

RESEARCH

Open Access



CD19⁺CD24^{hi}CD38^{hi} regulatory B cells deficiency revealed severity and poor prognosis in patients with sepsis

Chunmei Wang^{1,2†}, Huihui Xu^{3,4†}, Rui Gao⁵, Fengying Leng², Fangjie Huo⁶, Yinzhen Li^{2,7}, Siting Liu², Mingzheng Xu^{2*} and Jianwen Bai^{1,2*}

Abstract

Background: Sepsis still remains a major challenge in intensive care medicine with unacceptably high mortality among patients with septic shock. Due to current limitations of human CD19⁺CD24^{hi}CD38^{hi} Breg cells (Bregs) studies among sepsis, here, we tried to evaluate Bregs in severity and prognostic value in patients with sepsis.

Methods: Peripheral blood from 58 patients with sepsis and 22 healthy controls was analyzed using flow cytometry to evaluate the frequency and number of Bregs. All cases were divided into non-survived or survived group after 28 days followed up. Spearman's correlation analysis was performed on Bregs frequency and clinical indices. The area under the curve was acquired using the receiver operating characteristic analysis to assess the sensitivity and specificity of Bregs for outcome of sepsis. Survival curve analysis and binary logistic regression were applied to estimate the value of Bregs in prognosis among cases with sepsis.

Results: Sepsis patients had decreased proportions and number of Bregs. Sepsis patients with low frequency of Bregs were associated with an increased risk of septic shock. Bregs frequency is inversely associated with lactate, SOFA, and APACHE II and positively correlated with Tregs frequency. Low levels of Bregs closely correlated with septic outcomes. Numbers of Bregs were prediction factors for poor prognosis.

Conclusions: Frequency and number of Bregs decreased, and Bregs deficiency revealed poor prognosis in patients with sepsis.

Keywords: Sepsis, Flow cytometry, B-lymphocytes, Interleukin-10, Regulatory B cells, Prognosis

Introduction

Sepsis is defined clinically as the presence of acute syndrome of infection with the development of new organ

dysfunction, an increase of 2 points in the Sequential (Sepsis Related) Organ Failure Assessment (SOFA) [1]. Sepsis remains one of the major challenges in intensive care medicine, its incidence increasing continuously over the past decades [2, 3]. Despite massive efforts in sepsis research, in-hospital mortality (20.3%) is broadly more common among patients with hospital-onset sepsis than those (18.3%) with community-onset sepsis [4, 5], mortality in patients with septic shock still remains unacceptably high [3]. Therefore, emphasis has been focused on identifying patients who are at a higher risk of developing septic shock, early treatment, and early prognosis

[†]Chunmei Wang and Huihui Xu have contributed equally to this work and share first authorship

*Correspondence: xzm2878@126.com; baijianwen1019@163.com

¹ Department of Emergency Medicine and Critical Care, Shanghai East Hospital, Nanjing Medical University, Nanjing 211166, Jiangsu Province, China

² Department of Emergency Medicine and Critical Care, Shanghai East Hospital, Tongji University School of Medicine, 150 Jimo Road, Shanghai 200120, China

Full list of author information is available at the end of the article



assessment. Research in sepsis has shown that the pathology of sepsis results not only from the presence of an infection, but also from the dysregulated host response to an infection [6]. Recent accumulating evidence depicts that sepsis is characterized by an initial overwhelming production of proinflammatory cytokines followed by immunosuppression, with increase of the regulatory T cells (Tregs) population [7–9]. Interleukin-10–producing B cells are a newly described subset of B cells with regulatory function. Mizoguchi and collaborators identified regulatory B cells (Bregs) as an IL-10–producing B-cell subpopulation and introduced the term “regulatory B cells [10].” In humans, Blair and coworkers described Bregs as CD19⁺CD24^{hi}CD38^{hi}, a phenotype that generally defines human transitional B cells [11, 12]. Since these seminal observations, a considerable body of evidence of CD19⁺CD24^{hi}CD38^{hi} has been shown to prevent tissue injury, autoimmunity, chronic graft-versus-host disease (cGVHD), infection, and cancer [13–17]. So far, Bregs in sepsis, however, remain incompletely understood.

An increased frequency of circulatory Tregs in patients diagnosed with septic shock is significantly associated with immunosuppression, meanwhile percentage of Tregs increased as early as 3 days after shock, but the absolute number still was lower than in healthy donors [18]. Recent study supports Bregs, like Tregs, capable of negatively modulating T-cell–dependent autoimmune responses in an Ag-specific manner [19–21]. However, the function and clinical relevance of Bregs during sepsis remains unclear and little is presently known about the survival of patients following diagnosis. The prime aim of this exploratory study was to investigate Bregs in septic shock development and prognosis using a whole blood staining approach by flow cytometry. Our study demonstrated that sepsis patients with high frequency of Bregs had a lower risk of septic shock development. Our results showed that low levels of Bregs were associated with an increased risk of non-survival in patients with sepsis. This association might be due to decreased IL-10–producing B cells, and elevated IL-10–producing T cells. Findings indicated that Bregs might be applied to identify sepsis patients who are at risk of septic shock development and evaluate outcome during sepsis state.

Materials and methods

Subjects

Sepsis inclusion criteria were based on the Surviving Sepsis Campaign definition 3.0 [1]. Overall, 58 septic patients, diagnosed with sepsis (n = 26) and septic shock (n = 32) at intensive care unit (ICU) of Shanghai East Hospital between May 2016 and May 2018 were enrolled into study (Fig. 1). Healthy controls inclusion criteria were as follows: Matched age to the sepsis group of

patients; good health; no history of chronic or metabolic diseases (e.g., hypertension, diabetes, coronary heart disease, hepatitis, and hyperthyroidism); and not using antibiotics within 3 days before enrollment. Healthy control group exclusion criteria were the same as those for sepsis.

The study was approved by the Institutional Ethics Authorities (No. 2015-028, Tongji University, China). All enrolled patients and healthy controls signed informed consent forms.

Eight mL of peripheral venous blood samples collected from septic patients at day 1 after admission and from healthy controls were subjected to lymphocyte separation within 3 h and flow cytometry analysis. The following general clinical data were collected and recorded at day 1: demographic characteristics (age and sex), main diagnosis, vasopressor use, ICU days, mechanical ventilation, infection site, and pathogenic bacterial agent [based on qualitative culture or metagenomic next generation sequencing (mNGS)] (Table 1). The white blood cell (WBC), creatinine, bilirubin, C-reactive protein (CRP), procalcitonin (PCT), blood lactate (Lac), Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and Sequential Organ Failure Assessment (SOFA) score of patients on the day of admission were recorded (Additional file 1: Table S1). Twenty eight-day follow-up was complete. All patients with sepsis were divided into survivors (Alive) and non-survivors (Dead).

CD19⁺CD24^{hi}CD38^{hi} Bregs analysis by flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using standard Ficoll gradient centrifugation. The isolated PBMCs were stained at room temperature for 15 min with antibodies against CD45 (OKT3), CD3 (OKT3), CD19 (SJ25C1), CD20 (2H7), CD24 (ML5) and CD38 (HIT2). Cells were gated on CD19⁺ B cells and CD19[−] fraction after gating out CD3⁺ T cells. B cells were selected on a biparametric CD3[−]CD19⁺ dot-plot and Bregs were selected on CD24^{hi}CD38^{hi} circled from the CD19⁺ gate.

300 μL fresh whole blood was stained with antibodies against CD45 (OKT3) and CD33 (WM53) after lysing red blood cells. Then we selected WBC as CD45⁺ cells based on a biparametric CD45/SSC dot plot. By gating out CD33^{lo}SSC^{hi} polymorphonuclear (PMN), PBMCs were selected and the percentage of PBMCs in CD45⁺ cells was calculated. Absolute numbers of CD45⁺ lymphocytes per microliter were then calculated. Next, B cells were selected based on a biparametric CD19/SSC dot plot and the percentage of CD19⁺ B cells in PBMCs was calculated. Then, Bregs were gated based on CD3[−]CD19⁺CD24^{hi}CD38^{hi} and the percentage of Bregs in CD19⁺ cells was calculated. Meanwhile, the

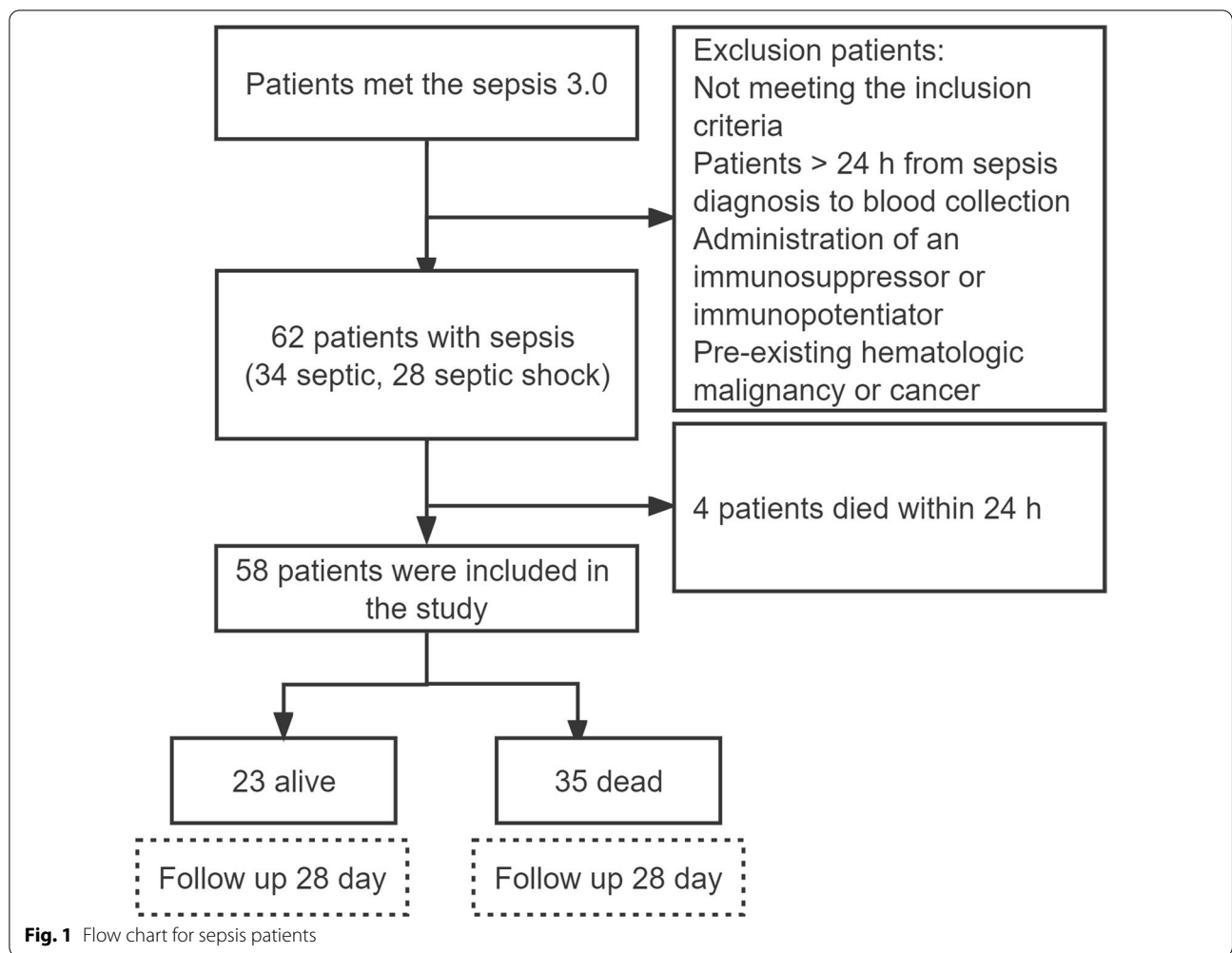


Table 1 Clinical data in elderly patients with sepsis and healthy donors

	Healthy	Sepsis	t/F	p
n	22	58		
Age (year)	75.2 ± 7.0	78.6 ± 8.1	1.832	0.0707
Male gender, n (%)	10 (45.5)	38 (65.5)	0.167	0.1281
Septic shock, n (%)		26 (44.8%)		
Mechanical ventilation, n (%)		6 (10.3%)		
Infection site, n(%)		Lung, 38 (65.5)		
		Biliary tract 9 (15.5)		
		Digestive tract 5 (8.6)		
		Urinary tract 3 (5.2)		
		Skin/ soft tissue 3 (5.2)		
Etiology, n (%)		Bacterial, 38 (65.5)		
		Fungal, 9 (15.5)		
		Viral, 11 (19.0)		

appropriate irrelevant anti-mouse isotype controls IgG1-FITC, APC and PE were used.

The absolute numbers of Bregs were calculated according to standard flow cytometry criteria for lymphocyte subset identification. The absolute number of CD19⁺ B lymphocytes was calculated by determining the percentage of CD19⁺ B cells in peripheral blood lymphocytes multiplied by the total number of lymphocytes per microliter measured using URIT-2900. The absolute number of CD19⁺CD24^{hi}CD38^{hi} was calculated by multiplying the total number of B lymphocytes calculated by the percentage of positive cells in CD19⁺ B cells. Absolute numbers were expressed as cells per milliliter.

Type 1 T helper (Th1) and Tregs analysis by flow cytometry

PBMCs were obtained from blood sample cells at day 1 after patients admission. The isolated PBMCs were stained for 15 min at room temperature with anti-CD3-BUV395, CD4-BV510, CD25-PE-Dazzle594, CD127-BV650, CXCR3-PECy7 and CCR6-BV785. PBMCs were suspended in staining buffer and detected by FACS Fortessa flow cytometry. T cells were selected on a biparametric CD3/SSC dot plot. Tregs were selected on CD4⁺CD25⁺CD127⁺ and Th1 were selected on CD4⁺CXCR3⁺CCR6⁻.

IL-10⁺ Breg cells analysis by flow cytometry

PBMCs were first stained with combinations of cell surface markers, including anti-human CD3, CD19, CD20 monoclonal antibody (BD) for 30 min at 4 °C, and then stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma), 1 µg/mL ionomycin (Sigma), and 5 µg/mL brefeldin A (Biolegend) for 5 h at 37 °C. Finally, cells were washed twice and stained with zombie yellowTM dye (Biolegend) to eliminate dead cells. Unstimulated cells were used as controls for the following analyses. Intracellular cytokine staining was then carried out using anti-human interleukin-10 (IL-10) monoclonal antibodies (BD) after permeabilization and fixation. Labeled cells were acquired on LSRFortessa flow cytometer (BD Biosciences), and data were processed using Flowjo software (Tree Star).

ELISA for plasma cytokine

A total of 150 µL plasma was collected after centrifugation on day 1 after diagnosis and then stored at -80 °C for further detection. Human plasma IL-10 was detected by ELISA kit (R&D Systems) per manufacturers instructions.

Statistical analysis

The clinical data were first tested for normality and homogeneity of variance. Data conforming to a normal distribution and homogeneity of variance were expressed as the mean ± SD, and the t-test or analysis of variance was used for statistical analysis. Data were shown as the mean ± SD. The χ^2 test was used to compare the enumeration data. Mann-Whitney U-test was used for the comparison of the two groups. One-way analysis of variance (ANOVA) was for comparison among multiple groups, followed by Kruskal-Wallis test with Dunns multiple comparison test when the values were not normally distributed. Correlation analysis was analyzed using Pearson's chi-square test. Statistical tests were performed using SPSS 20.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Frequency of Bregs decreased in patients with sepsis

Blood samples were collected at day 1 after patients were admitted in ICU and prepared within 3 h for flow cytometry after collection. The frequency of classical human Bregs was distinguished by CD3⁻CD19⁺CD24^{hi}CD38^{hi} (Fig. 2A). 58 septic patients (38 males, 20 females) and 22 healthy controls were recruited for the study (Table 1, Additional file 1: Table S1). There was no significant age or sex differences between healthy controls and septic ones (Table 1). Infection sites were defined as lung, biliary tract, urinary tract, digestive tract, and skin/soft tissue. Among the patients with sepsis, pneumonia represented the initiation of infection (38/58); bacterial infection was the main cause of sepsis (38/58) (Table 1). We found that the frequency and absolute number of Bregs were both significantly decreased in septic patients compared with healthy controls (Fig. 2B–C). Frequency and absolute number of Bregs did not differ significantly among infection sites (Fig. 2D–E). No significant differences were observed for the etiology of sepsis (Fig. 2F–G).

(See figure on next page.)

Fig. 2 Bregs were decreased in sepsis patients on day 1. **A** Gating strategy of Bregs in representative healthy and sepsis patients. > 1 million events in the lymphocyte gate were collected. **B** Bregs frequency in total B cells and **C** absolute number per microlitre of blood of Bregs in all healthy and sepsis subjects. Absolute number of Bregs per microlitre of blood was calculated by multiplying the total number of B lymphocytes calculated by the percentage of positive cells in Bregs in lymphocytes. **D** Frequency and **E** absolute number of Bregs in sepsis patients caused by digestive tract, lung, biliary tract, urinary tract, and skin/soft tissue infection. **F** Frequency and **G** absolute number of Bregs in sepsis patients with bacterial, viral, and fungal causes. Two-tailed t-test with unequal variance or Kruskal-Wallis test. **** $p < 0.0001$

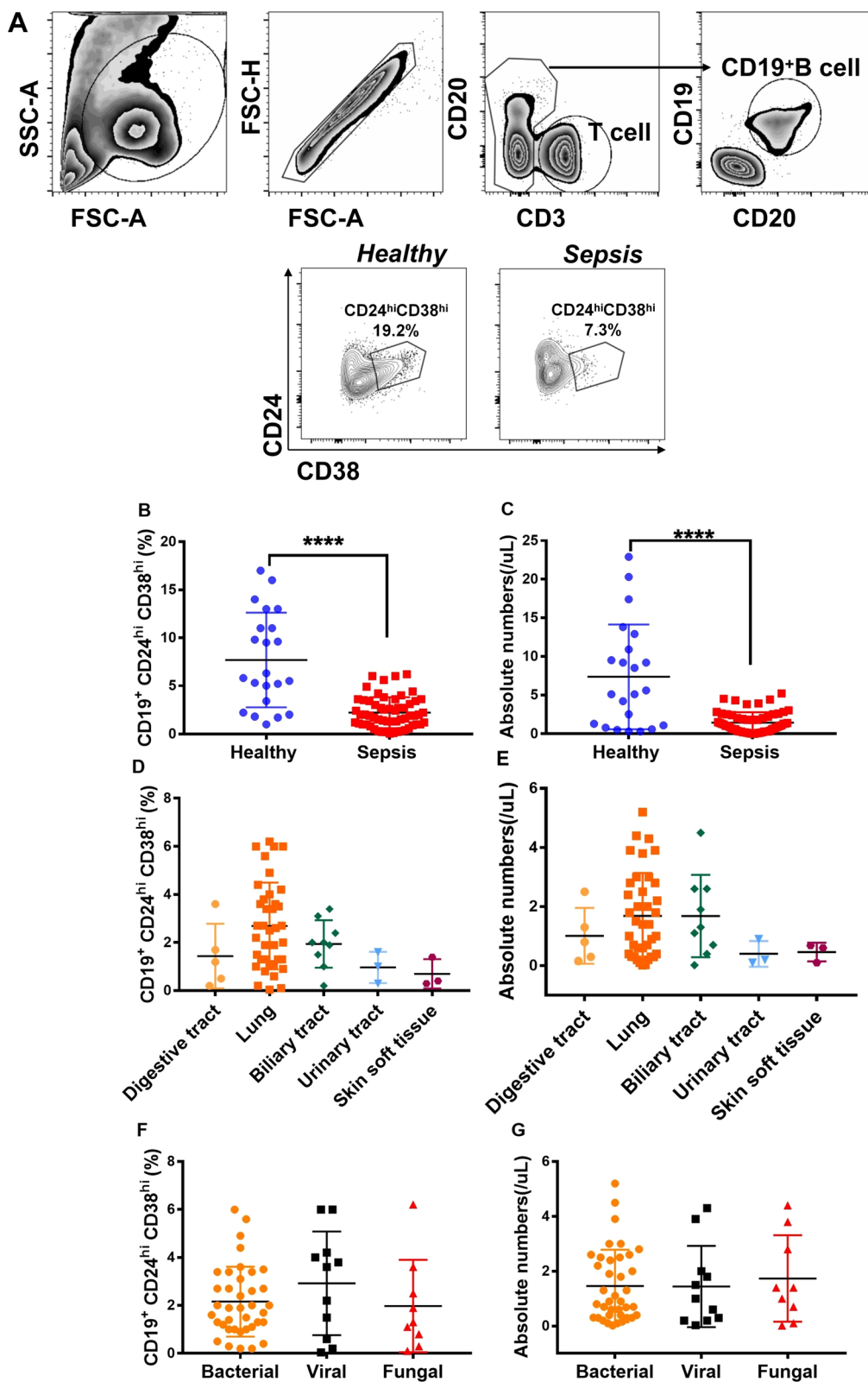


Fig. 2 (See legend on previous page.)

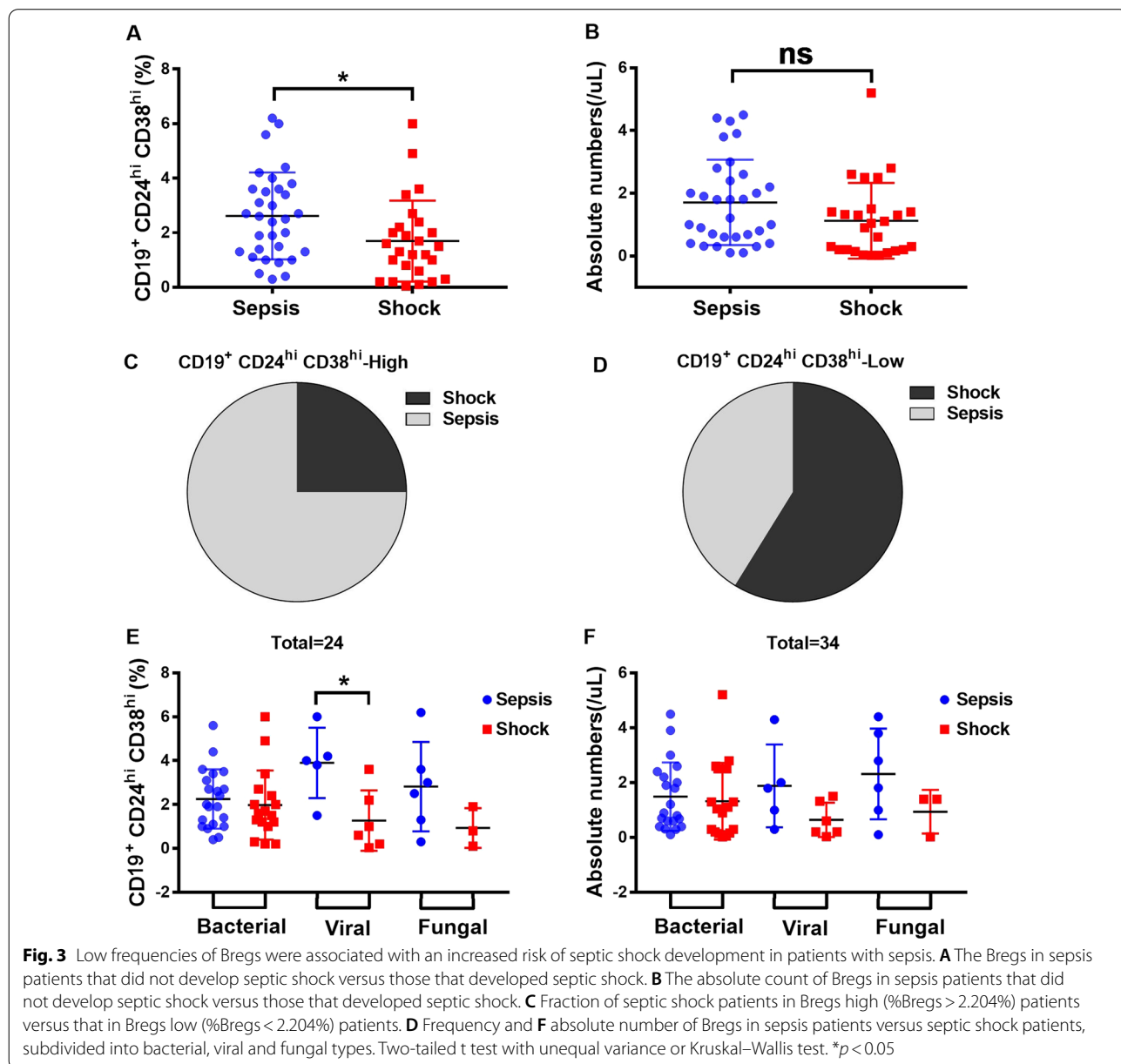
Low frequencies of Bregs were associated with development of septic shock in patients with sepsis

To investigate the potential role of Bregs in septic shock development in patients with sepsis, we assessed our study cohort for septic shock development (Additional file 2: Table S2). We found that the frequency of Bregs was markedly lower in septic shock group than that in sepsis group, but there was no difference in absolute number of Bregs (Fig. 3A–B). Sepsis patients with high Bregs had reduced risk of developing septic shock. Our study revealed that there was a significantly lower frequency of Bregs in patients who later developed septic

shock compared to patients who did not (Fig. 3C, D). When sepsis-related shock was stratified by etiology type, our study revealed that viral sepsis patients who later developed shock had significantly less Bregs frequency, while Bregs counts were not different (Fig. 3E, F).

IL-10⁺B cells did not match plasma IL-10 in septic patients

To identify circulating lymphocyte subsets producing IL-10, we detected IL-10 expressed on CD3 and CD19 between septic group and healthy one. Accordingly, we found the proportion of CD4⁺ T cells expressing IL-10 was significantly higher in septic patients than that in



healthy group (Fig. 4A, B), while the CD19⁺ B cells expressing IL-10 were lower (Fig. 4C, D). Concentration of plasma IL-10 was significantly higher among septic group when compared to healthy groups (median [IQR] 7.2 [3.4–17.7] vs 1.2 [0.7–2.4], $p=0.0211$) (Additional file 1: Table S1).

Bregs frequency was inversely associated with lactate, SOFA and APACHE II and positively correlated with Tregs

Clinical severity scores such as SOFA, APACHE II and available assays such as blood lactate reflect the severity of septic patients. Here, we found that there were no correlation between Bregs frequency at day 1 and PCT, CRP and plasma IL-10 (Fig. 5A, B, D). Bregs frequency at day 1 was inversely associated with lactate, SOFA, and APACHE II (Fig. 5C, E, F). We examined the correlation between Bregs and T cell subsets. There was no correlation between the frequency of Bregs and Th1 (Fig. 5G), while Bregs were positively correlated with Tregs (Fig. 5H) ($R^2=0.0897$, $p=0.0224$).

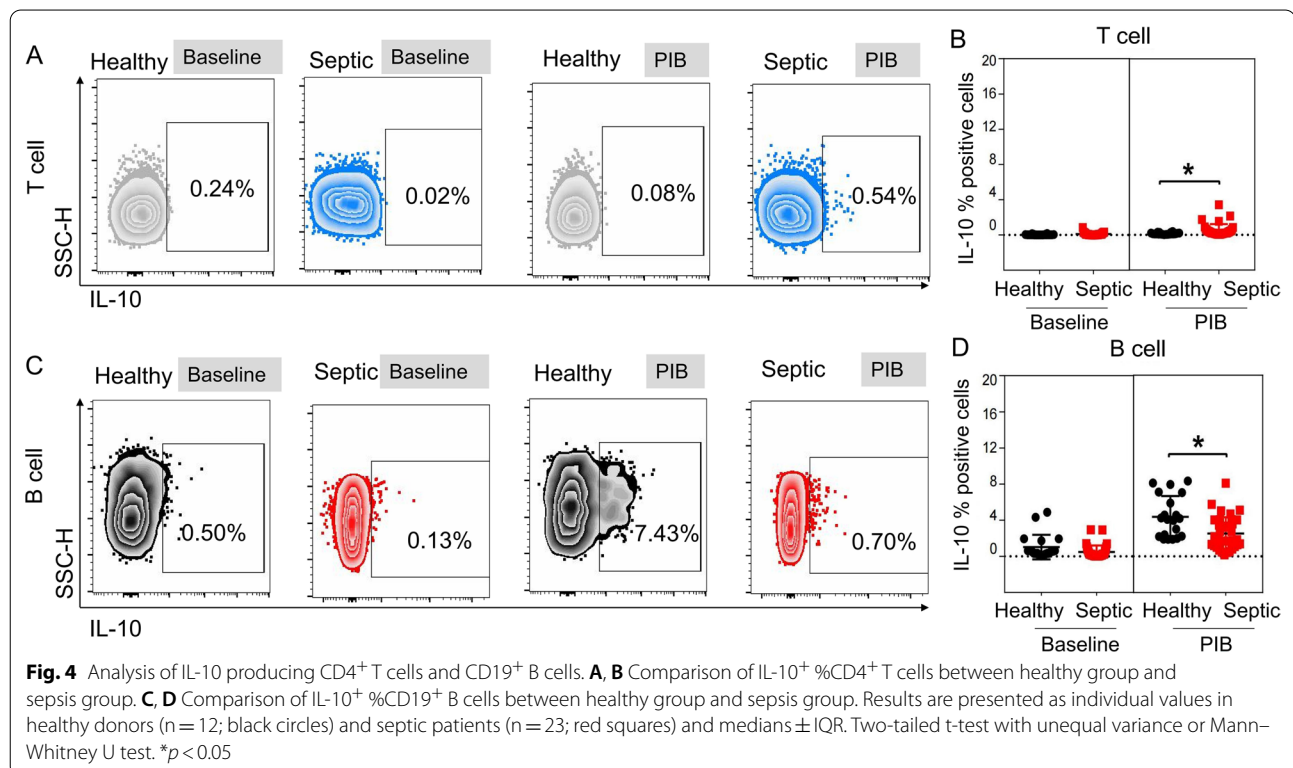
Low levels of Bregs were associated with an increased risk of non-survival in septic patients

Of the 35 patients, who died during hospitalization, 20 (57.1%) patients had septic shock during their course, meanwhile, 6 out of 23 patients who survived had septic shock (Additional file 3: Table S3, $p=0.036$). We observed

that patients non-survivor had decreased frequency and numbers of Bregs than survivor patients (Fig. 6A–B). Furthermore, lactate, SOFA, and APACHE II, established sepsis severity markers, were elevated in non-survivor group (Fig. 6E, H, I), consistent with a causal association of Bregs changes with non-survivor in septic patients. There were no significant differences in PCT, CRP, creatinine, and bilirubin between *non-survival* group and *survival* group (Fig. 6C, D, F, G).

Bregs exerted a good predictive value for 28-day mortality

Mortality predictions were explored for septic patients who died within 28 days. We used the factors mentioned above (Fig. 6) to develop a ROC (receiver operator characteristic curve) for predictive assessment for mortality in septic patients. The area under the ROC curve of PCT, CRP, and lactate were 0.53 (95% CI 0.37–0.69), 0.51 (95% CI 0.36–0.67), 0.67 (95% CI 0.52–0.81) (Fig. 7A–C). The area under the ROC curve of Bregs frequency, SOFA and APACHE II were 0.78 (95% CI 0.66–0.90), 0.67 (95% CI 0.53–0.81), 0.80 (95% CI 0.69–0.92) (Fig. 7D). Moreover, patients with higher frequency and number of Bregs levels ($>2.204\%$, $>1.444/\mu\text{L}$, respectively) had a significantly better outcome than those with lower levels ($<2.204\%$, $<1.444/\mu\text{L}$, respectively) ($p=0.0293$, $p=0.0052$, respectively) especially in 28-day mortality (Fig. 7E, F).



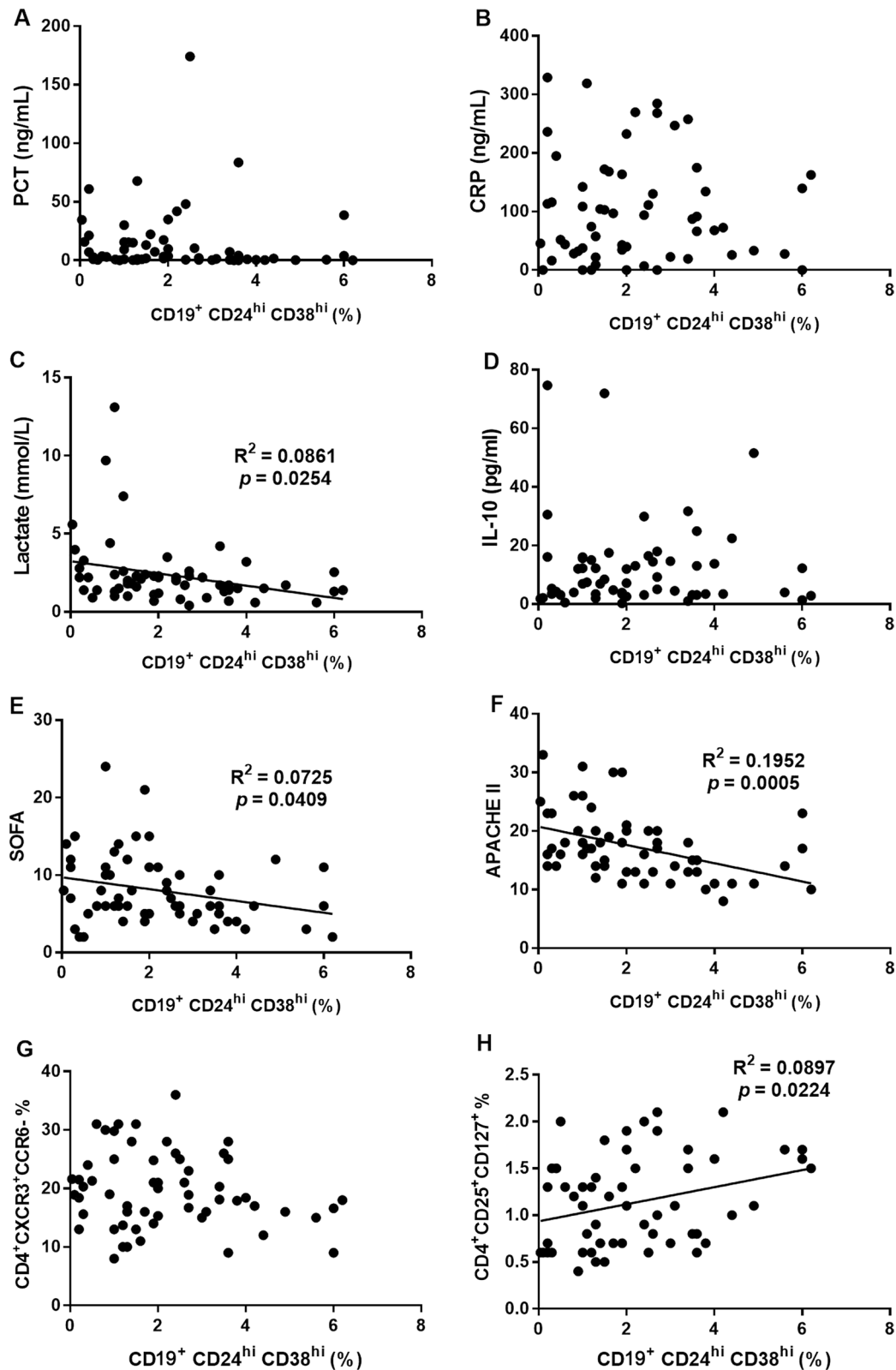


Fig. 5 The frequency of Bregs was inversely associated with lactate, SOFA, and APACHE II and positively correlated with Tregs frequency. **A–H** The correlations between the frequencies of Bregs and PCT, CRP, lactate, plasma IL-10, SOFA, APACHE II, CD4⁺CXCR3⁺CCR6⁻ T cells, and CD4⁺CD25⁺CD127⁺ T cells in all sepsis patients at day 1 were shown. *p* values represented Pearson’s correlation coefficient

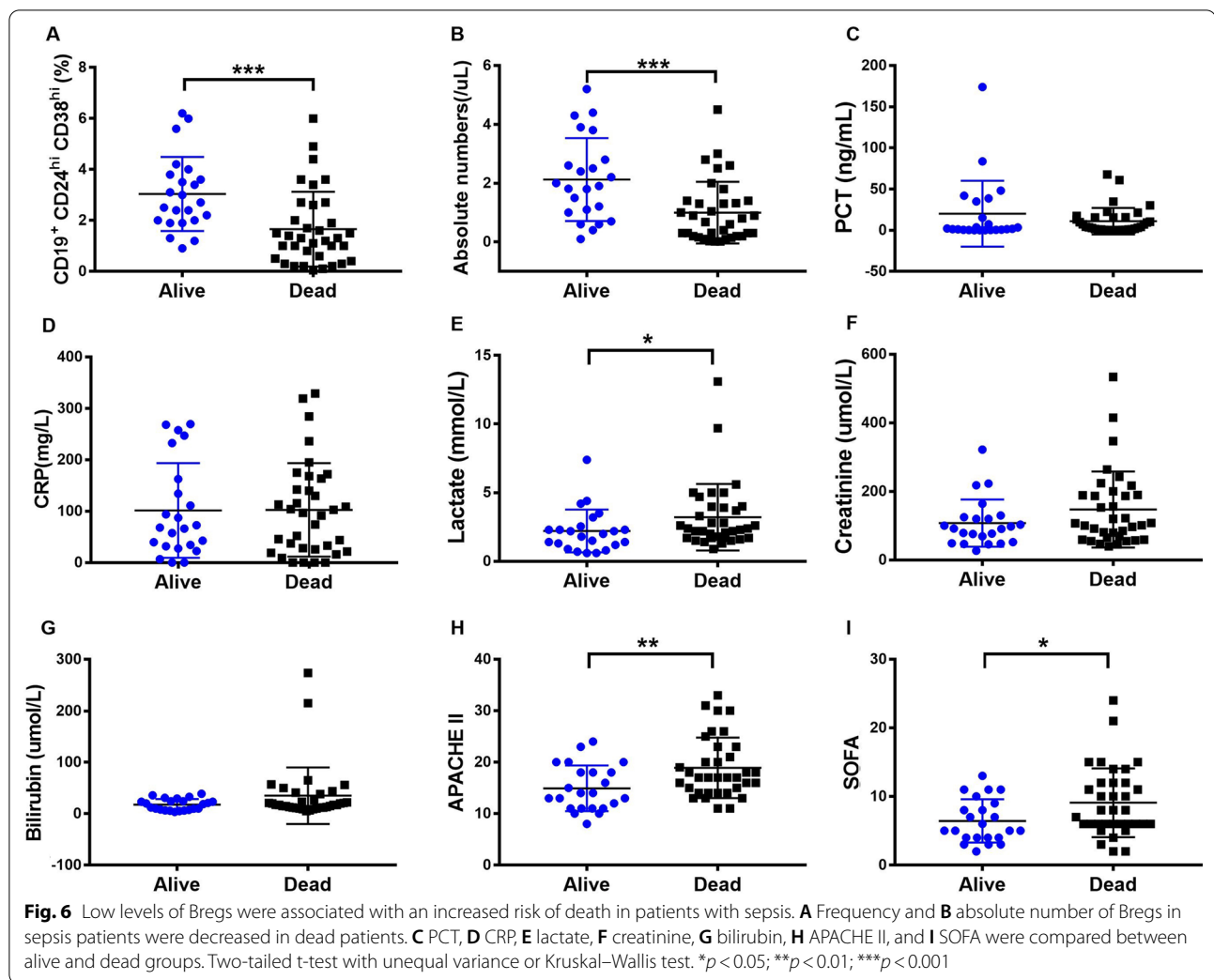


Fig. 6 Low levels of Bregs were associated with an increased risk of death in patients with sepsis. **A** Frequency and **B** absolute number of Bregs in sepsis patients were decreased in dead patients. **C** PCT, **D** CRP, **E** lactate, **F** creatinine, **G** bilirubin, **H** APACHE II, and **I** SOFA were compared between alive and dead groups. Two-tailed t-test with unequal variance or Kruskal–Wallis test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Frequency and numbers of Bregs were prediction factors for poor prognosis.

Binary logistic regression analysis (entry method) was performed on the frequency and numbers of Bregs, APACHE II score, and SOFA score, which had statistically significant differences between the survival and the non-survival group, to determine the independent outcome risk factors. The method of screening variables is forward: LR method and the regression coefficient of absolute Bregs value is -0.642 , showing a significance level of 0.023 (Table 2, $p < 0.05$), which means that the absolute Bregs number has a significant negative impact on the mortality in septic patients. And the odds ratio (OR value) was 0.526 , meaning that when the absolute number of Bregs increased by one unit, the mortality of septic patients decreased by 0.526 times. In a word, the results showed that the numbers of Bregs were independent risk factors affecting the prognosis of elderly septic patients.

Discussion

Sepsis is defined as a life-threatening organ dysfunction due to a dysregulated host response following an infection [1]. Sepsis is treatable, and timely implementation of targeted interventions can improve prognosis [22–24]. Because the mechanism of sepsis pathogenesis remains unclear, accurately identifying sepsis patients who are at higher risk of septic shock, while important for public health leaders, healthcare providers, medical personnel, and researchers, remains a great challenge. Recent studies suggest that Bregs have had an essential role in regulating inflammation and autoimmune diseases, very little is known about their involvement in sepsis. Here, we examined the frequency and absolute number of the Bregs population in septic patients at day 1 after admission. We found that septic patients had decreased Bregs frequency and absolute number compared to healthy controls. Moreover, we found a reduced levels of Bregs in sepsis patients who developed septic shock and in

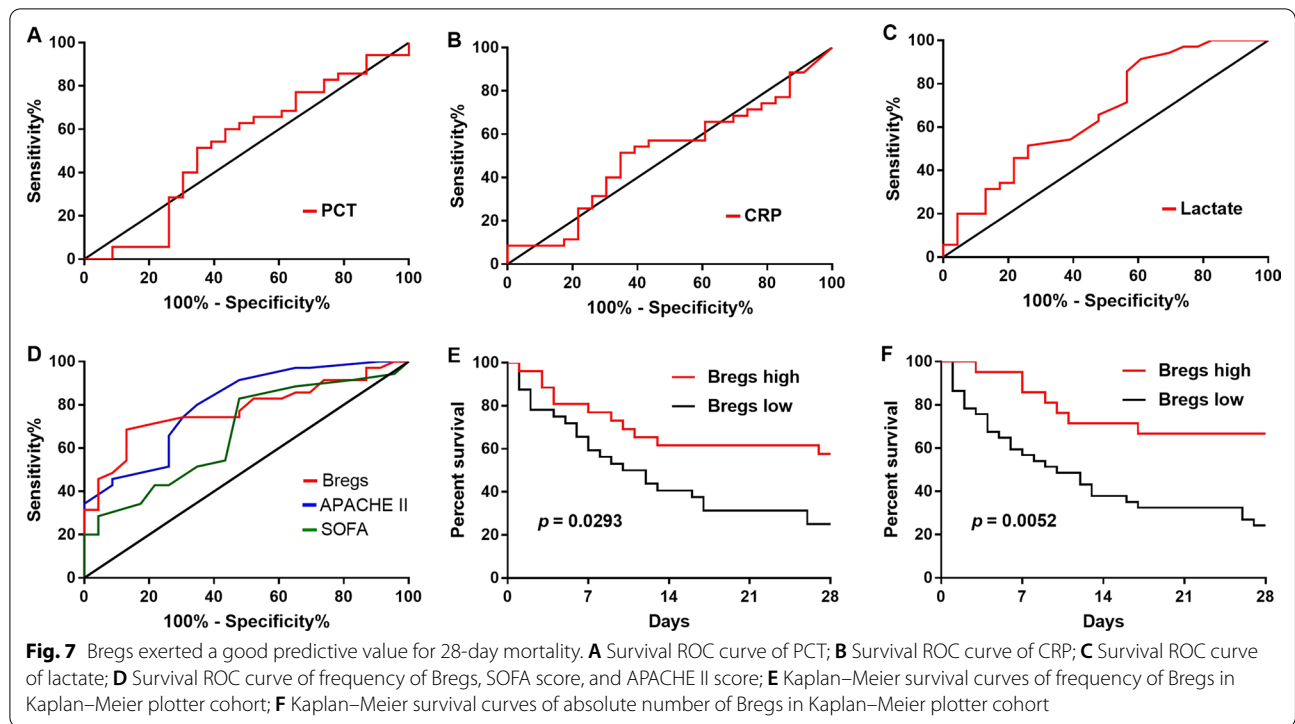


Table 2 The influence of independent variables on prognosis of patients with sepsis at 28 days

	β	S.E.	Wals	p	OR	OR 95% CI	
						Lower	Upper
SOFA score	0.080	0.103	0.608	0.436	1.083	0.886	1.325
Bregs frequency	−0.261	0.251	1.082	0.298	0.770	0.471	1.260
Bregs count	−0.642	0.282	5.169	0.023	0.526	0.303	0.915
APACHE II score	0.118	0.094	1.561	0.211	1.125	0.935	1.354
Stable	−0.553	1.664	0.110	0.740	0.575		

non-survival group compared to the survival one. Binary logistic regression analysis identified that Bregs absolute number represented an independent prognostic factor for poor outcome in septic patients.

Bregs can impair immune responses and be linked to increased disease severity during infections. Data on CD19⁺CD24^{hi}CD38^{hi} regulatory B cells are conflicting in cohort studies of patients with sepsis. Contrary to our results, Pan X et al. showed that the percentage of CD19⁺CD24^{hi}CD38^{hi} regulatory B cells was higher in neonatal patients with sepsis than healthy controls by using flow cytometry [25]. Whereas, the fraction of CD19⁺CD24^{hi}CD38^{hi} regulatory B cells on adult patients with sepsis was not altered [26]. Such differences may be attributed to the complexity of sepsis and physiological environments within individuals. In addition to the role in sepsis, a recent study showed Bregs revealed a

deficiency proportion of IgM memory and transitional subsets in chronic GVHD [27]. Two other studies confirmed the protective effect of Bregs on mouse cGVHD models [14, 28]. Meanwhile, CD19⁺CD24^{hi}CD38^{hi} regulatory B cells are also known to play an important role in the pathogenesis of systemic lupus erythematosus (SLE), because the percentage of CD19⁺CD24^{hi}CD38^{hi} regulatory B cells was significantly increased in peripheral blood from patients with SLE [29]. Cui et al. [30] have shown that Bregs significantly decreased in patients with RA.

Moreover, we found a reduced level of Bregs in sepsis patients who developed septic shock. Interestingly, when septic patients were divided into two layers, sepsis and septic shock patients, percent of Bregs was lower in viral septic shock compared to septic patients, indicating the lower the Bregs, the more likely they are to predict viral

septic. Peripheral blood CD19⁺CD24^{hi}CD38^{hi} regulatory B cells were significantly reduced in patients with severe COVID-19 compared with those with mild disease, which corresponds to the expansion of extrafollicular B cells [31]. We also found Bregs were closely correlated with the progression of sepsis. There was a close correlation between Bregs and progression of sepsis. We observed that there was a positive correlation between the frequency of Bregs and frequency of Tregs in peripheral blood. In particular, we also observed that the frequency of Bregs was inversely associated with artery Lac, SOFA, and APACHE II. It was suggested that the frequency of Bregs had a significant negative correlation with the disease process. Binary logistic regression analysis identified that Bregs absolute number represented an independent prognostic factor for poor outcome in septic patients. Altogether, our results demonstrated that Bregs have been proposed to mediate the anti-inflammatory function and may exert protective effects during sepsis. Bregs have been shown to maintain immune tolerance in humans and mice including secretion of IL-35, TGF- β , IL-10, and through the expression of FasL, GITRL, PD-1, and CD73 [32, 33]. IL-10, a major anti-inflammatory cytokine, suppresses both Th1 and type 2 T helper (Th2) polarization and inhibits antigen presentation and proinflammatory cytokine production by macrophages, lymphocytes, monocytes, dendritic cell and some epithelial cells [34, 35]. Anti-inflammatory response, including the anti-inflammatory cytokine IL-10, IL-LRA, SIL-LR, STNFR-I and STNFR-II, parallel the overproduction of proinflammatory cytokines, may induce an immunosuppressive state in patients with sepsis [36]. We revealed that plasma IL-10 was higher in septic patients than healthy donors, which indicated very early immunosuppression or immunoparalysis in elderly septic patients. Contrary to data from the literature [37], we observed that the ability of B cells secreting IL-10 decreased, which may be due to different stimulation conditions, B cell exhaustion, decreasing numbers and impaired function of B cells. Finally, we observed an increase in IL-10 secreting by CD4⁺ T cells in septic patients, which is consistent with previous published literature on Tregs in sepsis and septic shock [38, 39]. Previous findings by Roth et al. showed that increased IL-10 concentrations in sepsis may be associated with susceptibility of Th1 to apoptosis, leading to a prevalence of Th2 cells, which is known for their IL-10 production. CD14⁺ monocytes, not CD4⁺ T cells nor multipotent adult progenitor cells, are responsible for IL-10 production [40]. In addition, B cells from acute COVID-19 patients showed increased IL-6 and decreased IL-10 production compared with

healthy B cells. Age-related declines in the number and function of Bregs may contribute to "inflammation" or the "chronic inflammation" observed with ageing [41]. Age-associated reductions in Bregs frequency, as well as impaired IL-10 production, were observed in healthy older donors (>60 years) compared to healthy younger donors (20-40 years old) [41]. Patients that succumbed to shock might be due to B cells exhibited a decrease in IL-10 secretion, indicating that both anti-inflammatory cytokines, inhibition of B cells in the initial stage of sepsis in elderly patients, and exogenous IL-10 had protective effects in sepsis.

APACHE II score is a classical standard to evaluate the severity of the disease in critically ill patients, and patients can be classified according to the severity of the disease. Studies have shown that APACHE II score is negatively correlated with ICU length of stay, and positively correlated with mortality [42]. SOFA score is also a simple and effective method to evaluate organ dysfunction/failure in critically ill patients. High SOFA score is associated with high mortality [42]. Our study demonstrated that APACHE II score, SOFA score, lactate, and Bregs differed significantly between dead and alive patients. Moreover, percentage of Bregs were negatively correlated with severity scores, and significantly correlated with non-survival of septic patients. All this data indicates that changes in Bregs may predict the prognosis in septic patients.

Conclusions

Overall, our results indicate that patients with sepsis had a lower frequency and number of Bregs, which seemed to be closely associated with disease progression, predicting a shorter overall survival and poor prognosis. Circulating Bregs numbers may be used as a reliable supplementary resource in the determination of sepsis severity. A longitudinal study of Bregs activity in sepsis patients should be explored as a test for disease progression/resolution. It is noteworthy that Bregs provides a novel insight into the dynamics underlying sepsis progression. All these data suggest Bregs have a potential immunosuppressant function in sepsis.

Abbreviations

ANOVA: One-way analysis of variance; APACHE II: Acute physiology and chronic health evaluation II; Bregs: Regulatory B cells; cGVHD: Chronic graft-versus-host disease; CRP: C-reactive protein; HBV: Hepatitis B virus; ICU: Intensive care unit; IL-10: Interleukin-10; Lac: Lactate; mNGS: Metagenomic next generation sequencing; PBMCs: Peripheral blood mononuclear cells; PCT: Procalcitonin; ROC: Receiver operator characteristic curve; Th1: Type 1 T helper; Th2: Type 2 T helper; Tregs: Regulatory T cells; SOFA: Sequential organ failure assessment; WBC: White blood cell.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12865-022-00528-x>.

Additional file 1. Table S1. Clinical and laboratory data in septic patients and healthy donors.

Additional file 2. Table S2. Clinical and laboratory data between septic and septic shock patients.

Additional file 3. Table S3. Clinical and laboratory data between survivors and non-survivors of sepsis.

Additional file 4. All original data generated or analyzed in the study.

Acknowledgements

The authors thank Xiaoming Zhang for flow cytometry support (Key laboratory of Molecular Virology & Immunology, Institut Pasteur of Shanghai, Shanghai, China), Lunxian Tang, Hong Sun, Qian Yang, Xiandong Liu, Huijuan Ren, and Xiaowei Bao for clinical support (Shanghai East Hospital, Shanghai, China).

Author contributions

Jianwen Bai designed the research. Siting Liu, Mingzheng Xu and Chunmei Wang included and collected data. Mingzheng Xu and Jianwen Bai provided reagents, and quality control. Fengying Leng searched literature. Chunmei Wang, Rui Gao, and Huihui Xu wrote the manuscript. Chunmei Wang, Fangjie Huo and Huihui Xu performed the experiments, and analyzed data. All of the authors contributed to the article and approved the final version. All authors read and approved the final manuscript.

Funding

This study was funded by the National Natural Science Foundation of China (Grant Nos. 82003182, 81670067, and 82070073) and Shanghai Pudong New Area summit (emergency medicine and critical care) construction project (Grant No. PWYgf2021-03).

Availability of data and materials

All original data generated or analyzed in the study are included in this article/ supplementary materials (Additional file 4). Further inquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate

All experiments were performed according to relevant guidelines and regulations. The written informed consent was provided by all the recruited patients. This study was approved by the Research Ethics Board of East Hospital, Tongji University (Grant No. 2015–028).

Consent for publication

All authors have contributed to, read and approved the final version of this manuscript.

Competing interests

All authors have no relevant financial or non-financial interests to disclose.

Author details

¹Department of Emergency Medicine and Critical Care, Shanghai East Hospital, Nanjing Medical University, Nanjing 211166, Jiangsu Province, China. ²Department of Emergency Medicine and Critical Care, Shanghai East Hospital, Tongji University School of Medicine, 150 Jimo Road, Shanghai 200120, China. ³Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China. ⁴University of Chinese Academy of Sciences, Beijing, China. ⁵Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Jining Medical University, Jining 272067, Shandong Province, China. ⁶Department of Respiratory Medicine, Xi'an No. 4 Hospital, Xi'an 710004, Shanxi Province, China. ⁷Medical School, Tongji University, Shanghai 200120, China.

Received: 24 March 2022 Accepted: 20 October 2022

Published online: 10 November 2022

References

- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*. 2016;315:801–10.
- Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. *VIRULENCE*. 2014;5:4–11.
- Walkey AJ, Wiener RS, Lindenauer PK. Utilization patterns and outcomes associated with central venous catheter in septic shock: a population-based study. *CRIT CARE MED*. 2013;41:1450–7.
- Rhee C, Dantes R, Epstein L, Murphy DJ, Seymour CW, Iwashyna TJ, Kadri SS, Angus DC, Danner RL, Fiore AE, Jernigan JA, Martin GS, Septimus E, Warren DK, Karcz A, Chan C, Menchaca JT, Wang R, Gruber S, Klompas M. Incidence and trends of sepsis in US Hospitals using clinical vs claims data, 2009–2014. *JAMA*. 2017;318:1241–9.
- Baghdadi JD, Brook RH, Uslan DZ, Needleman J, Bell DS, Cunningham WE, Wong MD. Association of a care bundle for early sepsis management with mortality among patients with hospital-onset or community-onset sepsis. *JAMA Intern Med*. 2020;180:707–16.
- Jones SL, Ashton CM, Kiehne LB, Nicolas JC, Rose AL, Shirkey BA, Masud F, Wray NP. Outcomes and resource use of sepsis-associated stays by presence on admission, severity, and hospital type. *Med Care*. 2016;54:303–10.
- Hotchkiss RS, Coopersmith CM, McDunn JE, Ferguson TA. The sepsis seesaw: tilting toward immunosuppression. *Nat Med*. 2009;15:496–7.
- Daviaud F, Grimaldi D, Dechartres A, Charpentier J, Geri G, Marin N, Chiche JD, Cariou A, Mira JP, Pene F. Timing and causes of death in septic shock. *Ann Intensive Care*. 2015;5:16.
- Hiraki S, Ono S, Tsujimoto H, Kinoshita M, Takahata R, Miyazaki H, Saitoh D, Hase K. Neutralization of interleukin-10 or transforming growth factor-beta decreases the percentages of CD4+ CD25+ Foxp3+ regulatory T cells in septic mice, thereby leading to an improved survival. *Surgery*. 2012;151:313–22.
- Mizoguchi A, Bhan AK. A case for regulatory B cells. *J Immunol*. 2006;176:705–10.
- Mauri C, Blair PA. The incognito journey of a regulatory B cell. *Immunity*. 2014;41:878–80.
- Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity*. 2010;32:129–40.
- DiLillo DJ, Matsushita T, Tedder TF. B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. *Ann NY Acad Sci*. 2010;1183:38–57.
- Le Huu D, Matsushita T, Jin G, Hamaguchi Y, Hasegawa M, Takehara K, Tedder TF, Fujimoto M. Donor-derived regulatory B cells are important for suppression of murine sclerodermatous chronic graft-versus-host disease. *Blood*. 2013;121:3274–83.
- Sarvaria A, Basar R, Mehta RS, Shaim H, Muftuoglu M, Khoder A, Sekine T, Gokdemir E, Kondo K, Marin D, Daher M, Alousi AM, Alsuliman A, Liu E, Oran B, Olson A, Jones RB, Popat U, Hosing C, Champlin R, Shpall EJ, Rezvani K. IL-10+ regulatory B cells are enriched in cord blood and may protect against cGVHD after cord blood transplantation. *Blood*. 2016;128:1346–61.
- Yang M, Rui K, Wang S, Lu L. Regulatory B cells in autoimmune diseases. *Cell Mol Immunol*. 2013;10:122–32.
- He Y, Qian H, Liu Y, Duan L, Li Y, Shi G. The roles of regulatory B cells in cancer. *J Immunol Res*. 2014;2014:215471.
- Hein F, Massin F, Cravoisy-Popovic A, Barraud D, Levy B, Bollaert PE, Gibot S. The relationship between CD4+CD25+CD127- regulatory T cells and inflammatory response and outcome during shock states. *Crit Care*. 2010;14:R19.

19. Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol.* 2012;30:221–41.
20. Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, St CE, Tedder TF. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood.* 2011;117:530–41.
21. Lin W, Cerny D, Chua E, Duan K, Yi JT, Shadan NB, Lum J, Maho-Vaillant M, Zolezzi F, Wong SC, Larbi A, Fink K, Musette P, Poidinger M, Calbo S. Human regulatory B cells combine phenotypic and genetic hallmarks with a distinct differentiation fate. *J Immunol.* 2014;193:2258–66.
22. Evans I, Phillips GS, Alpern ER, Angus DC, Friedrich ME, Kissoon N, Lemeshow S, Levy MM, Parker MM, Terry KM, Watson RS, Weiss SL, Zimmerman J, Seymour CW. Association between the New York Sepsis Care Mandate and in-hospital mortality for pediatric sepsis. *JAMA.* 2018;320:358–67.
23. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerf B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellingham GJ, Bernard GR, Chiche JD, Cooper Smith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving sepsis Campaign: International guidelines for management of sepsis and septic shock: 2016. *CRIT Care Med.* 2017;45:486–552.
24. Seymour CW, Gesten F, Prescott HC, Friedrich ME, Iwashyna TJ, Phillips GS, Lemeshow S, Osborn T, Terry KM, Levy MM. Time to treatment and mortality during mandated emergency care for sepsis. *N Engl J Med.* 2017;376:2235–44.
25. Pan X, Ji Z, Xue J. Percentage of peripheral CD19+CD24hiCD38hi regulatory B cells in neonatal sepsis patients and its functional implication. *Med Sci Monit.* 2016;22:2374–8.
26. Shankar-Hari M, Fear D, Lavender P, Mare T, Beale R, Swanson C, Singer M, Spencer J. Activation-associated accelerated apoptosis of memory B cells in critically ill patients with sepsis. *Crit Care Med.* 2017;45:875–82.
27. Khoder A, Sarvaria A, Alsuliman A, Chew C, Sekine T, Cooper N, Mielke S, de Lavallade H, Muftuoglu M, Fernandez CI, Liu E, Muraro PA, Alousi A, Stringaris K, Parmar S, Shah N, Shaim H, Yvon E, Molldrem J, Rouce R, Champlin R, McNiece I, Mauri C, Shpall EJ, Rezvani K. Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD. *Blood.* 2014;124:2034–45.
28. Weber M, Stein P, Pruffer S, Rudolph B, Kreft A, Schmitt E, Bopp T, Roers A, Schild H, Fillatreau S, Radsak MP. Donor and host B cell-derived IL-10 contributes to suppression of graft-versus-host disease. *Eur J Immunol.* 2014;44:1857–65.
29. Wang T, Li Z, Li X, Chen L, Zhao H, Jiang C, Song L. Expression of CD19+CD24highCD38high B cells, IL10 and IL10R in peripheral blood from patients with systemic lupus erythematosus. *Mol Med Rep.* 2017;16:6326–33.
30. Cui D, Zhang L, Chen J, Zhu M, Hou L, Chen B, Shen B. Changes in regulatory B cells and their relationship with rheumatoid arthritis disease activity. *Clin Exp Med.* 2015;15:285–92.
31. Woodruff M, Ramonell R, Cashman K, Nguyen D, Saini A, Haddad N, Ley A, Kyu S, Howell JC, Ozturk T, Lee S, Chen W, Estrada J, Morrison-Porter A, Derrico A, Anam F, Sharma M, Wu H, Le S, Jenks S, Tipton CM, Hu W, Lee FE, Sanz I. Dominant extrafollicular B cell responses in severe COVID-19 disease correlate with robust viral-specific antibody production but poor clinical outcomes. *medRxiv.* (2020).
32. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. *Immunity.* 2015;42:607–12.
33. Floudas A, Amu S, Fallon PG. New Insights into IL-10 dependent and IL-10 independent mechanisms of regulatory B Cell immune suppression. *J Clin Immunol.* 2016;36(Suppl 1):25–33.
34. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol.* 2010;10:170–81.
35. Moore KW, de Waal MR, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001;19:683–765.
36. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest.* 1997;112:235–43.
37. Gustave CA, Gossez M, Demaret J, Rimmele T, Lepape A, Malcus C, Poitevin-Later F, Jallades L, Textoris J, Monneret G, Venet F. Septic shock shapes B cell response toward an exhausted-like/immunoregulatory profile in patients. *J Immunol.* 2018;200:2418–25.
38. Jiang LN, Yao YM, Sheng ZY. The role of regulatory T cells in the pathogenesis of sepsis and its clinical implication. *J Interferon Cytokine Res.* 2012;32:341–9.
39. Fabri A, Kandara K, Coudereau R, Gossez M, Abraham P, Monard C, Cour M, Rimmele T, Argaud L, Monneret G, Venet F. Characterization of circulating IL-10-producing cells in septic shock patients: a proof of concept study. *FRONT IMMUNOL.* 2020;11:615009.
40. Valentin-Torres A, Day C, Taggart JM, Williams N, Stubblefield SR, Roobrouck VD, Beyens J, Ting AE. Multipotent adult progenitor cells induce regulatory T cells and promote their suppressive phenotype via TGFbeta and monocyte-dependent mechanisms. *Sci Rep.* 2021;11:13549.
41. Duggal NA, Upton J, Phillips AC, Sapey E, Lord JM. An age-related numerical and functional deficit in CD19(+) CD24(hi) CD38(hi) B cells is associated with an increase in systemic autoimmunity. *Aging Cell.* 2013;12:873–81.
42. Morkar DN, Dwivedi M, Patil P. Comparative study of sofa, Apache II, Saps II, as a predictor of mortality in patients of sepsis admitted in medical ICU. *J Assoc Physicians India.* 2022;70:11–2.
43. Bauer M, Gerlach H, Vogelmann T, Preissing F, Stiefel J, Adam D. Mortality in sepsis and septic shock in Europe, North America and Australia between 2009 and 2019- results from a systematic review and meta-analysis. *Crit Care.* 2020;24:239.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

