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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ABSTRACT

Purpose

To examine the relationship of early persistent lymphopenia with hospital survival in critically ill patients with and without sepsis to assess whether it can be considered a treatable trait.

Methods

Retrospective database analysis of patients with non-elective admission to ICUs during January 2015 to December 2018. Patients were classified as having sepsis if the Acute Physiology and Chronic Health Evaluation (APACHE) III admission diagnostic code included sepsis or coded for an infection combined with a Sequential Organ Failure Assessment (SOFA) score of ≥2. We defined early persistent lymphopenia at two thresholds (absolute lymphocyte count [ALC] <1.0 and <0.75x10^9/L) based on two qualifying values recorded during the first four days in ICU. The main outcome measure was time to in-hospital death.

Results

Of 8507 eligible patients, 7605 (89.4%) had two ALCs recorded during their first four days in ICU, 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. Of 3030 patients with persistent lymphopenia (ALC<1.0) 562 (18.5%) died compared with 439/4575 (9.6%) without persistent lymphopenia. Persistent lymphopenia was an independent risk factor for in-hospital death in all patients. The hazard ratios for death at ALC<1.0 were 1.89 (95%CI 1.32–2.71, p=0.0005) and 1.17 (1.02–1.35, p=0.0246) in patients with and without sepsis respectively.

Conclusions

Early persistent lymphopenia is common in critically ill patients and associated with increased risk of death in patients with and without sepsis. Although the association is stronger in patients with sepsis, lymphopenia is a candidate to be considered a treatable trait; drugs that reverse lymphopenia should be trialled in critically ill patients.

Keywords

Shock, immunosuppression, lymphocyte, trauma, mortality

INTRODUCTION

Persistent lymphopenia is known to be associated with increased mortality in critically ill patients with sepsis [1] and trauma [2-4] but whether this association holds for other critically ill patients is unknown. Patients who survive critical illness often experience long term morbidity affecting physical function, cognition, and mental health [5,6] and these long-term health effects are similar in patients with and without sepsis [7]. The terms “post-intensive care syndrome” and “post-sepsis syndrome” describe these persisting adverse 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. This supports the hypothesis that sepsis survivors suffer persistent immunosuppression.[8] In patients with sepsis multiple factors influence the immune system, including the microbiome,[9] which impacts the post-sepsis metabolic and epigenetic immune composition [9,10]. This results in complex alterations to cellular immunity with consequent immunodeficiency. The immune dysfunction, which can be predicted by serum soluble programmed cell death ligand-1 concentration,[11] can often be prolonged, [12-15] and is postulated to be a leading cause of late mortality in patients who have survived sepsis [13,16].

Published data on the prevalence and prognostic value of persistent lymphopenia in critically ill patients 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 the risk of death and longer-term morbidity in critically ill patients regardless of their sepsis status [17,18]. We hypothesized that early persistent lymphopenia would be common and associated with an increased risk of death in critically ill patients

without sepsis making it potentially a treatable trait, an observable biologic abnormality potentially present in different disease states whose modification results in improved outcomes.[19]

Consequently, we analysed data from two intensive care units (ICUs) 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. The present study represents one of the largest studies to date examining the impact of lymphopenia on mortality in critically ill patients with and without sepsis.

MATERIALS AND METHODS

Study setting and population

Ethics approval for data collection and analysis was granted by Metro South, Human Research Ethics Committee, Queensland (Australia). We interrogated the clinical information datasets (MetaVision, *i*MD*soft*®) from the Intensive Care Units (ICU) of two tertiary hospitals (total of 2,019 beds) in Queensland, Australia.

We accessed data for adult patients, admitted to the study ICUs between January 2015 – December 2018. We defined early 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. Patients admitted to ICU after elective surgery were excluded as they were expected to have a short ICU stay and a low risk of death.

We used the Acute Physiology and Chronic Health Evaluation (APACHE) III [20] 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 [21] of two or more. 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 risk of 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 early persistent lymphopenia defined at the two thresholds, the association of early 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. The multivariable models included a set of covariates that were defined a-priori with no automatic selection; these were age, sex, sepsis, early persistent lymphopenia, presence of comorbidities, SOFA score and quartiles of illness severity using the APACHE III score.

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 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). 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.

RESULTS

PATIENTS

We identified 8,507potentially eligible patients, of who 902 (10.6%) were excluded as they did not have two ALCs recorded within the first four days of admission, 848 of the 902 (94.01%) had an ICU stay of less than 48 hours, leaving 7605 eligible patients (Fig. 1).

Baseline Characteristics

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. APACHE III comorbidities were present in 15.9% of patients with sepsis and 9.5% of patients without sepsis.

OUTCOMES

Proportions of patients with early persistent lymphopenia

Early persistent lymphopenia (ALC < 1.0 x 10^9/L) was present in 728/1482 (49.1%) vs 2302/6123 (37.6%) of patients with and without sepsis, respectively. The odds ratio (OR) for persistent lymphopenia in sepsis versus non-sepsis was 1.60 (95% CI 1.43-1.80; p<0.0001).

Persistent lymphopenia at an ALC < 0.75 x 10^9/L was present in 487/1482 (32.9%) vs 1125 /6123 (18.4%) of patients with and without sepsis, respectively, OR for sepsis versus non-sepsis was 2.17 (95% CI 1.92 – 2.47; p<0.0001). ALCs on days 1-to-4 for patients with and without sepsis are shown in Fig. 2.

In-hospital death and its association with early persistent lymphopenia

Of the 3030 patients with early persistent lymphopenia, defined by an ALC < 1.0 x 10^9/L, 562 (18.5%) died during their hospital stay. 1612/3030 patients had early persistent lymphopenia, defined by an ALC < 0.75 x 10^9/L; of these, 334 (20.7%) died in hospital. Early persistent lymphopenia (ALC < 1.0 x 10^9/L) was an independent risk factor for in hospital death in those with and without sepsis. The association was stronger in patients with sepsis, Hazard Ratio (HR) for death 1.89 (95% CI 1.32 – 2.71; p=0.0005) compared to 1.17 (95% CI 1.02 – 1.35; p=0.0246) in patients without sepsis (Table 2 and Figure 3). A similar pattern was observed for ALC < 0.75 x 10^9/L. Patients with sepsis, HR 1.90 (95% CI 1.39 – 2.61; p<0.0001), patients without sepsis, HR 1.12 (95% CI 0.96 -1.31; p=0.149) (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.

SECONDARY OUTCOMES

Association of persistent lymphopenia with length of stay in hospital.

In all patients, early 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; p<0.0001) compared to patients without early persistent lymphopenia. Patients without sepsis had an increase in hospital stay of 6.18 days (95% CI 4.63 – 7.73; P<0.0001). For patients with sepsis the increase

was 1.79 days (95% CI -1.04 – 4.62; P=0.2152) (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; P<0.0001) compared to patients with sepsis, 1.52 days (95% CI -1.47 – 4.50; P=0.3184) (Table 3b).

Association of persistent lymphopenia with length of stay in ICU.

Early persistent lymphopenia at an ALC threshold of < 1.0 x 10^9/L was associated with an increase in lCU length of stay of 1.22 days (95% CI 0.89 – 1.55; P<0.0001) in patients without sepsis and 1.14 days (95% CI 0.36 – 1.92; P=0.0044) in patients with sepsis (Table 3a).

Early persistent lymphopenia at an ALC threshold of < 0.75 x 10^9/L was associated with increased ICU length of 1.25 days (95% CI 0.84 – 1.66; P<0.0001) in patients without sepsis and 0.98 days (95% CI 0.15 – 1.80; P=0.0200) in patients with sepsis (Table 3b).

Association of persistent lymphopenia with treatment with mechanical ventilation

For all patients, early persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with mechanical ventilation, adjusted odds ratio (OR) 1.15 (95% CI 1.01 – 1.30; P=0.0309). In patients without sepsis, an ALC < 1.0 was associated with an OR for treatment with mechanical ventilation of 1.20 (95% CI 1.04 – 1.39; P=0.0157). In patients with sepsis, the OR was 0.99 (95%. CI 0.78 – 1.27; P=0.9654), (Table 3a). A similar pattern was observed for those with an ALC < 0.75 x 10^9/L: OR 1.27 (95% CI 1.04 – 1.54; P=0.0176), in patients without sepsis, compared to OR 0.91 (95% CI 0.70 – 1.17; P=0.4515), in patients with sepsis (Table 3b).

Patients without sepsis and an ALC < 1.0 were treated with mechanical ventilation for 1.24 extra days (95% CI 0.88 – 1.61: P<0.0001) compared to non-sepsis patients with an ALC > 1.0. Patients with sepsis were treated for 1.65 extra days (95% CI 0.61 – 2.68; P=0.0018) (Table 3a). A similar pattern was observed for ALC < 0.75 x 10^9/L. Non-sepsis patients were

treated for 1.10 extra days (95% CI 0.65 – 1.55; P<0.0001), compared to 1.57 extra days (95% CI 0.49 – 2.65; P=0.0045) for patients with sepsis (Table 3b).

Association of persistent lymphopenia with treatment with vasopressors or inotropes

For all patients, early persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with a vasopressor or inotrope, OR 1.38 (95% CI 1.21 – 1.57; P<0.0001). In patients without sepsis, an ALC < 1.0 was associated with a OR for treatment with a vasopressor or inotrope of 1.43 (95% CI 1.23 – 1.65; P<0.0001) compared to patients without sepsis with an ALC >1.0. For patients with sepsis and an ALC < 1.0 x 10^9/L the OR was 1.17 (95% CI 0.87 – 1.57; P=0.2894) 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 had a OR 1.47 (95% CI 1.22 – 1.78) compared to patients with sepsis, OR 1.02 (95% CI 0.74 – 1.39; P=0.9188).

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

For all patients, early persistent lymphopenia (ALC < 1.0 x 10^9/L) was associated with an increased likelihood of treatment with RRT, OR 1.89 (95% CI 1.55 – 2.32: P<0.0001). For patients with sepsis, OR 1.74 (95% CI 1.19 – 2.55; P=0.0043), for those without sepsis OR 1.95 (95% CI 1.53 – 2.48; P<0.0001) (Table 3a). For patients with early persistent lymphopenia defined as ALC < 0.75 x 10^9/L, there was an independent association between lymphopenia and treatment with RRT in patients without sepsis, OR 1.93 (95% CI 1.50 – 2.47; P<0.0001) but not those with sepsis OR 1.22 (95% CI 0.85 – 1.76; P=0.2842) (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; P=0.1878) (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; P=0.4446) compared to patients with sepsis who were treated for 2.43 days longer (95% CI 0.36 – 4.50; P=0.0218) (Table 3b).

DISCUSSION

The current study represents one of the largest studies to date that examines the association of lymphopenia with survival in critically ill patients with and without sepsis. We found that early persistent lymphopenia, defined as an ALC < 1.0 x 10^9/L on at least two of the first four days in ICU, was common in critically ill patients both with and without sepsis. The likelihood of having early persistent lymphopenia was significantly greater in patients with sepsis, this is consistent with the trend observed by Andreu-Ballester in a retrospective study conducted at two hospitals in Spain [22]. This difference was greater for patients with an ALC <0.75 x 10^9/L. Early persistent lymphopenia was associated with an increased risk of in-hospital death, again this association was stronger for patients with sepsis.

For secondary outcomes, early persistent lymphopenia was associated with increased likelihood of treatment with mechanical ventilation, vasoactive drugs, and renal replacement therapy. In the population studied these findings resulted from a strong association for patients without sepsis. Early persistent lymphopenia was associated with increased length of ICU and hospital length of stay. The increase in hospital length of stay in those with lymphopenia was much greater for patients without sepsis: this this was due to a shorter length of stay for patients who have neither sepsis nor lymphopenia.

We observed 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 [1,23], 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 being multicentre and including data from over 7000 patients. This adds very substantially to previously published data [1]. We excluded patients admitted to the ICU after elective surgery, focussing our study on a cohort at greater risk of death. 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.

Limitations of our study include that we classified patients as having sepsis or not based on admission APACHE III coding and were not able to identify patients who developed sepsis later in the ICU stay. This may have reduced apparent differences between patients classified as having or not having sepsis. Additionally, our data come from two similar Australian hospitals and they may not be representative of data in other settings.

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 by selecting a population of patients at increased risk of dying. Whether it is a treatable trait and can therefore be used for

predictive enrichment to select patients more likely to respond to particular treatments is currently unknown. Treating lymphopenia in a diverse population of critically ill patients aligns with the current trend to look for treatable traits [19]. 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.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Derick Adigbli - No conflicts of interest to disclose.

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.

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Amritendu Bhattacharya – The George Institute for Global Health. Funding for data analysis (PIVOTAL) from RevImmune Inc., - all 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., - all 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., - all paid to The George Institute for Global Health.

Richard Hotchkiss – 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.

Legends for figures:

Fig. 1 Patient flow

Fig. 2 Absolute lymphocyte counts for Day 1-to-4 in patients with and without s

Fig. 3 Association of persistent lymphopenia with in-hospital death

Fig. 4A Probability of survival Patients with sepsis

Fig. 4B Probability of survival Patients without sepsis

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Fig. 1 Patient flow (Australian cohort)

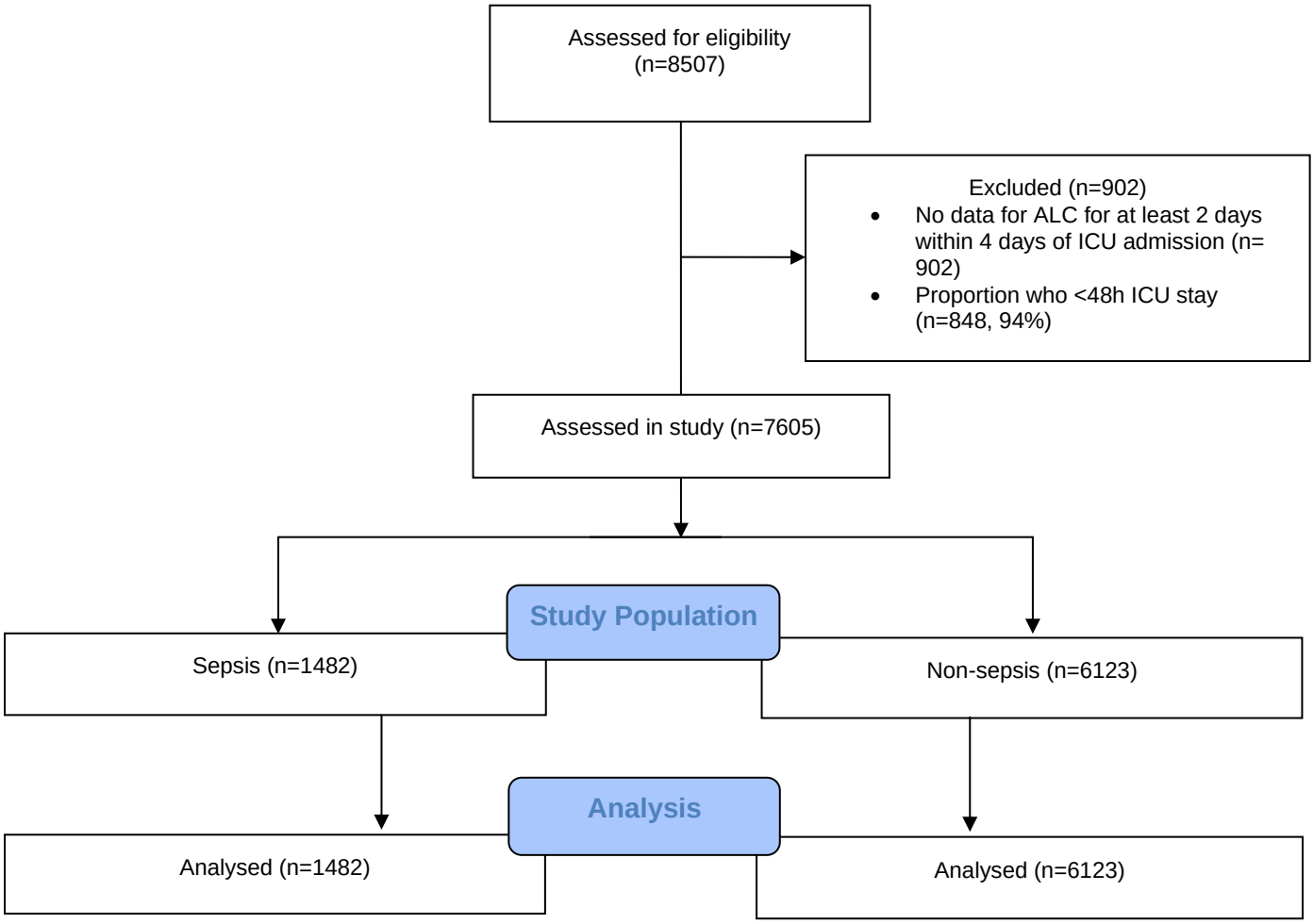


Fig. 2 Absolute lymphocyte counts for Day 1-to-4 in patients with and without sepsis.

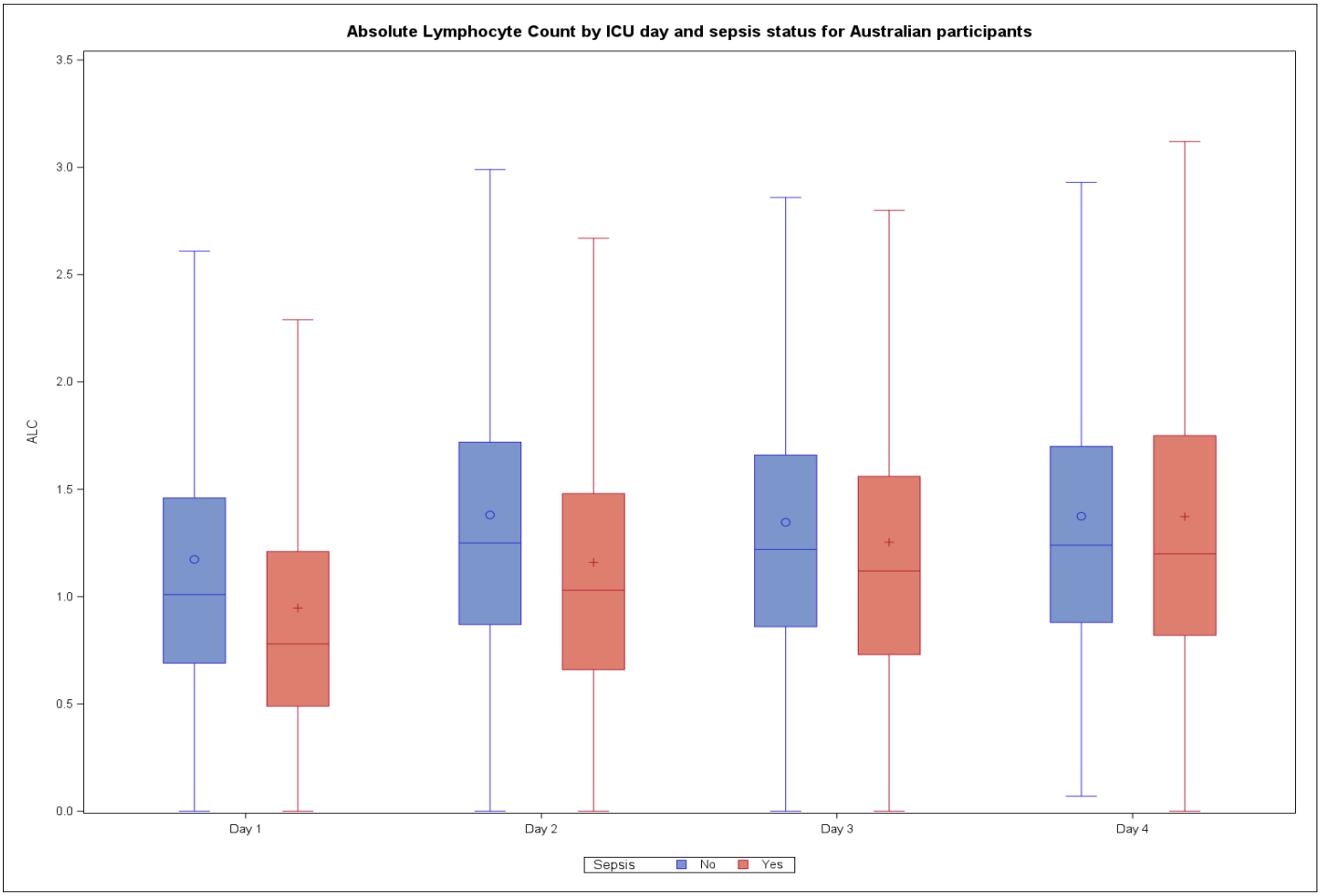


Fig. 3 Association of persistent lymphopenia with in-hospital death

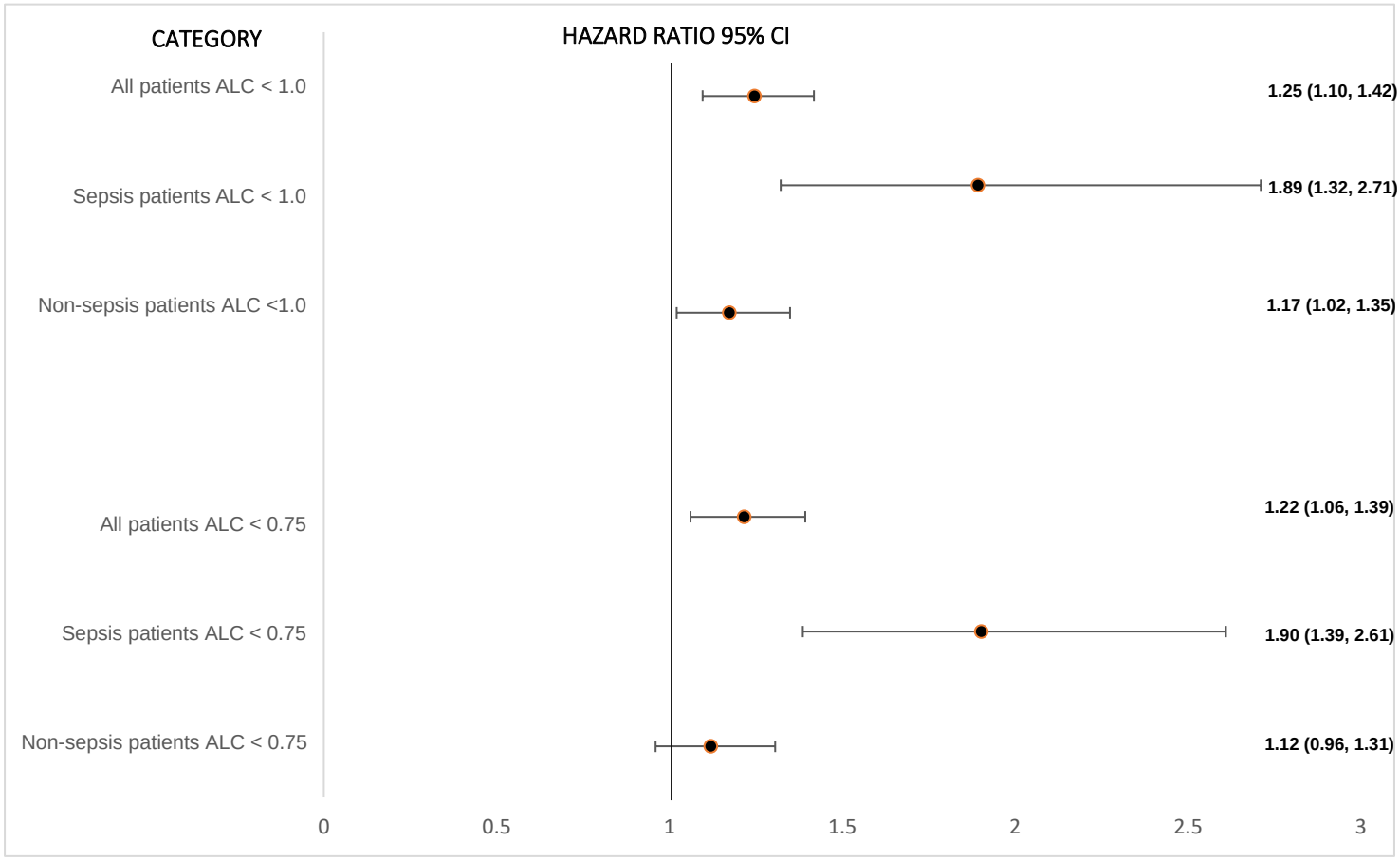
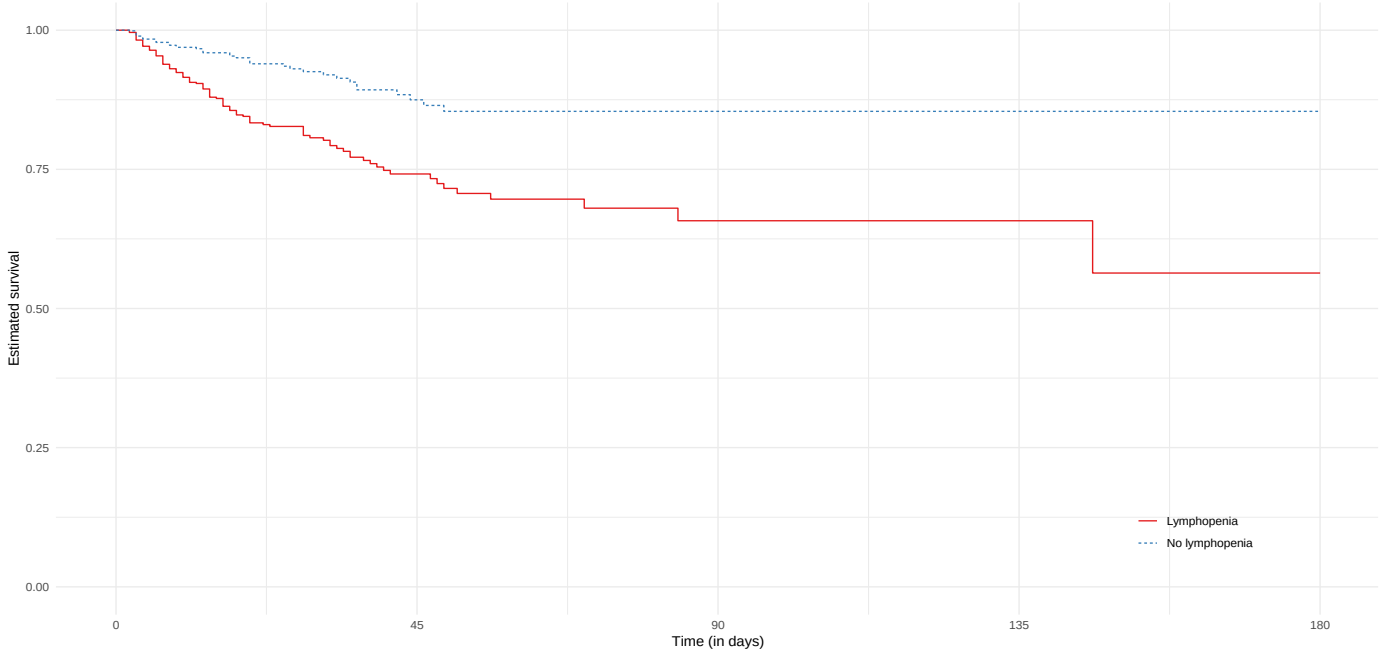


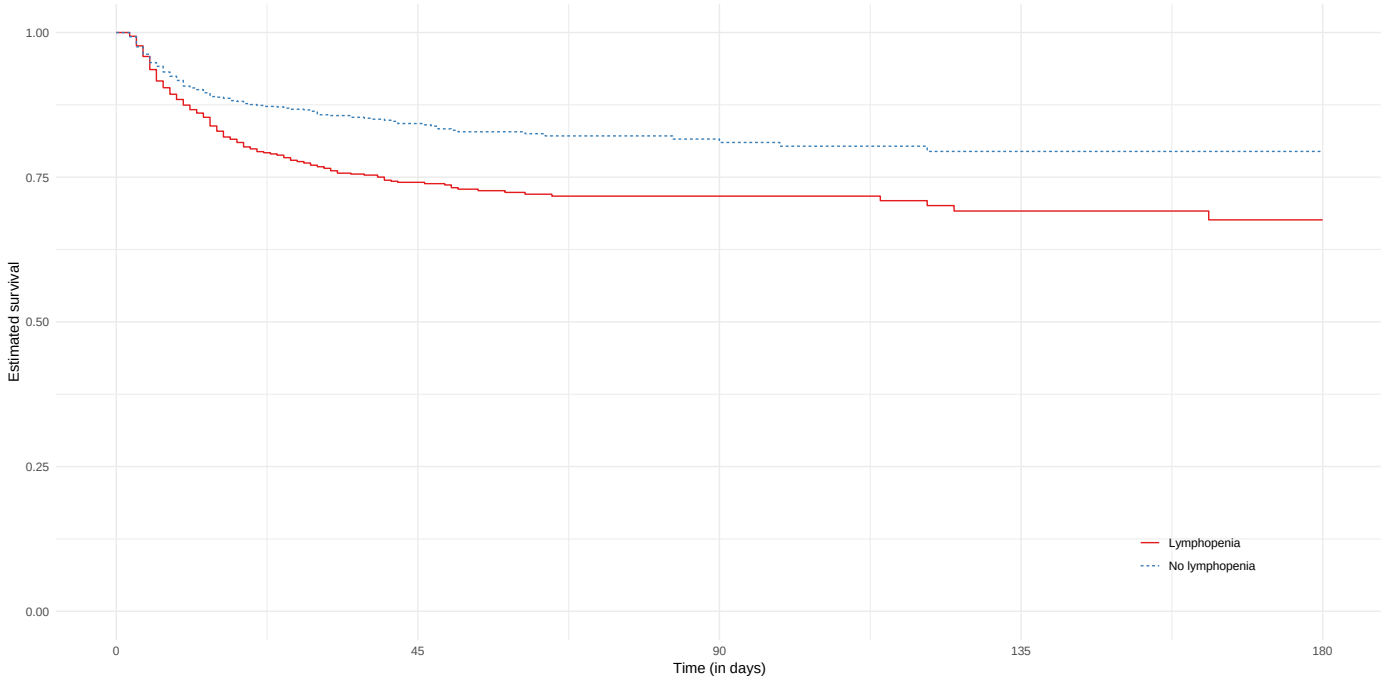
Fig. 4 Kaplan Meier survival graph. A) Sepsis patients. B) Non-sepsis patients.



Lymphopenia					
At risk	728	89	27	7	0
Censored	0	527	582	602	608
Events	0	112	119	119	120

No lymphopenia					
At risk	754	87	28	11	0
Censored	0	626	683	700	711
Events	0	41	43	43	43

a



Lymphopenia					
At risk	2302	338	128	65	0
Censored	0	1535	1736	1796	1860
Events	0	429	438	441	442
No lymphopenia					
At risk	3821	382	136	64	0
Censored	0	3055	3291	3361	3425
Events	0	384	394	396	396

b

Table 1 Characteristics of patients in the Australian cohort according to classification as sepsis or not.

Characteristic	Sepsis (N= 1482)	Non-sepsis (N=6123)
Median Age	61 (46-71)	54 (38-67)
Female	600 [40.5%]	2384 [38.9%]
Male	882 [59.5%]	3739 [61.1%]
Median APACHE II score (IQR)†	18 (14-24)	16 (11-22)
Median APACHE III score (IQR)‡	63 (49-80)	52 (37-73)
Median SOFA score (IQR)¶	7(4-9)	5 (3-8)
Persistent Lymphopenia < 1.0, *f*	728 [49.1%]	2302 [37.6%]
Persistent Lymphopenia < 0.75, *f*	487 (32.9)	1125 [18.4%]
No comorbidity	1247 [84.1%]	5541 [90.5%]
Mean ALC Day 1	0.95 ± 0.82	1.17 ± 0.85
Mean ALC Day 2	1.16 ± 0.80	1.38 ± 0.91
Mean ALC Day 3	1.25 ± 0.89	1.35 ± 0.79
Mean ALC Day 4	1.37 ± 0.89	1.38 ± 0.87

* Plus-minus values are means ±SD. The Sepsis group were defined based on an Acute Physiology and Chronic Health Evaluation (APACHE) III 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 2 Proportion of patients with Persistent lymphopenia at the two cut-off levels and its association with in-hospital death

In-hospital death and its association with persistent lymphopenia						
	ALC < 1.0	**ALC > 1.0**	**ALC < 1.0 vs ALC > 1.0 Hazard ratio**	**ALC < 0.75**	**ALC > 0.75**	**ALC < 0.75 vs ALC > 0.75 Hazard ratio**
Sepsis	120/728 (16.5%)	43/754 (5.7%)	1.89 (1.32, 2.71) n=1468*	92/487 (18.9%)	71/995 (7.1%)	1.90 (1.39, 2.61) n=1468*
Non-Sepsis	442/2302 (19.2%)	396/3821 (10.4%)	1.17 (1.02, 1.35) n=6088**	242/1125 (21.5%)	596/4998 (11.9%)	1.12 (0.96, 1.31) n=6088**

ALC: absolute lymphocyte count, HR; Hazard ratio, CI; confidence interval

* Acute Physiology and Chronic Health Evaluation (APACHE) III 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.

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 109/L.

Secondary outcomes for patients with sepsis and patients without sepsis (ALC < 1.0 x 10^9/L)						
	Sepsis:			**Non-sepsis:**		
	ALC < 1.0 (N=728)	**ALC > 1.0 (N=754)**	**ALC < 1.0 vs ALC > 1.0 * Difference; ^ HR**	**ALC < 1 (N=2302)**	**ALC > 1 (N=3821)**	**ALC < 1.0 vs ALC > 1.0 * Difference; ^ HR**
Mean (95% CI) Hospital stay, (Days)	24.6	23	1.79 (-1.04, 4.62)*	27.1	20.9	6.18 (4.63, 7.73)
Mean (95% CI) ICU Stay, (Days)	7.3	5.7	1.14 (0.36, 1.92)*	6.9	5.3	1.22 (0.89, 1.55)
Mechanical ventilation, HR	482/728 (66.2%)	482/754 (63.9%)	0.99 (0.78, 1.27) ^	1845/2302 (80.1%)	2743/3821 (71.8%)	1.20 (1.04, 1.39)
Vasopressor/inotrope, HR	532/728 (73.1%)	439/754 (58.2%)	1.17 (0.87, 1.57)^	1427/2302 (62.0%)	1717/3821 (44.9%)	1.43 (1.23, 1.65)
Renal replacement therapy, HR	134/728 (18.4%)	63/754 (8.4%)	1.74 (1.19, 2.55) ^	215/2302 (9.3%)	128/3821 (3.3%)	1.95 (1.53, 2.48)
Mean (95% CI) Mechanical ventilation, (Days)	7	5.7	1.65 (0.61, 2.68) *	5.7	4.3	1.24 (0.88, 1.61)
Mean (95% CI) Vasopressor/inotrope, (Days)	3.4	2.7	0.46 (-0.11, 1.02) *	3.1	2.9	0.22 (0.00, 0.45)
Mean (95% CI) Renal replacement therapy, (Days)	7.3	6.1	1.51 (-0.74, 3.75) *	5.4	3.8	1.45 (0.46, 2.44)

ALC: absolute lymphocyte count, HR; Hazard ratio, CI; confidence interval

Table 3b 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 <0.75 x 109/L.

Secondary outcomes for patients with sepsis and patients without sepsis (ALC < 0.75 x 10^9/L)						
	Sepsis			**Non-sepsis**		
	ALC < 0.75 (N =487)	**ALC > 0.75 (N=995)**	**ALC < 0.75 vs ALC > 0.75 * Difference; ^ HR**	**ALC < 0.75 (N=1125)**	**ALC > 0.75 (N=4998)**	**ALC <0.75 vs ALC >0.75 * Difference; ^ HR**
Mean (95% CI) Hospital stay, (Days)	24.4	23.5	1.52 (-1.47, 4.50)*	28.0	22.2	5.80 (3.88, 7.23)
Mean (95% CI) ICU Stay, (Days)	7.5	6.0	0.98 (0.15, 1.80)*	7.3	5.6	1.25 (0.84, 1.66)
Mechanical ventilation, HR	318/487 (65.3%)	646/995 (64.9%)	0.91 (0.70, 1.17) ^	925/1125 (82.2%)	3663/4998 (73.3%)	1.27 (1.04, 1.54)
Vasopressor/inotrope, HR	360/487 (73.9%)	611/995 (61.4%)	1.02 (0.74, 1.39) ^	753/1125 (66.9%)	2391/4998 (47.8%)	1.47 (1.22, 1.78)
Renal replacement therapy, HR	93/487 (19.1%)	104/995 (10.5%)	1.22 (0.85, 1.76) ^	137/1125 (12.2%)	206/4998 (4.1%)	1.93 (1.50, 2.47)
Mean (95% CI) Mechanical ventilation, (Days)	7.3	5.4	1.57 (0.49, 2.65)*	6.0	4.6	1.10 (0.65, 1.55)
Mean (95% CI) Vasopressor/inotrope, (Days)	3.6	2.8	0.50 (-0.07, 1.07)*	3.2	2.9	0.26 (0.00, 0.52)
Mean (95% CI) Renal replacement therapy, (Days)	8.0	5.9	2.43 (0.36, 4.50)*	5.1	4.6	0.38 (-0.60, 1.37)

ALC: absolute lymphocyte count, HR; Hazard ratio, CI; confidence interval

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

In-hospital death and its association with persistent lymphopenia				
	Sepsis ALC < 1.0	**Sepsis ALC < 1.0 vs ALC > 1.0 HR**	**Non-Sepsis ALC < 1.0**	**Non-Sepsis ALC < 1.0 vs ALC > 1.0 HR**
Vasopressor	532/971 (54.7%)	1.95 (1.32, 2.89) n= 971	1427/3144 (45.4%)	1.12 (0.96, 1.30) n=3140*
No Vasopressor	196/511 (38.4%)	1.25 (0.49, 3.15) n= 497**	875/2979 (29.4%)	1.36 (0.97, 1.90) n=2948***

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

In-hospital death and its association with persistent lymphopenia				
	Sepsis ALC < 0.75	**Sepsis ALC < 0.75 vs ALC > 0.75 HR**	**Non-Sepsis ALC < 0.75**	**Non-Sepsis ALC < 0.75 vs ALC > 0.75 HR**
Vasopressor	360/971 (37.1%)	1.99 (1.42, 2.80) n= 971	753/3144 (24.0%)	1.09 (0.93, 1.29) n=3140*
No Vasopressor	127/511 (24.9%)	0.99 (0.39, 2.54) n=497**	372/2979 (12.5%)	1.24 (0.84, 1.84) n=2948***

ALC: absolute lymphocyte count, HR; Hazard ratio, CI; confidence interval

* Acute Physiology and Chronic Health Evaluation (APACHE) III for 4 patients were missing and therefore excluded from HR calculation.

** Acute Physiology and Chronic Health Evaluation (APACHE) III for 14 patients were missing and therefore excluded from HR calculation.

*** Acute Physiology and Chronic Health Evaluation (APACHE) III for 31 patients were missing and therefore excluded from HR calculation.