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The Presence of Hypothermia within 24 Hours of Sepsis Diagnosis Predicts Persistent Lymphopenia

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Abstract

Objective—To determine whether hypothermia within 24 hours of sepsis diagnosis is associated with development of persistent lymphopenia, a feature of sepsis-induced immunosuppression

Design—Retrospective cohort study

Setting—1200-bed university-affiliated tertiary care hospital

Patients—Adult patients diagnosed with bacteremia and sepsis within 5 days of hospital admission between January 1, 2010 and July 31, 2012

Interventions—None

Measurements and main results—Leukocyte counts were recorded during the first four days following sepsis diagnosis. Persistent lymphopenia was defined as an absolute lymphocyte count less than $1.2 \text{ cells}/\mu\text{l} \times 10^3$ present on the fourth day after diagnosis. Of the 445 septic patients included, 64 (14.4%) developed hypothermia (defined as a body temperature less than 36.0°C) within 24 hours of sepsis diagnosis. Hypothermia was a significant independent predictor of persistent lymphopenia (adjusted OR 2.70 [95% CI 1.10, 6.60], $p = .03$) after accounting for age, disease severity, comorbidities, source of bacteremia, and type of organism. Compared to the non-hypothermic patients, hypothermic patients had higher 28-day (50.0% vs. 24.9%, $p < .001$) and 1-year mortality (60.9% vs. 47.0%, $p = .001$).

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Conflicts of Interest

Anne Drewry, Brian Fuller, and Lee Skrupky have no competing interests to declare.

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Conclusions—Hypothermia is associated with higher mortality and an increased risk of persistent lymphopenia in septic patients, and it may be an early clinical predictor of sepsis-induced immunosuppression.

Keywords

sepsis; hypothermia; fever; body temperature; lymphopenia; immunosuppression

Introduction

Fever is considered to be an adaptive response to infection, and *in vitro* and animal studies have shown that elevated temperatures augment several aspects of humoral and cellular immunity (1). Yet, 20% of septic patients present to the hospital with hypothermia rather than fever. These patients have twice the mortality of febrile patients even after accounting for factors such as age, disease severity, and comorbidities (2). Although there is limited data to explain why these patients have worse outcomes, clear evidence links body temperature to infection risk. Clinical studies have demonstrated increased rates of surgical wound infections in hypothermic perioperative patients, and a recent meta-analysis showed an association between therapeutic hypothermia and sepsis in post-cardiac arrest patients (3,4). Additionally, a trial of systemic hypothermia in neonates with hypoxic ischemic encephalopathy found persistently depressed levels of total leukocytes and lymphocytes in patients who were cooled (5).

To date, most clinical investigations of the effect of hypothermia on immune function have been limited to patients undergoing induced therapeutic hypothermia rather than in septic patients presenting with spontaneous hypothermia. Only two previous studies have attempted to assess the immune status of hypothermic septic patients. Marik et al found no differences in circulating levels of IL-6, TNF α , or soluble TNF α receptors between hypothermic and febrile septic patients, while Arons et al observed increased plasma levels of IL-6 and TNF α as well as increased urinary excretion of cyclooxygenase-derived lipid mediators in hypothermic patients, suggesting a dysregulated inflammatory response (6,7). It is unknown whether hypothermic patients exhibit immune dysfunction that is not apparent with quantitative cytokine measurements.

Sepsis activates both pro- and anti-inflammatory mechanisms and can lead to extended periods of immunosuppression (8). One feature of sepsis-induced immunosuppression is apoptotic loss of immune cells, including T and B cells. Persistent lymphopenia has been associated with increased risks of mortality and nosocomial infection (9,10). Although patients treated with induced therapeutic hypothermia have been shown to develop lymphopenia (5), investigation of lymphopenia as a potential link between spontaneous hypothermia and increased mortality in septic patients has not been performed. Thus, the objective of this study was to examine the relationship between hypothermia and persistent lymphopenia in septic patients. We hypothesized that patients who presented with hypothermia within 24 hours of sepsis diagnosis would be more likely to develop persistent lymphopenia than non-hypothermic patients.

Materials and methods

Study design, setting, and population

This was a *post-hoc* analysis of a retrospective cohort study conducted at a 1,200-bed university-affiliated hospital between January 1, 2010 and July 31, 2012. It was approved by the Human Research Protection Office at our institution with waiver of informed consent. All patients with positive blood cultures drawn within five days of admission to the hospital and a diagnosis of sepsis were eligible for inclusion. Sepsis was diagnosed by the presence of at least two systemic inflammatory response syndrome (SIRS) criteria within 24 hours of the time the positive culture was collected (11). Exclusion criteria included: diagnosis of immunological disease or treatment with immunosuppressant medication within 6 months prior to or during the hospitalization (see Supplemental Table 1, Supplemental Digital Content 1, which lists of the specific immunosuppressive medications and immunological diseases that led to exclusion).

Patients were divided into two cohorts, hypothermic or non-hypothermic, based on their lowest body temperature within 24 hours of sepsis diagnosis (identified by the time of their first positive blood culture). Hypothermia was defined as a temperature less than 36.0°C, consistent with the definition of hypothermia in the SIRS criteria (11).

Data collection

Baseline demographics, daily leukocyte counts, and clinical outcomes were collected by a research assistant blinded to the patients' temperatures. The first 24-hour 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 in a 24-hour period, the mean value was reported. Lymphopenia was defined as an absolute lymphocyte count less than $1.2 \text{ cells}/\mu\text{L} \times 10^3$, which is the lower limit of normal at our institution.

The primary outcome was development of persistent lymphopenia, defined as lymphopenia present on day 4 following sepsis diagnosis. Day 4 was chosen based on previous work from our group demonstrating that septic patients with lymphopenia persisting to day 4 after sepsis diagnosis had increased mortality compared to those whose absolute lymphocyte counts recovered to normal (9). Secondary outcomes included 28-day mortality, 1-year mortality, intensive care unit (ICU) length of stay (LOS), and hospital LOS.

Statistical analysis

Descriptive statistics, including mean (standard deviation) and median (interquartile range), were used to describe the patient cohorts. Normality was assessed using histograms and the Kolmogorov-Smirnov Test. Baseline characteristics were compared using independent-samples t-tests (for normally distributed data), Mann-Whitney U tests (for non-normally distributed data), or chi square tests (for categorical data).

Kaplan-Meier survival curves were compared with log-rank tests to assess the relationship between hypothermia and 28-day and 1-year mortality. To determine the independent effect of hypothermia on the development of persistent lymphopenia after accounting for multiple

confounders, multivariable logistic regression was used to model the odds of lymphopenia on day 4. Hypothermia, age, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and the presence of at least one comorbidity were chosen *a priori* to be included in the model to control for underlying characteristics which might predispose patients to persistent lymphopenia. Other independent variables were included in the multivariable model if significant at a *p* value of .05 during univariable comparisons of baseline characteristics in patients with and without persistent lymphopenia. Collinearity diagnostics, including variance inflation factors, tolerance statistics, and variance proportions, were evaluated to ensure variable independence.

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 445 patients met the inclusion and exclusion criteria during the study period. Sixty-four patients were included in the hypothermic group and 381 patients in the non-hypothermic group. In the hypothermic and non-hypothermic groups respectively, 23 (35.9%) and 87 (22.8%) patients died or were discharged prior to day 4 or did not have complete blood counts sampled on day 4 (detailed in Supplemental Figure 1, Supplemental Digital Content 2). This resulted in 41 patients in the hypothermic group and 294 patients in the non-hypothermic group who were available for analysis of persistent lymphopenia.

Table 1 reports baseline characteristics and outcomes of the two groups. Hypothermic patients had higher APACHE II scores (median 20.5 [IQR 15.0, 27.8] vs. 16.0 [13.0, 20.0], *p* < .001) and a higher incidence of a pulmonary source of bacteremia (29.7% vs. 14.7%, *p* = .03). Gram-negative infections occurred in 51.6% of hypothermic patients versus 34.6% of non-hypothermic patients (*p* = .053).

The median absolute lymphocyte count on day 1 was similar between the hypothermic and non-hypothermic groups (0.71 [IQR 0.48, 1.14] vs. 0.66 [0.42, 1.09], *p* = .52). By day 4, the median absolute lymphocyte count was significantly lower in the hypothermic group (0.82 [IQR 0.64, 0.97] vs. 1.02 [0.68, 1.45], *p* = .02), and a greater percentage of the hypothermic patients had developed persistent lymphopenia (82.9% vs. 59.9%, *p* = .004) (Table 1). Hypothermic patients were also more likely to die by day 28 (50.0% vs. 24.9%, *p* < .001) and 1 year (60.9% vs. 47.0%, *p* = .001) (Kaplan-Meier curves shown in Supplemental Figure 2, Supplemental Digital Content 3). No differences in ICU and hospital LOS were observed between the two groups (Table 1).

Comparisons of baseline characteristics in patients who did and did not develop persistent lymphopenia are shown in Supplemental Table 2 (Supplemental Digital Content 4). Incidence of hypothermia, APACHE II score, source of bacteremia, and type of organism were significantly different between these two groups. The multivariable analysis to model the odds of developing persistent lymphopenia is shown in Table 2. Significant predictors of persistent lymphopenia included hypothermia (adjusted OR 2.70 [95% CI 1.10, 6.60], *p* = .03) and APACHE II score (adjusted OR 1.07 [95% CI 1.02, 1.13], *p* = .006). A catheter-

related source of bacteremia was protective (adjusted OR 0.33 [95% CI 0.13, 0.83], reference pulmonary source, $p = .02$).

Discussion

Persistent lymphopenia is a feature of sepsis-induced immunosuppression and a predictor of mortality in septic patients (9). This study demonstrates that hypothermic septic patients are more likely to develop persistent lymphopenia. It also confirms the results of previous studies associating hypothermia with short-term mortality (2,12) and extends the data further by showing an increased risk of death at one year. These findings have prognostic and therapeutic implications and suggest areas for further study.

Sepsis-induced immunosuppression contributes to mortality in septic patients by inhibiting pathogen clearance and increasing susceptibility to nosocomial infections (3). Recent developments in immunostimulatory therapy suggest that these treatments have potential to improve clinical outcomes in septic patients when specifically targeted to patients with quantitative evidence of immune dysfunction (13,14). However, determining which patients are most likely to be immunosuppressed and optimizing timing of these treatments remains a challenge. Identifying early clinical predictors of sepsis-induced immunosuppression, such as hypothermia, could assist physicians in selecting the most appropriate candidates for additional immunological testing in future trials or clinical applications of immunostimulatory therapy.

This study has several limitations. First, a persistently low absolute lymphocyte count is just one feature of sepsis-induced immunosuppression. Sepsis-induced immunosuppression is characterized by numerous alterations in both the adaptive and innate immune systems including increased immune cell apoptosis, CD4 and CD8 T cell exhaustion, shift from a T helper 1 (T_H1) to T_H2 phenotype, expansion of the regulatory T cell population, decreased HLA-DR expression on antigen presenting cells, impaired phagocytosis, and altered cytokine secretion (3). As a retrospective analysis, our evaluation of the association between hypothermia and sepsis-induced immunosuppression was restricted to variables acquired during routine clinical care. We could not correlate hypothermia to other markers of immune dysfunction, such as decreased monocyte human lymphocyte antigen (HLA)-DR expression or ex vivo lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF α) levels. A future prospective study will provide insight into the association between hypothermia and these other measures of sepsis-induced immunosuppression.

Second, we cannot demonstrate causality with this retrospective analysis. Consistent with previous studies (2), the hypothermic patients in our study were sicker than the non-hypothermic patients, with significantly higher APACHE II scores. Thus, it is possible that the increased incidence of persistent lymphopenia in this population was due to increased disease severity. We attempted to control for this by performing a multivariable analysis that included potential confounders, such as age, APACHE II score, comorbidities and source of sepsis, as additional independent variables.

Third, methods of body temperature measurement were not standardized and not recorded in the electronic medical record from which our data was extracted. Given that most of the patients were diagnosed with sepsis in the emergency department where the usual method of temperature measurement is oral or axillary, we presume that most temperature measurements included in this study were peripheral rather than core. Since peripheral temperatures tend to be slightly lower than core, some patients with borderline hypothermia may have been misallocated to the hypothermic group. However, we were conservative in our definition of hypothermia, choosing a cutoff value of 36.0°C rather than 36.5°C which has used in other recent studies (2,12), so we feel our conclusions are sound.

Finally, this study does not address the impact of warming hypothermic septic patients on clinical outcomes or immune function. All the hypothermic patients included in this study were warmed to normothermia according to institutional protocol. Further optimal management of these patients is yet to be elucidated.

Conclusions

Hypothermia within 24 hours of presentation predicts persistent lymphopenia and is associated with increased short- and long-term mortality. Hypothermia may be a useful prognostic factor to identify patients at highest risk for sepsis-induced immunosuppression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Comparison of baseline characteristics and outcomes of hypothermic and non-hypothermic patients

	Hypothermic n = 64	Non-hypothermic n = 381	<i>p</i>
Baseline characteristics			
Age (years), mean (SD)	64.6 (16.9)	61.5 (15.2)	.20 ^a
Sex (male), n (%)	36 (56.3)	219 (57.5)	.85 ^b
APACHE II, median (IQR)	20.5 (15.0, 27.8)	16.0 (13.0, 20.0)	<.001 ^c
Source of bacteremia, n (%)			.03 ^b
Lung	19 (29.7)	56 (14.7)	
Abdomen	14 (21.9)	74 (19.4)	
Urinary tract	8 (12.5)	53 (13.9)	
Central line	3 (4.7)	52 (13.6)	
Bone or soft tissue	10 (15.6)	65 (17.1)	
Other/unknown	10 (15.6)	81 (21.3)	
Organism, n (%)			.053 ^b
Gram-positive	23 (35.9)	178 (46.7)	
Gram-negative	33 (51.6)	132 (34.6)	
Fungal	1 (1.6)	22 (5.8)	
Polymicrobial	7 (10.9)	49 (12.9)	
Hospital-acquired bacteremia, n (%)	9 (14.1)	53 (13.9)	.97 ^b
Time to appropriate antibiotic coverage (hours), median (IQR)	4.0 (1.4, 17.5)	4.3 (0.9, 16.6)	.92 ^c
Comorbidities, n (%)			
Coronary artery disease	23 (35.9)	115 (30.2)	.36 ^b
Cerebrovascular disease	15 (23.4)	61 (16.0)	.14 ^b
Congestive heart failure	15 (23.4)	110 (28.9)	.37 ^b
Diabetes	22 (34.4)	149 (39.1)	.24 ^b
Chronic renal insufficiency	20 (31.3)	93 (24.4)	.47 ^b
Liver disease	13 (20.3)	64 (16.8)	.49 ^b
COPD	21 (32.8)	122 (32.0)	.90 ^b
Temperature data			
Lowest body temperature within 24 hours of sepsis diagnosis (°C), median (IQR)	35.5 (34.4, 35.8)	36.5 (36.2, 36.7)	<.001 ^c
Highest body temperature within 24 hours of sepsis diagnosis (°C), median (IQR)	37.1 (36.6, 38.2)	38.3 (37.5, 39.2)	<.001 ^c
Outcomes			
Persistent lymphopenia ^{d,e} , n (%)	34 (82.9)	176 (59.9)	.004 ^b
28-day mortality, n (%)	32 (50.0)	95 (24.9)	<.001 ^f
1-year mortality, n (%)	39 (60.9)	179 (47.0)	.001 ^f
ICU length of stay ^g (days), median (IQR)	3.7 (1.9, 6.4)	3.1 (1.8, 6.9)	.84 ^c
Hospital length of stay ^g (days), median (IQR)	14.1 (8.2, 21.0)	11.0 (6.7, 20.7)	.44 ^c

^aIndependent-samples t test.

^bChi square test.

^cMann-Whitney U test.

^dAbsolute lymphocyte count less than $1.2 \text{ cells}/\mu\text{L} \times 10^3$ on the fourth day after sepsis diagnosis.

^ePercentage based on total number of patients available for evaluation on day 4 (hypothermic, n = 41; non-hypothermic, n = 294).

^fLog-rank test.

^gIncludes only patients who survived to ICU or hospital discharge.

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

Table 2Multivariable logistic regression analysis for persistent lymphopenia^a (n = 335)

	Adjusted OR ^b (95% CI)	<i>p</i>
Hypothermia ^c	2.70 (1.10, 6.60)	.03
Age	1.01 (0.99, 1.02)	.49
APACHE II ^d	1.07 (1.02, 1.13)	.006
Comorbidity ^e	1.05 (0.56, 1.99)	.87
Organism		
Gram-positive	Reference	--
Gram-negative	1.25 (0.67, 2.33)	.48
Fungal	2.96 (0.85, 10.23)	.09
Polymicrobial	0.46 (0.23, 1.06)	.07
Source of bacteremia		
Pulmonary	Reference	--
Intra-abdominal	0.95 (0.39, 2.28)	.90
Urinary tract	0.49 (0.19, 1.28)	.15
Catheter-related	0.33 (0.13, 0.83)	.02
Bone or soft tissue	0.43 (0.18, 1.02)	.06
Other/unknown	0.77 (0.33, 1.82)	.55

^a Absolute lymphocyte count less than 1.2 cells/ μ L $\times 10^3$ on the fourth day after sepsis diagnosis.^b For continuous variables, odds ratios reflect the increased odds of developing persistent lymphopenia for a one unit increase in the variable. For categorical variables, the reference category is absence of the condition, except where otherwise specified.^c Body temperature less than 36.0°C within 24 hours of sepsis diagnosis.^d Temperature and age components removed.^e At least one comorbidity present.

OR, odds ratio; CI, confidence interval; APACHE, Acute Physiology and Chronic Health Evaluation