune dysfunction can often be prolonged, [10-13] and is postulated to be a leading cause of late mortality in patients who have survived sepsis [11, 14]. Persistent lymphopenia is a candidate biomarker and mechanism for post-ICU and post-sepsis immunosuppression.

Published data on the prevalence and prognostic value of persistent lymphopenia in critically ill without sepsis or trauma are scarce. The longer-term outcomes of critically ill patients in general and those with sepsis are similar. Therefore, reversal of lymphopenia is a potential strategy to reduce risk of death and longer-term morbidity in both these patient groups [15, 16].

To inform the potential target population for a trial of reversing persistent lymphopenia following critically illness or sepsis we sought to analyse data from the publicly available MIMIC III database and the databases of two intensive care units in Queensland, Australia, to examine the relationship of persistent lymphopenia with risk of death and other outcomes in critically ill patients with and without sepsis.

METHODS

Study setting and population

Ethics approval for data collection and analysis was granted by Metro South, Human Research Ethics Committee, Queensland (Australia). Approval to access data from the US-based Medical Information Mart for Intensive Care (MIMIC) database was acquired following successful completion of the prerequisite, Collaborative Institutional Training Initiative. We interrogated the clinical information datasets (MetaVision, iMDsoft®) from the Intensive Care Units (ICU) of two tertiary hospitals (total of 2,019 beds) in Queensland, Australia, and the publicly available MIMIC database from a 719-bed, tertiary hospital in Boston, MA, USA [17].

We accessed data for adult patients, admitted to the study ICUs between January 2015 – December 2018. We defined persistent lymphopenia using two threshold values; an absolute lymphocyte count (ALC) < 1.0 x 10^9/L and < 0.75 x 10^9/L, recorded on at least two days during the first four days in the ICU. ICU admissions after elective surgery were excluded as patients were expected to have a short ICU stay with low risk of death.
For the Australian cohort we used the Acute Physiology and Chronic Health Evaluation (APACHE) III [18] admission diagnostic code to classify the patient as having sepsis or not. We classified patients as having sepsis if the admission diagnostic code specified sepsis, or the admission diagnosis was infection or pathology consistent with a diagnosis of sepsis (e.g. perforated bowel), combined with a Sequential Organ Failure Assessment (SOFA) score [19] of two or more. For the US cohort we used ICD-9 codes indicating the presence of sepsis or septic shock during the ICU stay to classify the patient as having sepsis or not. For both populations we classified as non-sepsis all eligible patients not meeting above criteria. Patients aged < 18 years and/or with a diagnosis of lymphoma, leukaemia or immunosuppression, including HIV/AIDS were also excluded.

**Data Collection and storage**

Individual data sets for eligible participants were extracted, de-identified and securely stored. These included: general demographics, primary diagnosis and co-morbidities, observational variables measured within the first 24 hours in the ICU.

**Outcomes**

The primary outcome was in-hospital death following a recorded ALC < 1.0 on at least two days within the first four days of ICU admission. Secondary outcomes were the proportion of patients with or without sepsis having persistent lymphopenia defined at the two thresholds, the association of persistent lymphopenia at the two thresholds with hospital mortality, and its association with use and duration of organ support (ventilation, vasopressor treatment and renal replacement therapy), and with length of stay in the hospital and ICU.

**Statistical Analysis**

Data were analysed by the Statistical Services Division of The George Institute for Global Health, Australia according to a prespecified statistical analysis plan. For continuous variables we report mean and standard deviation (SD), or medians and interquartile ranges, (IQR) as appropriate; for categorical variables we report proportions, with 95% confidence intervals calculated by Wilson’s method.

We assessed time to in-hospital death using uni- and multivariable Cox regression models using country as a log-normal frailty. The multivariable models included a set of covariates that were defined a-priori with no automatic selection; these were age, sex, sepsis, persistent lymphopenia, presence of comorbidities, SOFA score and quartiles of illness severity using the APACHE III score for Australian patients and the Simplified Acute Physiology Score (SAPS) – II [20] for US patients.

We checked the proportional hazard assumption by visual assessment of the log cumulative-hazard functions and by Kolmogorov-type Supremum test. We fitted hierarchical logistic models for binary outcomes (such as treatment with ventilation, renal replacement therapy [RRT], or vasopressors), for length of ICU and hospital stay we used mixed models with the same covariates set as described above. For subgroup analyses we explored by septic/non septic patients and lymphopenia presence/absence using the two thresholds pre-specified (<1.0 and <0.75 x 10^9/L). Post-hoc, after finding that only 15.0% of patients in the US dataset had two absolute
lymphocyte counts (ALCs) recorded within the first four days of their ICU stay, we compared the characteristics of the US patients with and without two ALCs recorded to assess whether ALCs were likely missing at random and whether those with two ALCs recorded could be considered representative of the whole US cohort. We performed complete case analyses with statistical significance set at alpha of 0.05 and we made no adjustments for multiple comparisons. We used SAS v9.3 for all statistical analyses.

Funding

The study was supported by The George Institute for Global Health (internal funding) and a grant from RevImmune Inc.

RESULTS

PATIENTS

We identified 41,035 potentially eligible patients, 8,507 in the Australian data sets and 32,528 in the US dataset. Of potentially eligible Australian patients, 902 (10.6%) were excluded as they did not have two ALCs recorded within the first four days of admission; 848/902 (94.01%) due to an ICU stay of less than 48 hours, leaving 7605 eligible patients (Fig. 1). Of 32,528 potentially eligible USA patients, 27,646 (85.0%) were excluded as they did not have two ALCs recorded within the first four days of admission; 13,756/27,646 (49.8%) were due to an ICU stay of less than 48 hours, leaving 4882 (15.0%) eligible patients. Thus 12,487 patients met the inclusion criteria for the study.

Due to the high proportion of US patients with missing ALC data we could not reliably calculate the proportion of patients with persistent lymphopenia in the US dataset. Within the US patient cohort disease severity was higher in patients with two or more ALC measurements compared to those with fewer than two ALC measurements; median SAPS II score 38.1 (95% CI - 37.7, 38.5) vs 34.5 (95% CI - 34.3, 34.6) (p<.0001), respectively. This is consistent with the contention that the patients with two ALC counts recorded were not representative of the whole US cohort as the ALCs were not likely to be missing at random. Consequently, we focus on the complete Australian dataset.
Fig. 1 Patient flow (Australian cohort)

Assessed for eligibility (n=8507)

- Excluded (n=902)
  - No data for ALC for at least 2 days within 4 days of ICU admission (n=902)
  - Proportion who <48h ICU stay (n=848, 94%)

Assessed in study (n=7605)

Study Population

- Sepsis (n=1482)
- Non-sepsis (n=6123)

Analysis

- Analysed (n=1482)
- Analysed (n=6123)
Baseline Characteristics of Australian patients

In total, 1482 (24.2%) patients had sepsis and 6123 (75.8%) did not (Table 1). The median age of patients with and without sepsis was 61 (IQR 46-71) and 54 (IQR 38-67) years, respectively. Female patients represented 40.5% and 38.9% of patients with sepsis and without sepsis, respectively. The median APACHE-3 score for patients with and without sepsis was 63 (IQR 49-80) and 52 (IQR 37-73), respectively. Comorbidities (APACHE-3) were present in 15.9% of patients with sepsis and 9.5% of patients without sepsis.

OUTCOMES
Proportions of patients with persistent lymphopenia

Persistent lymphopenia (ALC < 1.0 x 10⁹/L) was present in 728/1482 (49.1%) vs 2302/6123 (37.6%) with and without sepsis, respectively. The hazard ratio (HR) for persistent lymphopenia in sepsis versus non-sepsis was 1.60 (95% CI 1.43-1.80). Persistent lymphopenia at ALC < 0.75 x 10⁹/L was present in 487/1482 (32.9%) vs 1125/6123 (18.4%) with and without sepsis, respectively. HR for sepsis versus non-sepsis 2.17 (95% CI 1.92 – 2.47). ALCs on days 1-to-4 for sepsis verses non-sepsis patients are shown in Fig. 2.

In-hospital death and its association with persistent lymphopenia

Of the 3030 patients with persistent lymphopenia, defined by ALC < 1.0 x 10⁹/L, 562 (18.5%) died during their hospital stay. 1612/3030 patients had lymphopenia, defined by ALC < 0.75 x 10⁹/L. 334 (20.7%) of these patients died in hospital. Persistent lymphopenia (ALC < 1.0 x 10⁹/L) was an independent risk factor for in hospital death in those with and without sepsis. The association was stronger in patients with sepsis, HR for death 1.89 (95% CI 1.32 – 2.71) compared with to 1.17 (95% CI 1.02 – 1.35) in patients without sepsis (Table 2 and Figure 3). A similar pattern was observed for ALC < 0.75 x 10⁹/L; patients with sepsis, HR 1.90 (95% CI 1.39 – 2.61), patients without sepsis, HR 1.12 (95% CI 0.96 -1.31) (Table 2 and Figure 3). Kaplan-Meier curves for probability of survival for patients with and without lymphopenia with and without sepsis are given in Fig. 4.
Fig. 2 Absolute lymphocyte counts for Day 1-to-4 in patients with and without sepsis.
**Fig. 3** Association of persistent lymphopenia with in-hospital death

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>HAZARD RATIO 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients ALC &lt; 1.0</td>
<td>1.25 (1.10, 1.42)</td>
</tr>
<tr>
<td>Sepsis patients ALC &lt; 1.0</td>
<td>1.89 (1.32, 2.71)</td>
</tr>
<tr>
<td>Non-sepsis patients ALC &lt; 1.0</td>
<td>1.17 (1.02, 1.35)</td>
</tr>
<tr>
<td>All patients ALC &lt; 0.75</td>
<td>1.22 (1.06, 1.39)</td>
</tr>
<tr>
<td>Sepsis patients ALC &lt; 0.75</td>
<td>1.90 (1.39, 2.61)</td>
</tr>
<tr>
<td>Non-sepsis patients ALC &lt; 0.75</td>
<td>1.12 (0.96, 1.31)</td>
</tr>
</tbody>
</table>
**Fig. 4a** Kaplan Meier survival graph. Patients with sepsis.
Fig. 4b Kaplan Meier survival graph. Patients without sepsis.
SECONDARY OUTCOMES

Association of persistent lymphopenia with hospital length of stay

In all patients, persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increase in hospital length of stay of 5.41 days (95% CI 4.05 – 6.77) compared to patients without persistent lymphopenia. In patients without sepsis the increase in hospital stay was 6.18 days (95% CI 4.63 – 7.73), in patients with sepsis the increase was 1.79 days (95% CI 1.04 – 4.62) (Table 3a). The results using the ALC cut-off of 0.75 x 10^9/L; were similar, patients without sepsis, 5.80 days (95% CI 3.88 – 7.23), patients with sepsis, 1.52 days (95% CI 1.47 – 4.50) (Table 3b).

Association of persistent lymphopenia with ICU length of stay

In patients without sepsis, an ALC < 1.0 x 10^9/L was associated with an increase in ICU length of stay 1.22 days (95% CI 0.89 – 1.55), in patients with sepsis the increase was 1.14 days (95% CI 0.36 – 1.92) (Table 3a). For patients without sepsis and an ALC < 0.75 x 10^9/L, ICU length of stay was increased by 1.25 days (95% CI 0.84 – 1.66), in patients with sepsis the increase was 0.98 days (95% CI 0.15 – 1.80) (Table 3b).

Association of persistent lymphopenia with treatment with mechanical ventilation

For all patients, persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with mechanical ventilation, HR 1.15 (95% CI 1.01 – 1.30). In patients without sepsis, an ALC < 1.0 was associated with an HR for treatment with mechanical ventilation of 1.2 (95% CI 1.04 – 1.39). In patients with sepsis, the HR was 0.99 (95% CI 0.78 – 1.27) (Table 3a). A similar pattern was observed for ALC < 0.75 x 10^9/L; patients without sepsis, HR 1.27 (95% CI 1.04 – 1.54) patients with sepsis, HR 0.91 (95% CI 0.70 – 1.17) (Table 3b).

Non-sepsis patients with an ALC < 1.0 were treated with mechanical ventilation for 1.24 extra days (95% CI 0.88 – 1.61) compared to non-sepsis patients with an ALC > 1.0. Sepsis patients were treated for 1.65 extra days (95% CI 0.61 – 2.68) (Table 3a). A similar pattern was observed for ALC < 0.75 x 10^9/L; non-sepsis, 1.10 extra days (95% CI 0.65 – 1.55) and sepsis, 1.57 extra days (95% CI 0.49 – 2.65) (Table 3b).

Association of persistent lymphopenia with treatment with vasopressors or inotropes

For all patients, persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with a vasopressor or inotrope, HR 1.38 (95% CI 1.21 – 1.57). In patients without sepsis, an ALC < 1.0 was associated with an HR for treatment with a vasoactive drug of 1.43 (95% CI 1.23 – 1.65) compared to patients without sepsis with an ALC >1.0. For patients with sepsis and an ALC < 1.0 x 10^9/L the HR was 1.17 (95% CI 0.87 – 1.57) compared to patients with sepsis with and ALC > 1.0 (Table 3a). A similar pattern was observed for ALC < 0.75 x 10^9/L; patients without sepsis, HR 1.47 (95% CI 1.22 – 1.78), patients with sepsis, HR 1.02(95% CI 0.74 – 1.39). In patients treated with a vasoactive drug the association of lymphopenia (ALC < 1.0) with in-hospital mortality persisted in those with sepsis, (HR for in-hospital death of 1.95 [95% CI 1.32 – 2.89 for ALC < 1.0; HR 1.99 [95% CI 1.42 – 2.80]) (Table 4a), but not for patients without sepsis treated with a vasoactive drug; HR for in-hospital mortality of 1.12 (95% CI 0.96 – 1.30) and 1.09 (95% CI 0.93 – 1.29) for ALC < 1.0 and ALC < 0.75, respectively.

Association of persistent lymphopenia with treatment with renal replacement therapy (RRT)

For all patients, persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with RRT, HR 1.89 (95% CI 1.55 – 2.32). For patients with sepsis, HR 1.74 (95% CI 1.19 – 2.55), for those without sepsis HR 1.95 (95% CI 1.53 – 2.48) [Table 3a]. For patients with lymphopenia defined as ALC < 0.75 x 10^9/L, there was a significant association between lymphopenia and treatment with RRT in patients without sepsis, (HR 1.93 [95% CI 1.50 – 2.47]) but not those with sepsis (HR 1.22 [95% CI 0.85 – 1.76]) (Table 3b). Patients without sepsis with an ALC < 1.0 were treated with RRT for 1.45 days longer (95% CI 0.46 – 2.44) compared to those with an ALC > 1.0. Patients with sepsis were treated with RRT for 1.51 days longer (95% CI -0.74 – 3.75) (Table 3a). Patients without sepsis with an ALC < 0.75 x 10^9/L were treated with RRT for 0.38 days longer (95% CI 0.60 – 1.37); patients with sepsis were treated for 2.43 days longer (95% CI 0.36 – 4.50) (Table 3b).

DISCUSSION

We found that early persistent lymphopenia, at the level of ALC < 1.0 x 10^9/L on at least two of the first four days in ICU, was common in critically ill patients with sepsis and in those without sepsis. The likelihood of having persistent lymphopenia was significantly greater in patients with sepsis. This difference was greater patients with an ALC <0.75 x 10^9/L. Early persistent lymphopenia was associated with an increased risk of in-hospital death with a stronger association observed in patients with sepsis.

For secondary outcomes, early persistent lymphopenia was broadly associated with increased likelihood of treatment with mechanical ventilation, vasoactive drugs, and renal replacement therapy. In the total population studied, these findings resulted from a strong association in patients without sepsis. Our finding that a greater proportion of patients with sepsis had persistent lymphopenia is consistent with the trend observed by Andreu-Ballester in a retrospective study conducted at two hospitals in Spain [21], who observed 56.8% of patients with sepsis presented to hospital with lymphopenia compared to 43.2% of patients without sepsis.

We found that early persistent lymphopenia is an independent risk factor for in-hospital death in patients with and without sepsis. While this association has been reported for patient with sepsis [1, 22], we are not aware of it being reported in a heterogeneous population of critically ill patients without sepsis.

The strengths of our study include its design as a multicentre, database analysis involving data from 7605 patients, which is substantially more than previously published data [1]. We excluded patients admitted to the ICU after elective surgery, thus focussing our study on a cohort at greater risk of dying. Additionally, we selected a patient centred primary outcome, pre-selected a broad set of clinically relevant variables for multivariate analysis and followed a prespecified statistical analysis plan. Our data were obtained from two tertiary centres in Queensland, Australia, where daily blood sampling routinely includes a differential white cell count. Consequently, only 54 of the 902 (6%) of the patients excluded from the study were excluded due to missing lymphocyte counts. We therefore have confidence that the Australian data provide a reliable estimate of the proportion of patients with and without sepsis who exhibit early persistent lymphopenia in the Australian healthcare setting.
A limitation of our intended study is the low proportion of patients in the MIMIC dataset who had absolute lymphocyte counts available which prevented us replicating the findings of the Australian dataset. Due to these missing data, the US cohort did not contribute to the analysis. This may impact the strength of our findings. Furthermore, the generalisability of our findings is unclear, and our analysis should be repeated in other populations.

Our study was conceived as part of a planning process for a clinical trial to determine whether reversing lymphopenia will improve outcomes in critically ill patients. Given lymphopenia is associated with an increased risk of death in critically ill patients with and without sepsis it can be used for prognostic enrichment of clinical trials but as yet, it is unclear whether it can be used for predictive enrichment. Treating lymphopenia in a diverse population of critically ill patients aligns with the current trend to look for treatable traits [23]. However, given the stronger association of lymphopenia with risk of death in patients with sepsis, these patients may be considered the most appropriate population in which to conduct first trials of treatments designed to reverse lymphopenia.

CONCLUSION

Persistent lymphopenia is common in critically ill patients with and without sepsis and is associated with increased risk of death with the strength of the association being greater in patients with sepsis. Trials designed to examine the impact of reversing lymphopenia are warranted and should initially target patients with sepsis.
DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

**Derick Adigbli** – Honorary Research Fellow at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., fees for methodological support from Gilead Sciences Inc., - all paid to The George Institute for Global Health.

Rebecca Liu – No conflicts of interest to disclose.

Jason Meyer – No conflicts of interest to disclose.

Jeremy Cohen – No conflicts of interest to disclose.

Gian Luca Di Tanna – Previous employee at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., fees for methodological support from Gilead Sciences Inc., - all paid to The George Institute for Global Health.

Chris Gianacas – Senior Biostatistician at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., paid to The George Institute for Global Health.

Amrtendo Bhattacharya – The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., paid to The George Institute for Global Health.

Naomi Hammond – Associate Professor and Program Lead, Critical Care Division at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., paid to The George Institute for Global Health.

James Walsham – No conflicts of interest to disclose.

Bala Venkatesh – Professorial Fellow at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., paid to The George Institute for Global Health.

Richard Hotchiss – No conflicts of interest to disclose.

Simon Finfer – Professorial Fellow at The George Institute for Global Health. Funding for data analysis (PIVOTAL) and consulting fees for design of a clinical trial of interleukin 7 in sepsis from Revimmune Inc., - all paid to The George Institute for Global Health.


**Table 2** Proportion of patients with Persistent lymphopenia at the two cut-off levels and its association with in-hospital death

<table>
<thead>
<tr>
<th></th>
<th>ALC &lt; 1.0 (N=728)</th>
<th>ALC &gt; 1.0 (N=754)</th>
<th>ALC &lt; 1.0 vs ALC &gt; 1.0 Hazard ratio</th>
<th>ALC &lt; 0.75 (N=2302)</th>
<th>ALC &gt; 0.75 (N=3821)</th>
<th>ALC &lt; 0.75 vs ALC &gt; 0.75 Hazard ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
<td>120/728 (16.5%)</td>
<td>43/754 (5.7%)</td>
<td>1.89 (1.32, 2.71) n=1468**</td>
<td>92/487 (18.9%)</td>
<td>71/995 (7.1%)</td>
<td>1.90 (1.39, 2.61) n=6088**</td>
</tr>
<tr>
<td>Non-Sepsis</td>
<td>442/2302 (19.2%)</td>
<td>396/3821 (10.4%)</td>
<td>1.17 (1.02, 1.35) n=6088**</td>
<td>242/1125 (21.5%)</td>
<td>596/4998 (11.9%)</td>
<td>1.12 (0.96, 1.31) n=6088**</td>
</tr>
</tbody>
</table>

* Plus-minus values are means ±SD. The Sepsis group were defined based on an Acute Physiology and Chronic Health Evaluation (APACHE) II admission diagnostic code of sepsis, or an admission diagnostic code of infection or a pathology consistent with the diagnosis of sepsis combined with a Sequential Organ Failure Assessment (SOFA) score of ≥ two.
† Acute Physiology and Chronic Health Evaluation (APACHE) II score ranges from 0 to 71; with higher scores indicating increased risk of death.
‡ Acute Physiology and Chronic Health Evaluation (APACHE) III score ranges from 0 to 299; with higher scores indicating increased risk of death.
¶ Sequential Organ Failure Assessment (SOFA) integer score ranges from 0 to 24; higher scores indicate a greater degree of organ dysfunction.

Persistent lymphopenia was defined as an absolute lymphocyte count < 1.0 x 10^9/L and < 0.75 x 10^9/L, on at least two days within the first four days in ICU.

**Table 3a** Length of ICU and hospital stay, occurrence and duration of advanced organ support Comparing patience with and without Sepsis and with and without persistent lymphopenia defined by absolute lymphocyte threshold of <1.0 x 10^9/L.

<table>
<thead>
<tr>
<th></th>
<th>Sepsis:</th>
<th>Non-sepsis:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ALC &lt; 1.0 (N=728)</td>
<td>ALC &gt; 1.0 (N=754)</td>
</tr>
<tr>
<td>Mean (95% CI) Hospital stay, (Days)</td>
<td>24.6 (23)</td>
<td>23</td>
</tr>
<tr>
<td>Mean (95% CI) ICU Stay, (Days)</td>
<td>7.3 (5.7)</td>
<td>5.7</td>
</tr>
<tr>
<td>Mechanical ventilation, HR</td>
<td>482/728 (66.2%)</td>
<td>482/754 (63.9%)</td>
</tr>
<tr>
<td>Vasopressor/inotrope, HR</td>
<td>532/728 (73.1%)</td>
<td>439/754 (58.2%)</td>
</tr>
<tr>
<td>Renal replacement therapy, HR</td>
<td>134/728 (18.4%)</td>
<td>63/754 (8.4%)</td>
</tr>
<tr>
<td>Mean (95% CI) Mechanical ventilation, (Days)</td>
<td>7</td>
<td>5.7</td>
</tr>
<tr>
<td>Mean (95% CI) Vasopressor/inotrope, (Days)</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean (95% CI) Renal replacement therapy, (Days)</td>
<td>7.3</td>
<td>6.1</td>
</tr>
</tbody>
</table>

* Acute Physiology and Chronic Health Evaluation (APACHE) II for 14 patients were missing and therefore excluded from HR calculation.
** Acute Physiology and Chronic Health Evaluation (APACHE) III for 35 patients were missing and therefore excluded from HR calculation.
^ Hazard ratio
### Secondary outcomes for patients with sepsis and patients without sepsis (ALC < 0.75 x 10^9/L)

<table>
<thead>
<tr>
<th></th>
<th>Sepsis ALC &lt; 1.0</th>
<th>Sepsis ALC &lt; 1.0 vs ALC &gt; 1.0 HR</th>
<th>Non-Sepsis ALC &lt; 1.0</th>
<th>Non-Sepsis ALC &lt; 1.0 vs ALC &gt; 1.0 HR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vasopressor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>532/971 (54.7%)</td>
<td>1.95 (1.32, 2.89) n= 971</td>
<td>3427/3144 (45.4%)</td>
<td>1.12 (0.96, 1.30) n=3140**</td>
</tr>
<tr>
<td><strong>No Vasopressor</strong></td>
<td>106/511 (38.4%)</td>
<td>1.25 (0.49, 3.15) n= 493***</td>
<td>875/2979 (29.4%)</td>
<td>1.36 (0.97, 1.90) n=2948***</td>
</tr>
</tbody>
</table>

### Table 4a Proportion of patients with persistent lymphopenia (ALC < 1.0) +/- sepsis +/- vasopressor/inotrope and its association with in-hospital death

<table>
<thead>
<tr>
<th></th>
<th>Sepsis ALC &lt; 1.0</th>
<th>Sepsis ALC &lt; 1.0 vs ALC &gt; 1.0 HR</th>
<th>Non-Sepsis ALC &lt; 1.0</th>
<th>Non-Sepsis ALC &lt; 1.0 vs ALC &gt; 1.0 HR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vasopressor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>360/971 (37.1%)</td>
<td>1.99 (1.42, 2.80) n= 971</td>
<td>753/3144 (24.0%)</td>
<td>1.09 (0.93, 1.29) n=3140**</td>
</tr>
<tr>
<td><strong>No Vasopressor</strong></td>
<td>127/511 (24.9%)</td>
<td>0.99 (0.39, 2.54) n=493***</td>
<td>372/2979 (12.5%)</td>
<td>1.24 (0.84, 1.84) n=2948****</td>
</tr>
</tbody>
</table>

### Table 4b Proportion of patients with persistent lymphopenia (ALC < 0.75) +/- sepsis +/- vasopressor/inotrope and its association with in-hospital death