Is blood lymphocyte count a prognostic biomarker in *Staphylococcus aureus* bacteremia?

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ABSTRACT

Lymphopenia is common in patients with sepsis and associated with mortality. Immune-stimulatory therapies likely to restore T-cells count and function are under investigation in sepsis. Our study aimed to assess whether lymphopenia is a reliable prognostic biomarker in *Staphylococcus aureus* bacteremia. We conducted an ancillary study of the prospective VIRSTA Study including 574 patients with *S. aureus* bacteremia in two tertiary care centers. Neither lymphocyte count at the onset nor lymphocyte change during the first 4 days was associated with 12-week mortality. These results highlight the importance of characterizing the immune profile of patients with sepsis according to the cause before investigating immunostimulatory therapies to restore lymphocyte proliferation and function.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lymphopenia is common in patients with sepsis and associated with mortality.
⇒ Immune-stimulatory therapies likely to restore T-cells count and function are under investigation in sepsis.
⇒ Staphylococcus aureus bacteremia is a leading cause of sepsis, but whether lymphopenia is associated with a worse outcome is unknown.

WHAT THIS STUDY ADDS

⇒ S. aureus bacteremia was associated with a 25% case fatality at 12 weeks in our study.
⇒ Neither lymphocyte count at the onset, nor lymphocyte change during the first 4 days, was associated with 12-week mortality in S. aureus bacteremia.

HOW THIS STUDY MIGHT AFFECT CLINICAL RESEARCH AND PRACTICE

⇒ Lymphopenia is not a reliable prognostic biomarker in all causes of sepsis.
⇒ Heterogeneity of immune responses in patients with sepsis is thought to be one of the reasons behind the failure of immune-modulatory trials.
⇒ It is important to identify subgroups of patients with sepsis who are more likely to benefit from lymphocyte immune-stimulatory agents.

INTRODUCTION

Lymphopenia is common in patients with sepsis and associated with mortality.[1] Immune-stimulatory therapies likely to restore T-cells count and function are under investigation in patients with sepsis.[1 2] However, whether lymphopenia is a prognostic biomarker in all causes of sepsis, and whether it could be used to guide such immune-stimulatory therapies, must be investigated. Hence, we previously showed that cytopenia was associated with a poor outcome in pneumococcal bacteremic pneumonia.[3] Considering that *Staphylococcus aureus* bacteremia (SAB) is a leading cause of sepsis and associated with high mortality,[4] our study aimed to assess whether lymphopenia is a reliable prognostic biomarker in SAB.

METHODS

This is an ancillary study of the observational prospective cohort VIRSTA Study[4] including data from two French tertiary care centers (Dijon, Besançon). Consecutive patients were included if they had at least one positive blood culture specimen for *S. aureus* between April 2009 and October 2011. Exclusion criteria were age <18 years, pregnancy and adults under guardianship.[4] The VIRSTA Study is registered in the European Clinical Trials Database under the number: 2008-A00680-55. Standardized electronic case report forms were prospectively filled locally. An immunodepression was considered if one of these criteria was present: HIV infection with CD4 <500/mm^3, solid cancer, leukemia, lymphoma or immunosuppressive drug. Severe sepsis was defined by major organ dysfunction, or blood pressure <90 mm Hg or signs of hypoperfusion (confusion, oliguria, skin mottling, lactate elevation, metabolic acidosis), and septic shock by a severe sepsis requiring the use of vasopressive agents. White cell counts were retrospectively recorded the same day (or the day after if unavailable; D0–1) of the first positive blood culture for *S. aureus*, and 3 days later (or 4 if

Table 1 Characteristics of 574 patients with *Staphylococcus aureus* bacteremia (SAB), according to 12-week mortality

	Survivors	Non-survivors	
	429	145	P value
Demographics			
Age (y), median (IQR)	68 (56–79)	80 (72–86)	<0.001
Sex, male n (%)	169 (39)	63 (43)	0.434
Comorbidities			
Chronic lung disease, n (%)	81 (61)	52 (39)	<0.001
Chronic heart failure, n (%)	140 (33)	92 (63)	<0.001
Chronic renal insufficiency, n (%)	127 (30)	70 (48)	<0.001
Chronic liver disease, n (%)			
Diabetes mellitus, n (%)	120 (28)	39 (27)	0.101
Malignancy, n (%)	131 (31)	43 (30)	0.917
Immunodepression, n (%)	160 (37)	49 (34)	0.485
McCabe score, median (IQR)	3 (3–3)	3 (3–4)	<0.001
Presumed place of acquisition			
Nosocomial infection, n (%)	213 (50)	74 (51)	0.848
Presumed source of SAB			
Skin, n (%)	98 (23)	21 (14)	0.033
Vascular, n (%)	124 (29)	25 (17)	0.006
Surgery, n (%)	77 (18)	21 (14)	0.373
Urinary tract, n (%)	18 (4)	5 (3)	0.810
Other, n (%)	37 (9)	14 (10)	0.736
Unidentified, n (%)	74 (17)	58 (40)	<0.001
Clinical signs			
Temperature ≤38°C, n (%)	68 (16)	36 (25)	0.018
Cardiac insufficiency, n (%)	21 (5)	25 (17)	<0.001
Complicated bacteremia			
Endocarditis (certain or probable), n (%)	179 (42)	68 (47)	0.287
Severe sepsis, n (%)	53 (17)	45 (46)	<0.001
Septic shock, n (%)	17 (6)	35 (36)	<0.001
Origin of infection			
Osteoarticular, n (%)	77 (18)	14 (10)	0.018
Prosthetic infection, n (%)	30 (7)	4 (3)	0.068
Pneumonia, n (%)	21 (5)	21 (15)	<0.001
Central nervous system, n (%)	16 (4)	15 (10)	0.005
Other complication, n (%)	65 (15)	29 (20)	0.194
Biological findings			
C reactive protein (mg/dL), median (IQR) (421/139)	199 (132–289)	244 (162–342)	0.008
Procalcitonin (µg/L), median (IQR) (174/92)	2.33 (0.78–13.2)	8.11 (1.76–29.1)	<0.001
Hemoglobin (g/L), median (IQR) (336/113)	110 (96–123)	105 (90–120)	0.126
Leukocytes ($\times 10^9$/L), median (IQR) (336/113)	11.1 (7.7–16.0)	13.5 (8.3–19.1)	0.011
Neutrophils ($\times 10^9$/L), median (IQR) (247/86)	9.1 (5.7–13.7)	11.6 (6.8–15.9)	0.009
Eosinophils ($\times 10^9$/L) (247/86)	0 (0–100)	0 (0–30)	0.019
Lymphocytes ($\times 10^9$/L), median (IQR) (247/86)	750 (420–1180)	690 (300–1030)	0.132
Monocytes ($\times 10^9$/L), median (IQR) (247/86)	630 (370–970)	590 (330–900)	0.340
Platelets ($\times 10^9$/L, median (IQR) (336/113)	231 (154–317)	205 (130–292)	0.037
Microbiological findings			
Methicillin susceptibility, n (%)	343 (80)	106 (73)	0.079
Treatment			
Intensive care unit admission, n (%)	98 (23)	52 (36)	0.003
Antibiotic therapy duration, median (IQR)	22 (13–48)	7 (3–14)	<0.001
Mechanical ventilation, n (%)	69 (26)	32 (22)	0.103
Outcomes			
Persistent severe sepsis at 48 h, n (%)	41 (10)	42 (29)	<0.001
Persistent septic shock at 48 h, n (%)	17 (4)	37 (26)	<0.001

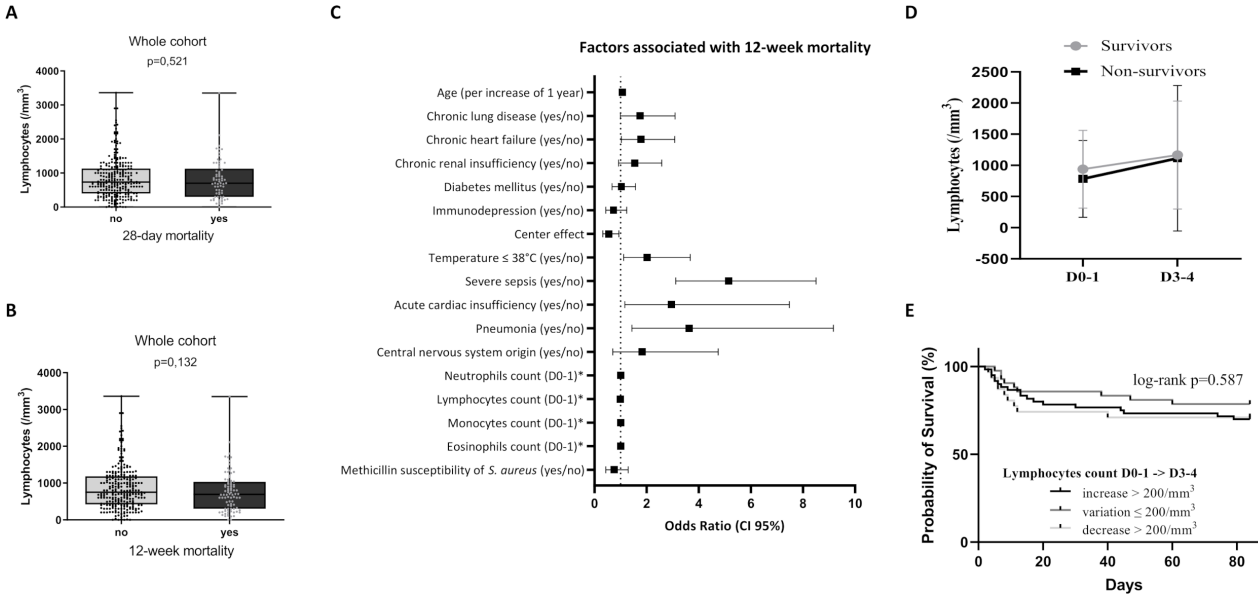

Figure 1 Lymphocyte count in patients with *Staphylococcus aureus* bacteremia. Lymphocyte count according to 28-day mortality (A) and 12-week mortality (B) in the whole cohort of patients with *S. aureus* bacteremia. Multivariable analysis of factors associated with 12-week mortality. For each variable included in the model, OR and 95% CIs are represented; multiple imputation was used for missing data in lymphocytes, neutrophils, monocytes and eosinophils count (n=241 patients with missing data, n=561 patients were considered in the analysis after imputation) (C). Lymphocyte count at D0–1 and D3–4 according to 12-week mortality, represented with the mean (SD) and connecting lines (D). Kaplan-Meier survival analysis comparing patients with *S. aureus* bacteremia and serial measurement of lymphocytes according to the variation in the lymphocyte count between D0–1 and D3–4 (increase >200/mm^3, variation <200/mm^3, decrease >200/mm^3) (E); (D,E: n=133 patients with lymphocytes data available at the two time points).

unavailable; D3–4). The main and secondary endpoints were, respectively, 12-week and 28-day mortality. For statistical analyses, continuous variables were compared with the Mann-Whitney test and categorical variables with the X^2 or Fisher's exact tests. Lymphocyte counts were presented using boxplots according to 12-week or 28-day mortality, in the whole cohort and after excluding immunocompromised patients. We used multivariable logistic regression to identify potential prognostic variables and including those with a p value of <0.20 in bivariate analysis, white cell count and immunodepression status. Multiple imputation by chained equations was used, with 30 imputations, for missing white cell counts. The distribution of imputed and observed data was compared and convergence of the imputation model was assessed. A p value lower than 0.05 was considered statistically significant. All analyses were performed using Stata V.13.1 or Prism V.8.0 software.

RESULTS

Out of the 574 patients included, 119 died before 28 days and 145 died before 12 weeks (case fatality 21% and 25%, respectively). Patient characteristics are shown in table 1. The lymphocyte count at the time of the first positive blood culture was not significantly different between 12-week survivors and non-survivors (median (IQR) 750 (420–1180) vs 690 (300–1033)/mm^3; p=0.132) and 28-day survivors and non-survivors (735 (400–1130) vs 700 (300–1125)/mm^3; p=0.521) (figure 1A,B). In the sensitivity analysis considering the 365 immunocompetent patients, the lymphocyte count was significantly higher in 12-week survivors compared with non-survivors (935 (600–1293) vs 700 (300–1020)/mm^3; p=0.007), but it was not significantly different for 28-day mortality (online supplemental figure 1A,B). In the multivariable analysis, age, chronic heart failure, center effect, temperature ≤38°C, severe sepsis, acute cardiac insufficiency and pneumonia were independently associated with 12-week mortality, but the lymphocyte count was not (figure 1C, online supplemental table 1). In addition, there was no significant difference in the overall change in the lymphocyte count over the 4-day period between 12-week survivors and non-survivors (figure 1D) and no significant difference in survival between the three groups according to the variation in lymphocyte count between D0–1 and D3–4 (increase >200/mm^3, variation ≤200/mm^3 and decrease >200/mm^3) (figure 1E).

DISCUSSION

In patients with sepsis, it is crucial to obtain immune phenotype before investigating immune-modulatory agents such as adjunctive therapies with antibiotics. While the heterogeneity of immune responses in patients with sepsis is thought to be one of the biggest reasons behind the failure of immune-modulatory trials,[5] the source of sepsis and microbiological etiology are two main factors that could be taken into account to capture this heterogeneity. Here, we focused on SAB, a leading cause of sepsis and associated with a 25% case fatality at 12 weeks in our study.

Several immunostimulatory agents likely to restore lymphocyte proliferation and function are currently being investigated in sepsis. For instance, interleukin 7 (CYT107) was found to be effective in reversing profound sepsis-induced lymphopenia in a randomized double-blind

placebo-controlled phase IIb trial in patients with septic shock and severe lymphopenia,[2] a well-known biomarker of sepsis-induced immune suppression. Lymphopenia at the onset of sepsis has been associated with poor outcomes in some studies[6] but not in others.[7] However, persistent lymphopenia on the fourth day following admission for sepsis was found to predict mortality.[7] In patients with SAB, we showed that neither lymphocyte count at the onset of sepsis nor lymphocyte change between D0–1 and D3–4 was associated with 12-week or 28-day mortality.

Several hypotheses can be put forward to explain our findings. First, factors related to bacterial virulence may account for such differences. For example, while neutropenia is frequently observed in severe forms of invasive pneumococcal diseases,[3] it is rarely observed in SAB, except in *S. aureus*-necrotizing pneumonia related to Panton-Valentin leukocidin expression.[8] The source of sepsis could thus account for the varying outcomes, since lymphopenia was independently associated with mortality in community-acquired pneumonia.[9] However, we observed no significant difference according to the origin of the infection (for instance, osteoarticular, lung, central nervous system, or endocarditis) (data not shown). Finally, immunosuppression-associated comorbidities could also explain the discrepancies. Accordingly, after we excluded immunocompromised patients, the lymphocyte count was associated with 12-week mortality. However, the lymphocyte count was not associated with 12-week mortality after adjusting on immunodepression status. Another remaining question is whether lymphocyte depletion is merely an epiphenomenon or whether it plays a central role in the lethality of sepsis.

Our study has several limitations. First, missing values of white cell count may have biased the results, even if multiple imputation was used. There was also a center effect, as shown in the multivariable analysis. Finally, even though we included 574 patients with SAB, our analysis may lack power.

In conclusion, unlike in patients with other types of sepsis, neither lymphocyte count at the onset nor lymphocyte change during the first 4 days was associated with a poor outcome in patients with SAB. These data underscore the importance of identifying subgroups of patients with sepsis who are more likely to benefit from lymphocyte immune-stimulatory agents.

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