HHS Public Access

Author manuscript

Shock. Author manuscript; available in PMC 2015 November 01.

Published in final edited form as:

Shock. 2014 November ; 42(5): 383–391. doi:10.1097/SHK.0000000000000234.

Persistent Lymphopenia after Diagnosis of Sepsis Predicts Mortality

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Abstract

Objective—To determine whether persistent lymphopenia on the fourth day following the diagnosis of sepsis predicts mortality.

Methods—Single-center, retrospective cohort study of 335 adult patients with bacteremia and sepsis admitted to a large university-affiliated tertiary care hospital between January 1, 2010 and July 31, 2012. All complete blood cell count profiles during the first four days following the diagnosis of sepsis were recorded. The primary outcome was 28-day mortality. Secondary outcomes included development of secondary infections, 1-year mortality, and hospital and intensive care unit lengths of stay.

Results—76 (22.7%) patients died within 28 days. Lymphopenia was present in 28-day survivors (median 0.7 cells/μl × 10^3 [IQR 0.4, 1.1]) and non-survivors (median 0.6 cells/μl × 10^3 [IQR 0.4, 1.1]) at the onset of sepsis and was not significantly different between the groups (p = .35). By Day 4, the median absolute lymphocyte count was significantly higher in survivors compared to non-survivors (1.1 cells/μl × 10^3 [IQR 0.7, 1.5] vs 0.7 cells/μl × 10^3 [IQR 0.5, 1.0], p < .0001). Using logistic regression to account for potentially confounding factors (including age, APACHEII score, comorbidities, surgical procedure during the study period, and time until appropriate antibiotic administration), day 4 absolute lymphocyte count was found to be independently associated with 28-day survival (adjusted OR 0.68 [95% CI 0.51, 0.91]) and 1-year survival (adjusted OR 0.74 [95% CI 0.59, 0.93]). Severe persistent lymphopenia (defined as an absolute lymphocyte count ≤ 0.6 cells/μL × 10^3 on the fourth day after sepsis diagnosis) was associated with increased development of secondary infections (p = .04).

Conclusions—Persistent lymphopenia on the fourth day following the diagnosis of sepsis predicts early and late mortality and may serve as a biomarker for sepsis-induced immunosuppression.

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Conflicts of Interest

Anne Drewry, Lee Skrupky, Brian Fuller, Navdeep Samra, and Stephanie Compton have no competing interests to declare.

Keywords

immunosuppression; immunomodulation; bacteremia; survival; absolute lymphocyte count

Introduction

Sepsis is a devastating illness that accounts for over 210,000 deaths annually in the United States (1). Previously believed to be a syndrome of uncontrolled inflammation, sepsis is now recognized to initiate both pro- and anti-inflammatory immune mechanisms that, in many cases, result in prolonged periods of immunosuppression (2–5). Sepsis-induced derangements of innate and adaptive immunity prevent pathogen clearance and predispose to secondary infections, as evidenced by autopsy studies that have demonstrated ongoing foci of infections and functional defects in immune effector cells in patients who have died of sepsis (6,7). This immune dysfunction is a primary cause of late mortality in sepsis (3,8).

An important feature of sepsis-induced immunosuppression is apoptosis-related loss of immune cells, including CD4 and CD8 T, B, and follicular dendritic cells (3,9). Clinical studies have demonstrated that circulating levels of lymphocytes fall during the onset of sepsis and can remain depressed for up to 28 days (10–16). Anti-apoptotic therapies which prevent lymphocyte death have successfully improved survival in animal models of sepsis (17,18), and immunomodulatory therapy may be a promising area of future clinical research (19,20). However, human phenotypic variability in the immune response to infection suggests that the broad application of immunomodulatory therapy across a broad cohort of septic patients is unlikely to show benefit (21). Ideally, immune-stimulating therapy would be applied only to those patients who are known to be persistently immunosuppressed. Therefore, strategies to better identify these patients in the clinical setting should be pursued.

Prolonged lymphopenia is a candidate marker of persistent immunosuppression in septic patients, and absolute lymphocyte counts are easily measured during routine care. Previous studies have shown that low absolute lymphocyte counts are predictive of postoperative sepsis and are a better predictor of bacteremia than conventional infection markers in the emergency care unit (22,23). Studies have also shown that septic non-survivors have sustained reductions in specific T and B cell populations during the first week after sepsis diagnosis (11–16). However, data is limited, and these studies did not often evaluate the absolute lymphocyte count. We therefore aimed to determine whether persistent lymphopenia over the first four days following the diagnosis of sepsis predicts mortality. This time point was chosen, in part, to avoid including patients in the non-survivor group who died during the initial hyperinflammatory stage of sepsis rather than during the immunosuppressive phase. Also, studies of septic patients have shown significantly decreased levels of lymphocyte subpopulations in adult non-survivors compared to survivors at days 3 and 7, suggesting that day 4 absolute lymphocyte counts could potentially discriminate between these two groups (11–13). Finally, previous literature has demonstrated that persistent lymphopenia on the fourth day after presentation was predictive of death in trauma patients, another group of critically ill patients at risk for developing persistent immunosuppression (24). We hypothesized that patients with persistently low

lymphocyte counts would be more likely to die and to develop secondary infections compared to patients whose lymphocyte counts recovered to normal by the fourth day after sepsis diagnosis.

Materials and methods

Study design

We conducted a retrospective observational cohort study and reported its results in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (25). Data collection and analysis were approved by the Human Research Protection Office at our institution with waiver of informed consent.

Study setting and population

This study was conducted at a 1,167-bed university-affiliated adult tertiary care hospital between January 1, 2010 and July 31, 2012. All patients admitted to the hospital with sepsis and blood cultures positive for bacteria and/or fungal organisms within five days after hospital admission were evaluated for inclusion. Sepsis was defined according to consensus criteria (26). For the purpose of this study, the diagnosis of sepsis required the presence of at least two systemic inflammatory response syndrome (SIRS) criteria within 24 hours of the time the positive blood culture was collected. Further, patients were included only if they survived until at least the fourth day after the positive blood culture was drawn and also had a complete blood cell count with differential (including lymphocyte, neutrophil, and monocyte counts) collected on that day. Each patient was only included in the study once. For patients who had multiple hospital admissions with positive blood cultures during the study period, only data from the most recent hospitalization were included. For patients with multiple positive blood cultures during a single admission, data collection began at the time of the first positive culture.

Exclusion criteria included: age less than eighteen years, blood cultures positive only for common contaminates (for example, coagulase-negative *Staphylococcus* species, *Propionibacterium* species, *Corynebacterium* species), diagnosis of hematological or immunological disease, and treatment with chemotherapy agents or corticosteroids within 6 months prior to or during the hospitalization.

Data collection

Patient identification—A computer query was used to identify all adult patients with positive blood cultures admitted during the study period. Detailed chart review was then performed to exclude patients based on inclusion and exclusion criteria and to collect demographic, microbiology, and outcome data. Leukocyte counts were collected by a separate research assistant blinded to the study hypothesis, the patients' baseline characteristics, and outcomes.

Definitions—The first 24-hour time period following the culture collection time was considered to be Day 1; the next 24-hour period, Day 2; etc. If multiple leukocyte counts were collected within any 24-hour period, the mean value was documented for that period.

Lymphopenia was defined as an absolute lymphocyte count less than 1.2 cells/μL × 10^3, which is the lower limit of normal at our institution. Severe lymphopenia was defined as an absolute lymphocyte count less than 0.6 cells/μL × 10^3, which is half of the lower limit of normal. Severe sepsis and septic shock were defined per consensus criteria (26).

Baseline characteristics—Baseline demographics included age, sex, source of bacteremia, presence of co-morbidities, and modified Acute Physiology and Chronic Health Evaluation (APACHE) II score (excluding the Glascow Coma Score) (27,28). The source of bacteremia was determined by the presence of concurrent cultures growing the same organism from another site or from documentation in the medical record by the treating physician. Requirement for vasopressors, requirement for mechanical ventilation, and surgical procedures prior to or during the four-day study period were documented. Microbiology and antibiotic susceptibility data were collected for each positive blood culture. Antibiotic administration times were recorded, and length of time to appropriate antibiotic coverage was calculated.

Leukocyte counts—For every patient, the white blood cell count (WBC), neutrophil count, lymphocyte count, and monocyte count were collected for the first four days following the culture collection date.

Outcomes—The primary outcome was 28-day mortality. Secondary outcomes included the development of secondary infections, 1-year mortality, hospital length of stay, and intensive care unit (ICU) length of stay. Secondary infections were defined as culture-positive infections identified greater than 48 hours after the primary bacteremia and arising from a secondary source. Identification of secondary infections was based on culture growth of a new organism at any site and documentation of a new infection by the treating physician in the medical record.

Statistical analysis

Descriptive statistics, including mean (standard deviation), median (interquartile range defined as the 25th and 75th percentile), and frequency distributions were used to describe the patient cohort. Normality was assessed using histograms and the Kolmogorov-Smirnov test. Variables which failed tests of normality were presented and analyzed in a nonparametric fashion. Comparison of baseline characteristics between 28-day survivors and non-survivors was done using unpaired t-tests, Mann-Whitney U tests, or chi-square tests, as appropriate.

Because not every patient had complete blood cell counts collected on days 1 through 3, longitudinal analyses of the blood cell counts (WBC, absolute neutrophil count, absolute lymphocyte count, and absolute monocyte count) were based on a repeated measures mixed model analysis of variance with an autoregressive covariance structure as implemented by the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). The primary focus of these analyses was on the significance of the interactions that tested hypotheses regarding the equality of changes over time in the two groups. Within the framework of the mixed model analysis and when the interaction was significant, the appropriate statistical contrasts

were used to test the null hypotheses that: (a) changes between days 1 and 4 in survivors were equal to corresponding changes in non-survivors; (b) day 1 values were equal for survivors and non-survivors; and (c) day 4 values were equal for survivors and non-survivors. P-values were not adjusted for multiple comparisons. Data were log-transformed prior to analysis.

To determine the independent effect of the day 4 absolute lymphocyte count on mortality after accounting for significant confounders, multivariable logistic regression with a forced entry method was used to model the odds of death at 28 days and 1 year. All variables that were significant at a *p* value of .05 in univariable analysis were included in the model. For all continuous variables (except absolute lymphocyte count), the odds ratios (ORs) reflect the increased odds of death for a one unit increase in the baseline variable. For absolute lymphocyte count, the OR indicates the increased odds of death for every increase in the absolute lymphocyte count by 0.5 cells/μL × 10^3. This value was chosen to improve clinical interpretation of the OR given that a normal absolute lymphocyte count is only 1.2 cells/μL × 10^3. For categorical variables, the reference category for the OR was absence of the condition. Collinearity diagnostics were evaluated to ensure variable independence. Multivariable models report ORs adjusted for all variables in the model.

Based on previous literature (11–13,24), day 4 absolute lymphocyte count was selected *a priori* to be an independent variable in analysis of the primary and secondary outcomes. However, to determine whether earlier absolute lymphocyte counts could also predict 28-day and 1-year mortality, we performed additional *post hoc* univariable and multivariable logistic regression analyses using the day 3 absolute lymphocyte count as an independent variable.

Patients were divided into three groups based on their day 4 absolute lymphocyte count. Group 1 consisted of patients with normal lymphocyte counts (≥ 1.2 cells/μL × 10^3) by day 4; Group 2 included patients with moderate persistent lymphopenia (day 4 count 0.7 to 1.1 cells/μL × 10^3); and Group 3 consisted of patients with severe persistent lymphopenia (day 4 count ≤ 0.6 cells/μL × 10^3). The reason for separating persistently lymphopenic patients into moderate and severe groupings was to facilitate observation of graduated changes in outcomes associated with increasingly severe lymphopenia. Kruskal-Wallis one-way analysis of variance and chi square tests were used for comparisons of baseline characteristics between the three lymphocyte groups. Kaplan-Meier survival curves were created and compared using the log-rank test. Additionally, univariable logistic regression was used to model the odds of developing secondary infections using group assignment as the independent variable. The reference category for the reported ORs was absence of persistent lymphopenia.

A receiver operating characteristic (ROC) plot was created to illustrate the ability of day 1 and day 4 absolute lymphocyte counts to predict 28-day mortality. Lower values of absolute lymphocyte counts were chosen to indicate a more positive test, such that higher areas under the curve (AUCs) indicated increased discriminatory ability. Confidence intervals on AUCs were calculated using non-parametric assumptions.

Except where otherwise stated, all statistical tests were carried out using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). All tests were two-tailed, and *p* values less than .05 were considered statistically significant.

Results

A total of 335 patients were included in the study (Figure 1). Seventy-six patients (22.7%) died within 28 days. Table 1 reports the baseline characteristics for the entire patient cohort. Non-survivors were older and had more severe illness, longer time to antibiotic administration, and higher incidences of congestive heart failure, chronic renal insufficiency, and liver disease.

Boxplots demonstrating the distributions of the WBC, absolute lymphocyte count, absolute monocyte count, and absolute neutrophil count in survivors and non-survivors for the first four days after the diagnosis of sepsis are shown in Figure 2. On day 1, the absolute lymphocyte counts were well below the lower limit of normal and not significantly different between the survivors (median 0.7 [0.4, 1.1] cells/μL × 10^3) and non-survivors (median 0.6 [0.4, 1.1] cells/μL × 10^3) ($p = .35$). By day 4, the absolute lymphocyte count was significantly higher in the survivors (median 1.1 [0.7, 1.5] cells/μL × 10^3) compared to the non-survivors (median 0.7 [0.5, 1.0] cells/μL × 10^3) ($p < .0001$), and the overall increase in absolute lymphocyte count over the 4-day period was significantly greater in the survivors ($p = .0004$). There was no significant difference in the overall change in the WBC ($p = .06$) or absolute monocyte count ($p = .11$) over the four-day period between the two groups. The absolute neutrophil count at day 4 was significantly lower in the survivors (median 8.2 [5.9, 13.1] cells/μL × 10^3) compared to the non-survivors (median 9.2 [7.2, 14.1] cells/μL × 10^3) ($p = .04$), and the overall decrease in absolute neutrophil count over the four day period was significantly greater in survivors ($p = .03$).

Table 2 shows the multivariable analysis of factors associated with 28-day mortality. Day 4 absolute lymphocyte count was significantly associated with 28-day mortality ($p = .009$) with an adjusted odds ratio of 0.68 (95% CI 0.51, 0.91). Day 4 absolute lymphocyte count was also associated with 1-year mortality ($p = .008$) with an adjusted odds ratio of 0.74 (95% CI 0.59, 0.93). In the subset of patients with septic shock, day 4 absolute lymphocyte count continued to be a significant predictor of both 28-day (OR 0.69 [95% CI 0.48, 0.98], $p = .04$) and 1-year mortality (OR 0.71 [95% CI 0.51, 0.97], $p = .03$).

Mortality at 28 days and 1 year was significantly higher in patients with severe persistent lymphopenia (39.5%, 57.9%) and moderate persistent lymphopenia (24.6%, 40.3%) compared to those without persistent lymphopenia (10.4%, 28.8%). The Kaplan-Meier survival analyses comparing patients based on day 4 lymphocyte groups are shown in Figure 3.

In the 305 patients who had complete blood cell counts drawn on day 3, univariable analysis demonstrated that day 3 absolute lymphocyte count was also a significant predictor of 28-day mortality (OR 0.62 [95% CI 0.46, 0.83], $p = .001$) and 1-year mortality (OR 0.71 [95% CI 0.57, 0.89], $p = .002$). However, the adjusted odds ratios for day 3 absolute lymphocyte

count were not statistically significant for death at 28 days or 1 year when other predictors of mortality were included in multivariable analyses.

Table 3 summarizes the baseline characteristics and outcomes of patients stratified by day 4 absolute lymphocyte count. Patients with persistent lymphopenia had higher APACHE II scores, while patients without persistent lymphopenia had a lower incidence of diabetes. There were no differences between the groups in hospital or ICU lengths of stay. Table 4 shows the occurrence of secondary infections based on the presence of persistent lymphopenia. The presence of severe persistent lymphopenia (OR 2.11 [95% CI 1.02, 4.39], $p = .04$), but not moderate persistent lymphopenia (OR 1.60 [95% CI 0.83, 3.11], $p = .16$) was significantly associated with development of secondary infections compared to those without persistent lymphopenia.

The discriminatory ability of day 1 and day 4 absolute lymphocyte counts to predict 28-day mortality is demonstrated in figure 4 using ROC plots. Day 4 absolute lymphocyte count, but not day 1 absolute lymphocyte count, demonstrated a significantly higher AUC compared to a reference of 0.5. The performance of specific day 4 absolute lymphocyte count thresholds to predict 28-day mortality is indicated on the graph. The most accurate discriminatory threshold was calculated to be 1.0 cells/μL × 10^3 which demonstrated a sensitivity of 76% and a specificity of 56%.

Discussion

Information regarding immune phenotype in septic patients is vital before any consideration of immunomodulatory therapeutic interventions can occur. The current study provides new findings in this domain and offers potential insight into areas for further research. Our findings have several implications.

After initial predominance of a proinflammatory cytokine-driven response, many septic patients develop persistent and profound immunosuppression prior to succumbing to the syndrome (3,7,8,15). The results of this study demonstrate that a persistently low level of circulating lymphocytes on the fourth day following the diagnosis of sepsis independently predicts short- and long-term survival and may serve as a biomarker for sepsis-induced immunosuppression. The immune response to sepsis is extremely variable and can change dramatically as the syndrome progresses. Patients who die early do so as a result of overwhelming hyperinflammation marked by cardiovascular collapse and multiple organ failure (4,29). Many of the patients who survive this phase, however, go on to develop a compensatory anti-inflammatory response characterized by increased inhibitory receptors on T cells and antigen presenting cells, decreased production of pro-inflammatory cytokines, expansion of myeloid-derived suppressor cells, and apoptosis-related loss of lymphocytes and dendritic cells (7,9,30–32). In the current study, only 33 patients (who otherwise would have met inclusion criteria) died prior to the fourth day after the diagnosis of sepsis. This accounted for only 30.3% of the total deaths due to sepsis, signifying that the majority of patients who ultimately died were at risk for sepsis-induced immunosuppression.

Previous studies have demonstrated persistently decreased levels of specific sub-populations of B and T cells in adult septic non-survivors compared to survivors, but have not shown significant differences in the overall absolute lymphocyte count (11–13,16). Our study, which included a large number of septic non-survivors, demonstrated that whereas absolute lymphocyte counts decrease to similarly low levels in survivors and non-survivors at the onset of sepsis, non-survivors' absolute lymphocyte counts remain persistently low while survivors experience lymphocyte recovery. We theorize that the initial fall in circulating lymphocytes at the onset of infection in septic patients reflects two separate processes. Firstly, lymphocytes are recruited out of the peripheral circulation to areas of infection and inflammation. And secondly, sepsis induces a number of stimuli that trigger lymphocyte apoptosis (18). Further, we postulate that the persistent lymphopenia seen in non-survivors is most likely due to ongoing sepsis-induced lymphocyte apoptosis secondary to continued release of pro-apoptotic stimuli during the immunosuppressive phase of sepsis (12,32–34). These findings are especially striking because the optimal host response to infection should be to increase lymphocyte proliferation and thereby augment the number of lymphocyte effector cells.

Another significant finding of our study is that patients with severe persistent lymphopenia had a significantly higher incidence of secondary infections compared to patients whose absolute lymphocyte counts had recovered to normal by day 4, and there was also a strong trend toward increased secondary infections in the moderate persistent lymphopenia group. These results suggest that increased susceptibility to new infections may be contributing to the higher mortality seen in persistently lymphopenic patients.

Similar to prior studies (10), we found no differences in the absolute monocyte counts of septic survivors compared to non-survivors; the median monocyte counts of the entire cohort of septic patients were within the normal range throughout the four-day study period. It is well known, however, that monocytes show severe functional deficits which contribute to the immunosuppressive state in septic patients (34–36). Laboratory procedures designed to identify monocytic defects (such as measurement of monocyte human lymphocyte antigen (HLA)-DR expression or ex vivo lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-_) levels) have successfully been shown to predict survival in septic patients (36–39). These tests have also been used to guide therapy in randomized controlled trials of granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine which enhances monocyte proliferation and phagocytosis, in adult and pediatric septic patients (19, 20). However, these tests are not widely available in typical clinical settings and therefore cannot be routinely used by clinicians to assess the prognosis or immune status of septic patients. In contrast, absolute lymphocyte counts are easily measured in most clinical laboratories and are already frequently obtained in infected patients. It is a routine test that could be used both clinically and in future trials of sepsis therapies to identify the patients at highest risk for immunosuppression and death.

For this study, we chose to focus on day 4 absolute lymphocyte counts based on previously published evidence that indicated that lymphocyte counts on this day would discriminate between survivors and non-survivors (11–13,24). The time point at which septic patients transition from a predominantly pro-inflammatory state to one of hypoinflammation is

unknown, and is likely extremely variable due to host-, pathogen-, and disease-related factors. Therefore, we also analyzed day 3 lymphocyte counts in the subset of patients who had complete blood counts measured on that day to determine whether persistent lymphopenia at an earlier time point could also predict death. We found that day 3 absolute lymphocyte counts were not associated with short- or long-term mortality after accounting for other covariates. Despite this, determining the time at which continued lymphopenia becomes most clinically relevant is difficult to ascertain, and there are likely subsets of patients (for example, elderly patients) in whom the lymphocyte pattern diverges between survivors and non-survivors at an earlier or later time point than seen in this study. Similarly, lymphopenia was defined *a priori* as an absolute lymphocyte count less than 1.2 cells/μL × 10^3 for this study because this was the lower limit of normal specified by the laboratory at our institution. Based on our ROC analysis, however, a day 4 absolute lymphocyte count less than 1.0 cells/μL × 10^3 may be a more accurate discriminatory threshold to optimally predict 28-day mortality. In the future, larger studies, which could stratify patients by age or disease severity, may clarify whether different absolute lymphocyte cutoff values would be more useful in specific subpopulations of septic patients.

By including only culture-positive bacteremic patients, we aimed to limit heterogeneity in the cohort by exclusively studying known septic patients. Patients without positive blood cultures were excluded to avoid including patients incorrectly diagnosed with sepsis based on growth of colonizing bacteria in respiratory, urine, or other cultures. Additionally, we limited our study population to patients whose positive cultures were drawn within five days of admission to the hospital to avoid biasing our study with chronically hospitalized patients who might be more likely to have underlying immunological dysfunction as well as a greater risk of death. To improve generalizability, we did not restrict our population to only patients with severe sepsis or septic shock. Despite this, approximately 93% of our patients were identified as having severe sepsis, suggesting that the patient cohort was fairly homogenous in terms of physiological perturbation. To further validate our results, we performed multivariable analyses to identify predictors of 28-day and 1-year mortality in the subset of patients with septic shock, and these yielded similar odds ratios for absolute lymphocyte count as in the multivariable analyses that included the entire cohort.

This study has several limitations. As a retrospective study, it was prone to limitations inherent in this study design, such as imbalance between the study groups. In contrast to previous studies of lymphopenia in septic patients, though, we attempted to account for major care-related and patient-specific determinants of survival by adjusting for these factors in a multivariable model. In fact, an important aspect of this study was that we included a sufficient number of non-survivors to show that persistent lymphopenia increased the risk of death at 28 days and 1 year even after accounting for other known predictors of mortality such as age, APACHE II score, time until appropriate antibiotic coverage, and co-morbidities. As a retrospective study, data collection was limited to variables that were obtained during the usual clinical care of the patients, so we were unable to correlate persistent lymphopenia with other known biomarkers of sepsis-induced immunosuppression such as decreased monocyte HLA-DR expression, overexpression of PD-1 on T cells, or decreased ex vivo LPS-induced TNF_ levels. Instead, we used the development of

secondary infections as a clinically-relevant surrogate marker of immunosuppression. Going forward, future clinical trials in this area should measure not only clinical outcomes, but biomarkers of immunosuppression to further establish causation.

Another limitation of this study is that the exact time of sepsis onset could not be precisely determined. For the purpose of this study, we defined the onset of sepsis to be the time that the first positive culture was ordered by the treating physician. The majority of included patients, however, had blood cultures drawn in the emergency department, so potential delays in presentation to the hospital further complicate interpretation of timing in this study. Furthermore, we excluded patients who expired or were discharged prior to day 4. While this may limit some of the generalizability of our results, it allowed us to focus on sepsis-induced immunosuppression by eliminating patients experiencing early death (which is typically due to cardiovascular collapse and not immunosuppression) or early clinical recovery.

Finally, the main limitation of this study is that the results do not allow us to conclude whether persistent lymphopenia directly contributes to mortality in septic patients or whether it is simply a marker of disease severity. We are also not able to presume that immune-stimulatory therapy aimed at reversing lymphopenia would alter patient outcomes. We postulate that ongoing lymphocyte apoptosis caused by the continued release of pro-apoptotic stimuli contributes to morbidity and mortality in septic patients by inhibiting clearance of primary infections and increasing susceptibility to secondary infections. This hypothesis is supported by multiple animal studies which have suggested that lymphocyte apoptosis is a key pathogenic mechanism in sepsis and that prevention of lymphocyte apoptosis improves survival (40–43). Still, prospective studies are needed to establish lymphopenia as a causative factor in late mortality in sepsis.

The clinical value of persistent lymphopenia is yet to be elucidated. Because of the enormous overlap in lymphocyte counts among survivors and non-survivors, day 4 absolute lymphocyte count would most likely be useful as a predictor of mortality in groups of patients rather than an accurate predictor of death in any individual patient. More importantly, perhaps, is the potential value of day 4 absolute lymphocyte count in predicting an increased risk of secondary infection and sepsis-induced immunosuppression. Currently, in the clinical setting, a persistently low absolute lymphocyte count in septic patients should prompt clinicians to reevaluate their patients' response to therapy and assess for the presence of new or untreated infections. In the future, sepsis therapies could be tailored to individuals' immunological phenotypes. Potential immunotherapeutic agents, such as interleukin-7 (IL-7) and anti-PD-1 antibody, act to increase CD4 and CD8 T cell production, block lymphocyte apoptosis, and prevent T cell exhaustion. These therapies may be most effective if administered selectively to patients with evidence of lymphocyte dysfunction or loss. Identification of patients with persistent lymphopenia will not only select for those at high risk for short- and long-term mortality, but also for those with the specific immunological derangements that these therapies aim improve.

Acknowledgments

Sources of Funding: Anne Drewry and Brian Fuller were each supported by the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences. Richard Hotchkiss was supported by National Institute of Health grants GM 44118 and GM 55194. Richard Hotchkiss reports receiving grant support from MedImmune, Bristol-Myers Squibb, Agennix, and Aurigene.

The authors thank Karen Steger-May, with the Division of Biostatistics at Washington University in St. Louis, for her assistance with statistical analysis. Drs. Drewry and Fuller each acknowledge this publication was made possible by the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences. Dr. Hotchkiss was supported by National Institutes of Health (NIH) grants GM 44118 and GM 55194.

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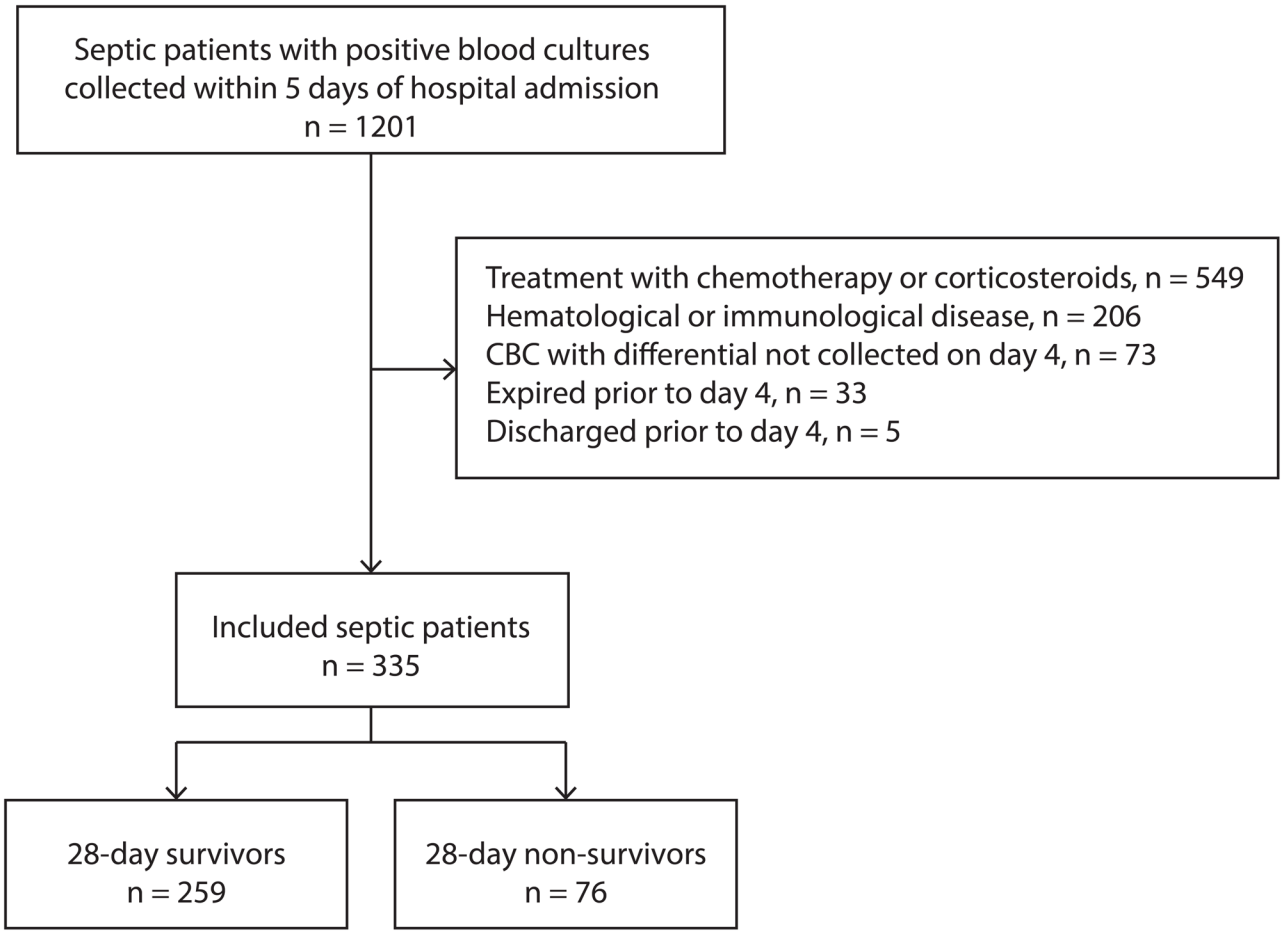

Figure 1. Flowchart of included and excluded patients
CBC, complete blood count.

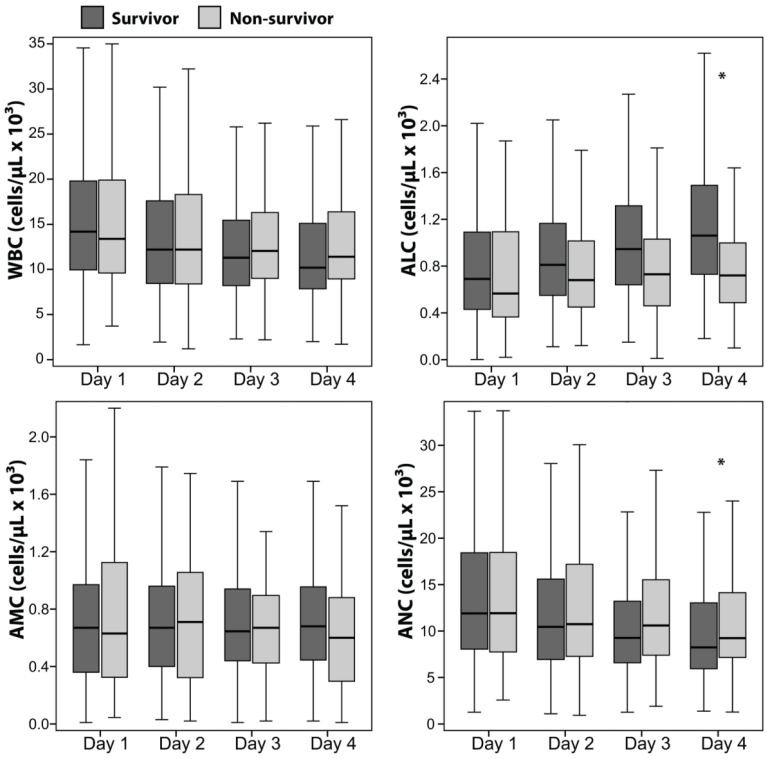

Figure 2. Boxplots of leukocyte counts in survivors and non-survivors during the first four days following sepsis diagnosis

Boxes represent 25th and 75th percentiles. Whiskers represent 5th and 95th percentiles. *$p < .05$ for the comparison of the day 4 counts between survivors and non-survivors and for the overall change in count over the four-day period. WBC, white blood cell count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count.

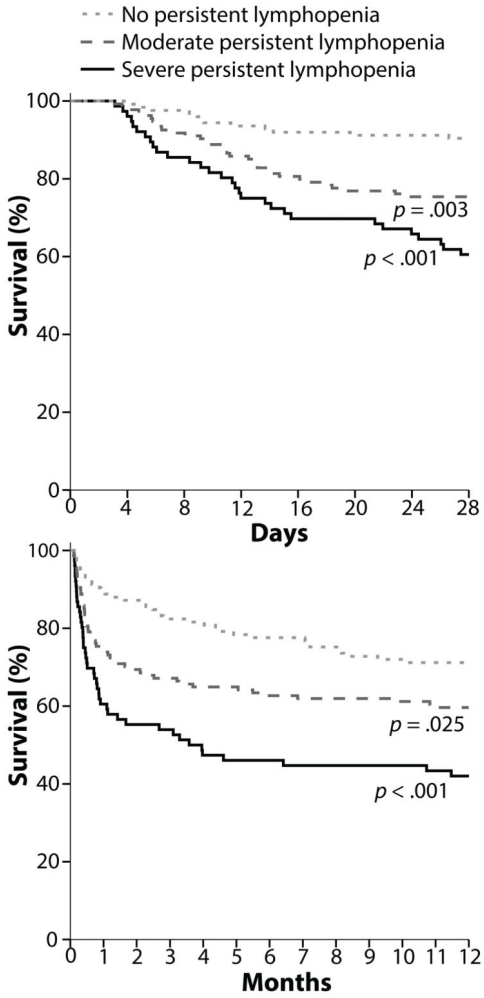

Figure 3. Kaplan-Meier survival analysis according to day 4 absolute lymphocyte counts

Severe persistent lymphopenia includes patients with day 4 absolute lymphocyte counts ≤ 0.6 cells/μL × 10^3; moderate persistent lymphopenia, those with day 4 counts of 0.7 to 1.1 cells/μL × 10^3; no persistent lymphopenia, those with day 4 counts ≥ 1.2 cells/μL × 10^3. P values were generated with log-rank tests comparing each lymphopenic group to the group without persistent lymphopenia.

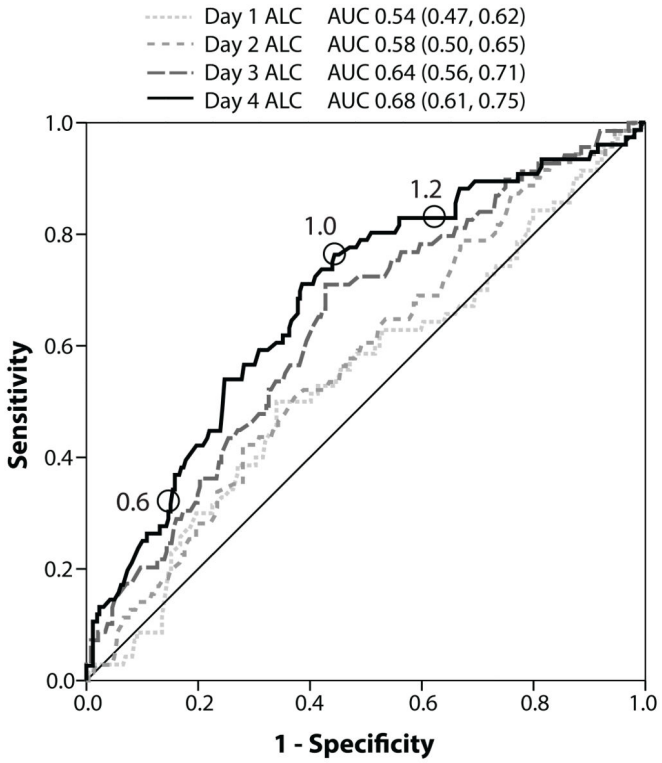

Figure 4. Receiver operator characteristic plot comparing the ability of absolute lymphocyte counts on the first four days after sepsis diagnosis to predict 28-day mortality

Circles on the curve indicate the position of specific absolute lymphocyte counts (in cells/μL × 10^3). Areas under the curve with 95% confidence intervals are displayed above the graph. ALC, absolute lymphocyte count; AUC, area under the curve.

Table 1

Baseline characteristics of survivors and non-survivors

	Survivors n = 259	Non-survivors n = 76	*p*
Age (years), mean (SD)	60.8 (15.3)	66.3 (12.8)	.004
Sex (male), n (%)	146 (56.4)	45 (59.2)	.66
APACHE II, mean (SD)	17.1 (5.5)	22.5 (6.6)	<.001
Source of bacteremia, n (%)			.07
Lung	33 (12.7)	18 (23.7)	
Abdomen	50 (19.3)	14 (18.4)	
Urinary tract	35 (13.5)	8 (10.5)	
Central line	37 (14.3)	9 (11.8)	
Bone or soft tissue	52 (20.1)	7 (9.2)	
Other	52 (20.1)	20 (26.3)	
Organism, n (%)			.11
Gram-positive	135 (52.1)	30 (39.5)	
Gram-negative	99 (38.2)	35 (46.1)	
Mixed	10 (3.9)	2 (2.6)	
Fungal	15 (5.8)	9 (11.8)	
Time to appropriate antibiotic coverage (hours), median (IQR)	3.5 (0.83, 15.4)	11.9 (1.7, 32.5)	.001
Surgical procedure, n (%)	70 (25.4)	7 (10.2)	.01
Co-morbidities, n (%)			
Coronary artery disease	80 (30.9)	29 (38.2)	.23
Cerebrovascular disease	46 (17.8)	15 (19.7)	.70
Congestive heart failure	85 (32.8)	30 (39.5)	.05
Diabetes	101 (39.0)	35 (46.1)	.27
Chronic renal insufficiency	61 (23.6)	30 (39.5)	.006
Liver disease	37 (14.3)	22 (28.9)	.003
COPD	85 (32.8)	33 (43.4)	.09

SD, standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation; IQR, 25%, 75% interquartile range; COPD, chronic obstructive pulmonary disease

Table 2

Multivariable analysis of clinical features associated with 28-day mortality

	Adjusted OR[a] (95% CI)	Wald[b]	*p*
Sepsis (n = 335)			
Age	1.03 (1.00, 1.05)	5.68	.02
APACHE II[c]	1.11 (1.05, 1.17)	14.34	<.001
Surgical procedure	0.32 (0.12, 0.86)	5.15	.02
Liver disease	3.31 (1.62, 6.76)	10.72	.001
Chronic renal insufficiency	1.30 (0.68, 2.50)	0.64	.43
Congestive heart failure	1.54 (0.80, 2.94)	1.71	.19
Time to appropriate antibiotic coverage	1.01 (1.00, 1.02)	6.08	.01
Day 4 ALC	0.68 (0.51, 0.91)	6.81	.009
Septic shock (n = 172)			
Age	1.03 (1.00, 1.06)	2.77	.10
APACHE II[c]	1.14 (1.06, 1.23)	12.28	<.001
Surgical procedure	0.11 (0.02, 0.47)	8.73	.003
Liver disease	4.65 (1.67, 13.00)	8.57	.003
Chronic renal insufficiency	1.42 (0.59, 3.37)	0.62	.43
Congestive heart failure	1.08 (0.46, 2.57)	0.03	.86
Time to appropriate antibiotic coverage	1.03 (1.01, 1.04)	9.56	.002
Day 4 ALC	0.69 (0.48, 0.98)	4.21	.04

[a] The odds ratios for continuous variables (except day 4 absolute lymphocyte count) represent the increased odds of death for every increase in the variable by one unit. For absolute lymphocyte count, the odds ratio represents the increased risk of death for every increase in the count by 0.5 cells/μL × 10^3.

[b] The Wald statistic provides information about the impact of individual predictors.

[c] The age component was removed from the APACHE II score prior to analysis. CI, confidence interval; APACHE, acute physiology and chronic health; ALC, absolute lymphocyte count.

Table 3

Characteristics of patients stratified by day 4 absolute lymphocyte count

	No Persistent Lymphopenia[a] n = 125	Moderate Persistent Lymphopenia[b] n = 134	Severe Persistent Lymphopenia[c] n = 76	*p*
Age (years), mean (SD)	60.7 (14.8)	61.5 (16.1)	65.3 (12.5)	.07
Sex (male), n (%)	70 (56.0)	70 (52.2)	51 (67.1)	.11
APACHE II, mean (SD)	15.7 (5.3)	17.6 (5.9)	18.6 (5.8)	.002
Source of bacteremia, n (%)				.053
Lung	13 (10.4)	22 (16.4)	16 (21.1)	
Abdomen	18 (14.4)	28 (20.9)	18 (23.7)	
Urinary tract	16 (12.8)	17 (12.7)	10 (13.2)	
Central line	24 (19.2)	13 (9.7)	9 (11.8)	
Bone or soft tissue	29 (23.2)	18 (13.4)	12 (15.8)	
Other	25 (20.0)	36 (26.9)	11 (14.5)	
Organism, n (%)				.24
Gram-positive	70 (56.0)	66 (49.3)	29 (38.2)	
Gram-negative	45 (36.0)	52 (38.8)	37 (48.7)	
Mixed	5 (4.0)	4 (3.0)	3 (3.9)	
Fungal	5 (4.0)	12 (9.0)	7 (9.2)	
Time to appropriate antibiotic coverage (hours), median (IQR)	4.8 (0.9, 16.8)	4.3 (1.1, 19.2)	4.9 (0.9, 16.5)	.89
Surgical procedure, n (%)	27 (21.6)	21 (15.7)	11 (14.5)	.33
Co-morbidities, n (%)				
Coronary artery disease	35 (28.0)	44 (32.8)	30 (39.5)	.22
Cerebrovascular disease	25 (20.0)	23 (17.2)	13 (17.1)	.81
Congestive heart failure	39 (31.2)	37 (31.3)	26 (34.2)	.59
Diabetes	62 (49.6)	44 (32.8)	30 (39.5)	.02
Chronic renal insufficiency	25 (20.0)	40 (29.9)	26 (34.2)	.06
Liver disease	15 (12.0)	26 (19.4)	18 (23.7)	.09
COPD	43 (34.4)	42 (31.3)	33 (43.4)	.21
Severe sepsis, n (%)	114 (91.2)	124 (92.5)	73 (96.1)	.42
Septic shock, n (%)	61 (48.8)	64 (47.8)	47 (61.8)	.11
Hospital LOS[d] (days), median (IQR)	10.8 (6.7, 21.9)	12.9 (8.1, 20.3)	14.1 (8.8, 22.0)	.37
ICU LOS[d] (days), median (IQR)	3.1 (1.7, 6.6)	3.0 (2.0, 6.8)	4.4 (2.0, 9.1)	.15

[a] Day 4 absolute lymphocyte count ≥ 1.2 cells/μL × 10^3.

[b] Day 4 absolute lymphocyte count 0.7 to 1.1 cells/μL × 10^3.

[c] Day 4 absolute lymphocyte count ≤ 0.6 cells/μL × 10^3.

[d] Among hospital survivors. SD, standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation; IQR, 25%, 75% interquartile range; COPD, chronic obstructive pulmonary disease; LOS, length of stay; ICU, intensive care unit.

Table 4

Secondary infections

	No Persistent Lymphopenia n = 125	Moderate Persistent Lymphopenia n = 134	Severe Persistent Lymphopenia n = 76
Secondary Infection[a], n (%)	17 (13.6)	27 (20.1)	19 (25.0)
OR (95% CI)[b]	--	1.60 (0.83, 3.11)	2.11 (1.02, 4.39)
p	--	.16	.04
Site of secondary infection, n (%)			
Lung	6 (4.8)	9 (6.7)	7 (10.5)
Urinary tract	6 (4.8)	8 (6.0)	4 (5.3)
Blood	4 (3.2)	6 (4.5)	4 (5.3)
Abdomen	1 (0.8)	3 (2.2)	4 (5.3)
Other	0 (0)	1 (0.7)	0 (0)
Organism, n (%)			
Acinetobacter species	3 (2.4)	1 (0.7)	3 (3.9)
Candida species	4 (3.2)	3 (2.2)	3 (3.9)
Clostridium difficile	0 (0)	2 (1.5)	2 (2.6)
Escherichia coli	3 (2.4)	4 (3.0)	1 (1.3)
Enterococcus species	0 (0)	3 (2.2)	2 (2.6)
Klebsiella pneumoniae	0 (0)	1 (0.7)	1 (1.3)
Pseudomonas species	4 (3.2)	6 (4.5)	3 (3.9)
Serratia marcescens	1 (0.8)	0 (0)	1 (1.3)
Staphylococcus Aureus	0 (0)	3 (2.2)	1 (1.3)
Other *Staphylococcus* species	0 (0)	2 (1.5)	1 (1.3)
Other	2 (1.6)	2 (1.5)	1 (1)

[a] Secondary infections were defined as culture-positive infections identified greater than 48 hours after the primary bacteremia and arising from a secondary source.

[b] The reference category for the ORs was absence of persistent lymphopenia. OR, odds ratio; CI, confidence interval.