


Natural killer cell deficiency experiences higher risk of sepsis after critical intracerebral hemorrhage

International Journal of
Immunopathology and Pharmacology
Volume 35: 1–12
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DOI: 10.1177/20587384211056495
journals.sagepub.com/home/iji


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Abstract

Background: Lymphopenia is common in intracerebral hemorrhage (ICH) and may predispose to severe infections such as sepsis. However, what specific kind of lymphocytes subsets decreases is still unclear. We investigated the impact of lymphocytes subsets on post-critical ICH infections and mortality.

Methods: Consecutive ICH patients (admitted to a single center between January 2017 and January 2018) were prospectively assessed to evaluate the following main parameters: peripheral blood lymphocytes, infections, and clinical scores. Predicting factors of sepsis were measured using multivariate Logistic regressions analysis. A Kaplan–Meier survival curve was performed to compare the mortality between septic and nonseptic patients. Survival status was evaluated by multivariate Cox regression analysis.

Results: In total, 112 critical ICH cases were enrolled including 29 septic patients. Total counts of lymphocytes decreased accordingly with reduced lymphocyte subsets, especially natural killer (NK) cells and CD8⁺T lymphocytes after ICH. Septic patients had a higher incidence of pneumonia, a longer length of stay, higher 90-day mortality, and worse long-term outcomes. Multivariate Logistic regression analysis showed venous catheterization, high APACHE-II score (>15), low GCS score (3–5), and NK cells percentages on admission were independently associated with ensuing sepsis. After sepsis, the percentages of CD4⁺T and NK cells percentages decreased, CD8⁺T cells increased followed by a significantly decreased CD4/CD8 ratio. Bloodstream infection alone directly affected the survival status of patients with sepsis.

Conclusions: Critical ICH patients underwent immune dysfunction and NK cells deficiency could favor nosocomial threatening sepsis after ICH.

Keywords

NK cells, intracerebral hemorrhage, lymphocyte subsets, immune response, sepsis

Date received: 1 December 2020; accepted: 24 September 2021

Introduction

Intracerebral hemorrhage (ICH) remains the most severe form of cerebrovascular diseases with high mortality in acute period.¹ Global brain inflammation and lymphocyte migration into the brain play a crucial role in secondary brain injury following acute ICH.² Given prior evidence that immunodepression manifested by lymphopenia is common in hemorrhagic strokes, such as ICH or aneurysmal subarachnoid hemorrhage (aSAH).³ Lymphopenia may predispose to high risks of nosocomial infections, but

little is known about what specific lymphocyte subsets changed. Sepsis as the most severe infection complication

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was associated with poor functional outcome in the neurocritical intensive care unit (NICU). For patients with critical ICH, the diagnosis of sepsis is challenging due to the high incidence of early systemic inflammatory response syndrome (SIRS), complex clinical symptoms, and deep coma.⁴ Our study depicted the incidence of sepsis with the Sepsis-3 criteria and its impact on clinical outcomes of patients with critical ICH. Meanwhile, each person's immune function level is not equal. Defining the individual risk of sepsis in patients at neurosurgery ICU admission has become a well-recognized priority. We used an observational single cohort of ICH patients, screening for clinical parameters, especially T/B or natural killer (NK) lymphocytes subsets changes of critical ICH patients during hospitalization in NICU. We then explored the trigger factors of sepsis and the overall prognosis after critical ICH.

Methods

Design and setting

The institute is a reference center for cerebrovascular diseases and receives approximately 240–300 ICH patients per year from a public healthcare network. Initial criteria for inclusion were all adult patients (≥ 18 years) admitted to NICU in Wuhan University Zhongnan Hospital with critical spontaneous ICH confirmed by neuroimaging (Glasgow coma scale score of 8 or less on admission) between January 2017 to January 2018. Sepsis met the 2016 Society of critical care medicine (SCCM) Sepsis-3.0 criteria.⁵ Subjects were excluded if there were presumed hemorrhagic causes, namely, traumatic intracranial bleeding, aneurysm or other vascular malformation, or hemorrhagic conversion of acute brain infarction. For the present analyses, patients were further excluded in case of missing clinical variables or lack of follow-up data.

Statement of ethics

All procedures performed in studies involving human participants were under the ethical standards of Wuhan University Zhongnan Hospital (No.2020296) and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written consent was obtained from all individual participants or their next of kin.

Clinical assessment

Demographic data, medical history, and clinical features at presentation were obtained shortly after admission. Neurological status was assessed with the Glasgow Coma Scale (GCS), ICH score, and National Institute of Health stroke scale (NIHSS). Systemic disease severity was assessed with the Acute Physiology and Chronic Health Evaluation II (APACHE-II) score. Peripheral blood lymphocyte subsets

were analyzed on admission and after sepsis. Data were collected daily during the hospital stay by two investigators (WQ and ZTB). The presence of sepsis during the hospital stay was daily assessed according to the Sepsis-3 criteria and the sequential organ failure assessment (SOFA). The clinical variables and the diagnosis of infection and sepsis were validated both by the research study team and through adjudication by an independent infectious disease specialist.

Complications during hospitalization

The incidence of pneumonia, urinary tract infections (UTIs), blood stream infection or bacteremia, symptomatic or asymptomatic deep vein thrombosis (DVT), gastrointestinal hemorrhage, acute myocardial infarction, and hypoglycemia coma during the immediate post-ICH hospital stay was established through a retrospective review by two investigators. Pneumonia was diagnosed with the combinations of typical clinical presentation, positive sputum microorganism findings, and confirmatory chest CT change. Positive urine or blood pathogenic microorganism culture was required for the diagnosis of UTIs and bacteremia. Sepsis was diagnosed in the setting of a documented source of infection associated with evidence of acute end-organ dysfunction. Deep vein thrombosis was diagnosed by positive intravenous ultrasound findings, elevated D-Dimer with or without limb swelling. The diagnosis of hypoglycemia coma was based on severe hypoglycemia with worsening consciousness disorder and its complications, such as epilepsy. Acute myocardial infarction was diagnosed with typical electrocardiographic features, significantly elevated high sensitive troponin with or without hypotension in unconscious ICH patients.

Outcome assessment

The patients were divided into septic and nonseptic groups according to whether sepsis occurred. Further, 29 septic patients were divided into survival and dead groups according to whether they survived at discharge. The primary outcome was the neurological function assessing by the modified Rankin Scale (mRS) via phone or face-to-face follow-up at third month, sixth month, and 12th month. Any patient without data available after hospital discharge was considered lost to follow-up.

Flow cytometry

To analyze the lymphocyte subsets distribution, peripheral blood mononuclear cells (PBMCs) were stained with anti-CD4-FITC, CD3-FITC, CD19-PE, CD56-PE, CD16-APC, CD25-APC, and CD8-APC cocktail monoclonal antibodies (eBiosciences, San Diego, CA, USA) at room temperature in the dark for 20 min. After washing, the cells were resuspended

in a fixation/permeabilization (eBiosciences) working solution and incubated at 4°C in the dark for 30 min. After staining, the cells were washed and resuspended in phosphate-buffered saline for measurement by BD FACS Verse flow cytometry (BD Biosciences, San Jose, CA, USA). Data were analyzed by FlowJo data analysis software (FlowJo, LLC, Ashland, OR, USA).

Statistical analyses

Age, GCS, APACHE-II score, SOFA score, mRS, ICH score, NISHH, hematoma volume, length of stay, peripheral blood lymphocyte subsets counts, international normalized ratio (INR), and intervals from admission to sepsis were analyzed as a continuous variable, described as Median (interquartile range, IQR) as appropriate, and compared with the Mann–Whitney test. All other variables were analyzed as static categorical variables, expressed as count (%) and compared using the Chi-square test and Fisher exact probability.

Multivariate Logistic regressions analysis was performed to assess the predictors of sepsis in critical ICH patients including variables listed below (model 1: sex, age, diabetes, venous catheterization, emergency neurosurgery, GCS and APACHE-II score on admission, CD4/8 ratio, and proportions of B and NK cells). A Kaplan–Meier survival curve was derived from the log-rank test to compare the mortality between septic and nonseptic groups. We performed a multivariate Cox regression analysis using a comprehensive model (model 2: age, sex, diabetes, venous catheterization, emergency neurosurgery, random blood glucose, bloodstream infection, APACHE-II score, GCS on admission, complications, CD4/CD8 ratio, length of stay, and admission to sepsis time). Survival curves were plotted to demonstrate the effect of bloodstream infection on sepsis. All analyses were performed with commercially available statistical software (SPSS version 26.0; SPSS Inc., now part of IBM Corporation, Armonk, NY, USA). We considered results statistically significant for p values <0.05.

Results

Baseline characteristics

A total of 112 patients met the eligibility criteria (median age 57 years/o, 64.3% males). Hematoma volume varies with the location. Twenty-nine patients were complicated with sepsis (median intervals from admission to sepsis 12 days). Among them, 8 (27.5%) patients developed sepsis within one week, 13 (44.8%) patients developed sepsis within 3 weeks, and 8 (27.5%) patients developed sepsis 3 weeks later due to long-term NICU hospitalization. Hematomas were located in deep brain (74, 66.1%), lobe (11, 9.8%), brainstem (14, 12.5%), cerebellum (8, 7.1%), and primary IVH

Table 1. Demographics characteristics and clinical profile of 112 critical ICH patients.

Parameter	Value
Age, years	57 (51–65)
Sex	
Male	72 (64.3)
Female	40 (35.7)
Hematoma volume, mL	42.2 (31.8–55.5)
GCS on admission	8 (5.5–8)
ICH score on admission	3 (3–3)
NIHSS on admission	32 (31–33)
Diabetes mellitus	13 (11.6%)
Emergency neurosurgery	95 (84.8%)
Venous catheterization	34 (30.3%)
Sepsis	29 (25.9%)
Intervals from admission to sepsis, days	12 (6–21)
Bacteremia	11 (9.8%)
Pneumonia	84 (75%)
Urinary tract infection	12 (10.7%)
Postoperative intracranial infection	6 (5.3%)
Hospital mortality	15 (13.3%)
Mortality at 3th month	7 (7.3%)
Poor outcome at 3th month	76 (67.8%)
Mortality at 6th month	4 (4.5%)
Poor outcome at 6th month	51 (45.5%)
Mortality at 12th month	3 (3.5%)
Poor outcome at 12th month	46 (41%)
Lymphocyte subsets on admission	
CD3+T abs./ μ L	672 (421–976.3)
CD3+T lym %	66.5 (59.4–73.2)
CD 3+CD4+T abs./ μ L	373 (282.7–620.2)
CD 3+CD4+T %	46.6 (40.5–51.5)
CD 3+CD8+T abs./ μ L	184 (110.2–273.2)
CD 3+CD8+T %	18.9 (14.2–23.2)
CD4/CD8 ratio	2.5 (2.1–3.4)
CD19+ B abs./ μ L	190 (118–300.5)
CD19+ B lym %	18.9 (14.1–26.4)
CD16+CD56+ NK abs./ μ L	82 (57.2–141.2)
CD16+CD56+ NK %	9.2 (6–12.9)

Values are reported as N (%) or median (interquartile range, IQR). mRS: Modified Rankin Scale; GCS: Glasgow Coma Scale; NIHSS: National Institute of Health stroke scale; Abs: absolute counts; Lym: lymphocyte; NK: natural killer.

(5, 4.5%). The demographic and baseline characteristics of all patients are detailed in Table 1. Based on Sepsis-3 criteria, sepsis means severe infections combined with organ dysfunction (SOFA score increased by 2 points). Therefore, we estimated the high SOFA score after sepsis and represented by Δ SOFA. Overall Δ SOFA ranged from 2 to 11 in those septic patients. Compared with nonseptic patients, septic patients had a higher incidence of pneumonia, a longer length of stay, worse long-term prognosis, higher 90-day mortality, and lower CD8+T and B lymphocytes percentages in peripheral blood.

Table 2. Comparison between septic and nonseptic patients.

	Septic group (N = 29)	Nonseptic group (N = 83)	p
Age	55 (50–63)	58 (51–65)	0.654
Male	19 (65.5%)	53 (63.9%)	0.5
INR	1.3 (1.2–1.4)	1.11 (1.0–1.2)	0.08
Random blood glucose on admission	7.8 (5.6–10.8)	7.0 (5.8–8.7)	0.356
Diabetes mellitus	5 (17.24%)	8 (9.64%)	0.218
Pneumonia	27 (93.1%)	57 (68.7%)	0.011*
Length of stay	34 (25–66)	22 (17–57)	<0.001*
Volume of ICH	40 (31.8–49.1)	43.1 (31.9–58)	0.432
GCS on admission	6 (4–8)	8 (6–8)	0.203
ICH score on admission	3 (3–3)	3 (3–3)	0.503
NIHSS on admission	32 (31–33)	32 (31–33)	0.482
APACHE-II score on admission	18 (17–20)	18 (16–21)	0.722
Poor outcome at 3th month	25 (86.2%)	51 (61.4%)	0.02*
Poor outcome at 6th month	21 (72.4%)	30 (36.1%)	0.001*
Poor outcome at 12th month	19 (65.5%)	27 (32.5%)	0.002*
Hospital mortality	9 (31%)	8 (9.6%)	0.013*
Mortality at 3th month	4 (20%)	3 (4%)	0.034*
Mortality at 6th month	2 (12.5%)	2 (2.8%)	0.15
Mortality at 12th month	1 (7.1%)	2 (2.8%)	0.426
Hematoma location			
Lobe	4 (13.8%)	7 (8.4%)	0.306
Deep Ruptured into ventricle			
Yes	15 (51.7%)	42 (50.6%)	0.170
No	3 (10.3%)	14 (16.9%)	0.180
Brainstem	5 (17.2%)	9 (10.8%)	0.162
Cerebellum	2 (6.9%)	6 (7.2%)	0.322
IVH	–	5 (6%)	0.216

Values are reported as N (%) or median (IQR).

*p <0.05 significant.

IVH: Intraventricular hemorrhage; APACHE-II: Acute Physiology and Chronic Health Evaluation II.

Comparison results of other variables without statistical significance between septic and nonseptic groups are shown in [Table 2](#).

Infections and complications

All the infection events were classified as follows: 84 episodes of nosocomial pneumonia, 11 bloodstream infections, 12 urinary tract infections, and six meningitis/ventriculitis. Pathogenic pneumonia bacteria mainly included 80 Gram-negative bacterial infections (*Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) and only four patients with methicillin-resistant *Staphylococcus aureus* (MRSA). Eleven patients had positive blood microorganism results including 4 cases of *K. pneumoniae*, four *A. baumannii*, two *Coagulase-negative Staphylococcus*, and one *Enterobacter cloacae* infection. Six septic patients combined with postoperative meningitis/ventriculitis were proven to be 2 cases of *K. pneumoniae*, two *A. baumannii*, one *Staphylococcus*

capitatus, and one *Candida albicans* infections. As shown in [Table 3A](#), septic patients had a higher incidence of pneumonia ($p = 0.011$) and bacteremia ($p = 0.001$) compared with nonseptic patients. All complication events were classified as follows: 15 cases with gastrointestinal hemorrhage, three symptomatic DVT, nine asymptomatic DVT, two hypoglycemia coma, and two acute myocardial infarctions. There were no differences in all variables between the two groups ([Table 3B](#)).

Outcomes at hospital discharge and long-term survival analyses

Follow-up data were acquired for 79 telephone interviews and 33 face-to-face assessments. Overall mortality of critical ICH at discharge was 13.3% (31% septic group vs. 9.6% nonseptic group). Compared to nonseptic patients, 90-day mortality of septic patients was much higher (20% vs. 4%). As time went on, the mortalities had no differences between two groups at the 6th and 12th months.

Table 3. Infections and complications in septic and nonseptic groups (N/percentage).

	Septic group N = 29	Nonseptic group N = 83	p
A. Infections			
Pneumonia	27 (93.1%)	57 (68.7%)	0.011*
<i>K. pneumoniae</i>	13 (44.8%)	18 (21.7%)	
<i>P. aeruginosa</i>	7 (24.1%)	21 (25.3%)	
<i>A. baumannii</i>	13 (44.8%)	20 (24.1%)	
MRSA	–	4 (4.8%)	
Mixed infection	9 (31.0%)	15 (18.1%)	
Bacteremia	10 (34.4%)	1 (1.2%)	<0.001*
<i>K. pneumoniae</i>	4 (13.8%)	–	
<i>A. baumannii</i>	3 (10.3%)	1 (1.2%)	
<i>C. Staphylococcus</i>	2 (6.9%)	–	
<i>E. cloacae</i>	1 (3.4%)	–	
Urinary tract infection	5 (17.2%)	7 (8.4%)	0.21
<i>K. pneumoniae</i>	1 (3.4%)	1 (1.2%)	
<i>Escherichia coli</i>	1 (3.4%)	–	
<i>C. albicans</i>	3 (10.3%)	5 (6.0%)	
<i>Enterobacter aerogenes</i>	–	1 (1.2%)	
Postoperative intracranial infection	6 (20.7%)	–	–
<i>K. pneumoniae</i>	2 (6.9%)	–	
<i>A. baumannii</i>	2 (6.9%)	–	
<i>S. capitatus</i>	1 (3.4%)	–	
<i>C. albicans</i>	1 (3.4%)	–	
B. Complications			
Gastrointestinal hemorrhage	7 (24.1%)	8 (9.6%)	0.053
Symptomatic DVT	2 (6.9%)	1 (1.2%)	0.15
Asymptomatic DVT	6 (20.7%)	13 (15.6%)	0.313
Hypoglycemia coma	2 (6.9%)	–	
Acute myocardial infarction	2 (6.9%)	–	

* $p < 0.05$ significant.MRSA: methicillin-resistant *S aureus*; DVT: deep vein thrombosis.

MRS assessed functional outcomes and poor outcome rate dropped month by month. Figure 1 shows detail distributions of mRS. Compared with the nonseptic group, septic patients had worse functional outcomes. As shown in Figure 2, the Kaplan–Meier survival curve depicted that sepsis was correlated with a poor prognosis after critical ICH.

We performed a multivariable Logistic regression analysis including established predictors of sepsis after critical ICH (model 1: sex, age, history of diabetes, bacteremia, emergency neurosurgery, GCS, APACHE-II score on admission, CD4/CD8 Ratio, and B and NK cells percentages). As shown in Table 4, risk factors of sepsis were independently associated with venous catheterization, high APACHE-II score (>15), low GCS score (3–5), and NK cell percentages (<7.92%) on admission.

Twenty-nine septic patients were divided into dead and survival groups according to whether they died at discharge. Deterioration after sepsis was evaluated by Δ APACHE-II score and Δ SOFA score. APACHE-II scores

on admission and Δ SOFA scores in dead group were higher than those of survival group ($p=0.049$ and $p=0.028$, respectively). Among them, five patients died within one week after sepsis, two patients died within three weeks, and two patients died three weeks later. Correspondingly, the length of stay in dead septic patients was shorter than that in survival patients ($p = 0.011$). Other variables with no significant differences were presented in Table 5.

The survival curve of septic patients was obtained with a multivariate Cox regression analysis (Figure 3(a)). Adjusted by age, sex, GCS and APACHE-II score on admission, history of diabetes, bloodstream infection, venous catheterization, emergency neurosurgery, Δ APACHE-II score, complications, CD4/CD8 ratio, and B and NK cells percentages, only bloodstream infection was closely related to the survival statuses of septic patients. We found that the survival time of septic patients without bloodstream infection was significantly longer than that of patients with bloodstream infection ($p=0.019$) (Figure 3(b)).

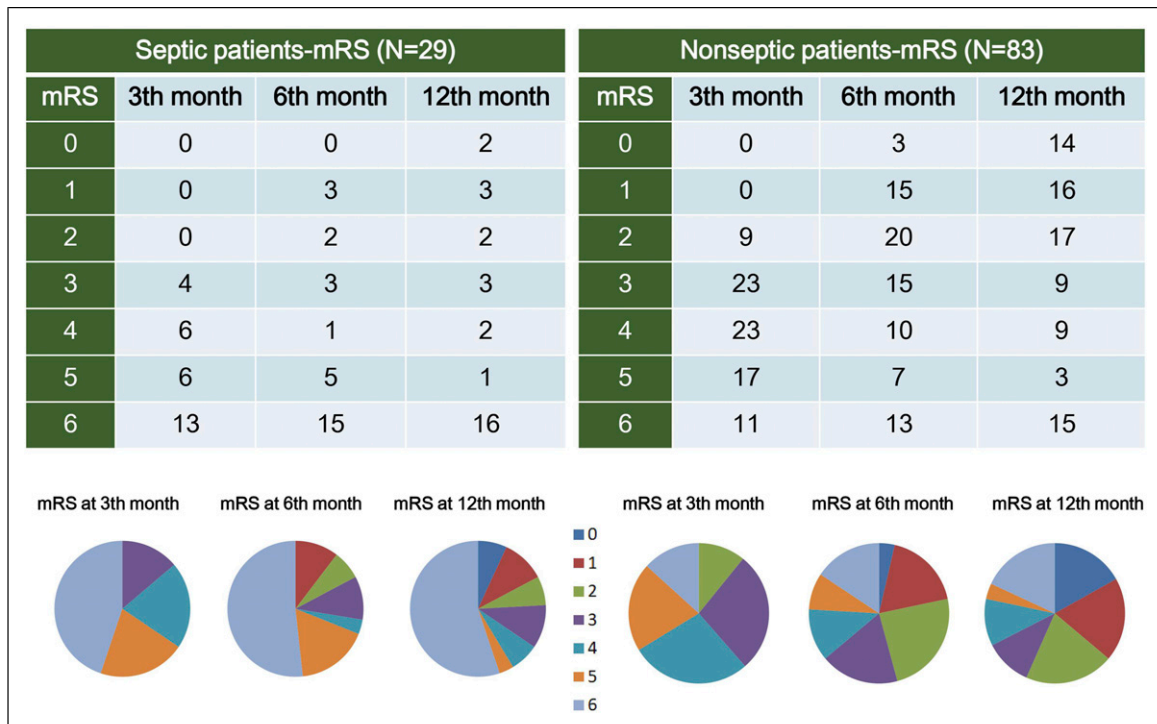


Figure 1. Long-term outcomes of septic and nonseptic patients. Compared with nonseptic group, septic patients had worse functional outcomes.

Peripheral blood lymphocyte subsets (PBLs) characteristics in critical ICH patients

Total absolute counts of lymphocytes in peripheral blood decreased significantly according with reduced lymphocyte subsets in critical ICH patients. Specifically, absolute counts of CD3+T, CD8+T (cytotoxic T-cells), and B and NK cells were decreased, especially NK and CD8+T cells. Even though CD4+T (T-helper) cells kept in the normal range, the median CD4/CD8 ratio (normal range: 0.9–2.05) increased to 2.5, which was higher than 2.05.

First, we compared the lymphocyte subsets between septic and nonseptic patients on admission. As shown in Table 6A, CD4/CD8 ratios were higher than the upper normal limit. Compared with nonseptic patients, the percentages of CD8+T, B, NK cells on admission were significantly lower in septic patients. The immune system might be activated after critical ICH. Patients suffered from the inhibited innate immune system and weak adaptive immune system-mediated cell lethality.

Next, we counted the changes of lymphocytes subsets of 29 septic patients. As shown in Table 6B, the percentages of CD4+T cells decreased and CD8+T cells increased followed by a dramatically decreased CD4/CD8 ratio after sepsis ($p < 0.001$). There were no significant differences in B and NK cells. These results indicated that sepsis mainly suppresses the adaptive immune response in critical ICH patients.

Finally, we investigated the association of peripheral immune cells subsets with septic outcomes. As shown in Table 6C, the percentages of CD3+T, CD4+T, CD8+T, and B and NK cells in survival and dead groups had no significant differences. Combined with the results of Logistic and Cox regression analyses, only low NK cells percentages on admission were associated with ensuing sepsis.

Discussions

Our study demonstrated a high incidence of sepsis in critical ICH patients with NK cells deficiency, independent of known risk factors for poor functional outcomes and high mortality death at 90 days. Under acute stress after ICH, patients often had a particular activated adaptive immune system at onset presented by elevated CD4/CD8 ratio. If they had sepsis, unfortunately, those patients suffered from a profound deteriorated immune function.

ICH is one of the most common and severe stroke events that may lead to lifelong disabilities, especially those with low GCS scores. Almost one-third patients with ICH developed infectious complications during hospitalization.⁶ Sepsis, as a complex syndrome, may damage the inflammatory response, leads to organ dysfunction, and be the most life-threatening complication.⁷ Our study found that

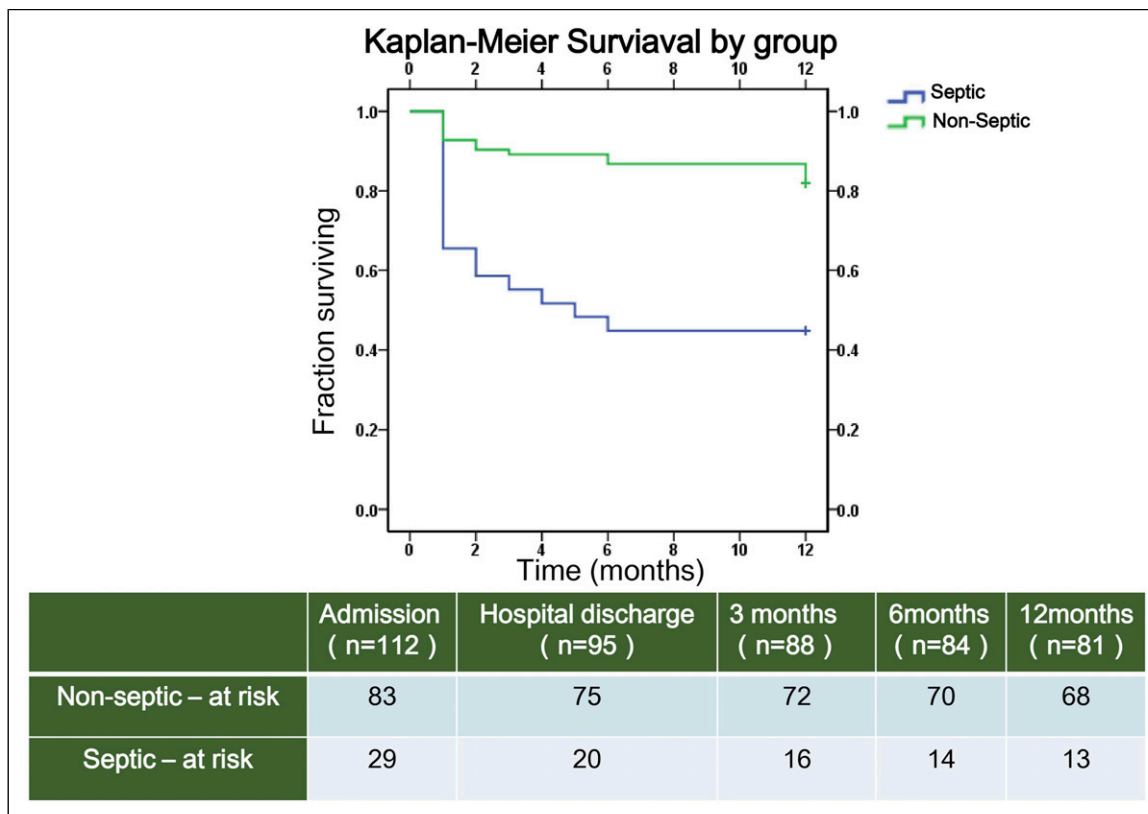


Figure 2. Kaplan–Meier survival curves of septic and nonseptic patients with critical ICH. Sepsis in critical ICH was correlated with a poor prognosis. Log-rank test $p < 0.001$. Time to death or last follow-up in months.

sepsis increased mortality from 9.6% to 31% after ICH, slightly higher than the sepsis-related mortality rate (25%) reported by PRISM meta-analysis data from USA, UK, and Australasia.⁸ Nosocomial pneumonia, urinary tract infection, and blood flow infection often occur in ICH patients and are often manifested as SIRS.⁹ Due to severe primary disturbance of consciousness in critical ICH, sepsis is often challenging to detect and might be covered by SIRS. Although some studies have shown an association between complications of infection and unfavorable outcomes,¹⁰ no one has reported the occurrence and clinical characteristics of sepsis (using Sepsis-3.0 criteria) after critical ICH. The previous definitions, which included the SIRS criteria, were less specific and did not discriminate sepsis from a systemic inflammatory response due to non-infectious causes.¹¹ What explains the poor outcomes and high mortality in critical ICH patients with sepsis? Our findings demonstrated that most septic patients had positive microbiological infections, which means those patients suffered from more severe infections before sepsis. In particular, six patients had postoperative intracranial infection caused by refractory multidrug-resistant bacteria. These patients were

hospitalized for a significantly longer time and prone to fulminant systemic infection. The use of a more aggressive treatment protocol might improve morbidity and mortality rates. In addition, a previous study found that septic patients suffered from sepsis-associated encephalopathy, which also worsened ICH manifestations.¹² Higher Δ SOFA score in the dead group implied severe secondary multiple organ failure. Intervals from admission to sepsis in dead groups were shorter than in survival groups, suggesting that patients with sepsis were more likely to have an early-onset inflammatory storm.

Brain tissue can reciprocally inhibit peripheral immune responses by regulating the hypothalamic–pituitary–adrenal (HPA) axis, sympathetic innervations.¹³ Stroke-associated immune suppression might inhibit the overwhelming brain inflammation but increase the risk of systemic infection because of the disruption of immune defense.^{14,15} The current study adds that immune modulators, such as fingolimod or immunoglobulin, should be administered early in ICH, as long-term use may exacerbate immune suppression.^{16,17} Lymphopenia is increasingly recognized as a consequence of acute illness and may predispose to infections.¹⁸ Most clinical and basic-science researches on the immune consequences of sepsis focused on the roles of macrophages,

Table 4. Predictors of sepsis for critical ICH patients multinomial Logistic regression analysis.

Parameters	B	SEM	Wald	df	Sig	RR	CI-RR	
							LB	UB
Sex-male	-0.106	0.761	0.019	1	0.889	0.9	0.203	3.996
No diabetes	-1.112	1.255	0.785	1	0.376	0.329	0.028	3.85
No bacteremia	-18.892	3453	0.000	1	0.996	6.24×10^{-9}	0.000	^c
No venous catheterization	-3.262	1.025	10.130	1	<0.001*	0.038	0.005	0.286
Emergency neurosurgery	1.383	1.042	1.763	1	0.184	3.988	0.518	30.711
Age								
<40	1.487	2.125	0.49	1	0.484	4.424	0.069	285.083
40-60	0.26	0.839	0.096	1	0.757	1.297	0.250	6.713
>60	0 ^b			0				
GCS score on admission								
3-5	-2.233	1.076	4.305	1	0.038*	0.107	0.013	0.884
6-8	0 ^b			0				
APACHE-II score on admission								
<15	1.927	1.372	1.972	1	0.160	6.866	0.466	101.045
15-20	2.486	1.076	5.337	1	0.021*	12.018	1.458	99.086
>20	0 ^b			0				
CD4/CD8 ratio								
<0.96	-30.252	4.344	0.000	1	0.995	7.2×10^{-14}	0.000	^c
0.96-2.05	-0.854	1.270	0.452	1	0.501	0.426	0.035	5.131
>2.05	0 ^b			0				
CD19+ B lym %								
<10.86%	-0.724	1.362	0.283	1	0.595	0.485	0.034	6.988
10.86%-28.03%	1.942	1.27	0.452	1	0.501	0.426	0.035	5.131
>28.03%	0 ^b			0				
CD16+CD56+ NK %								
<7.92%	10.557	0.124	0.004	1	<0.001*	0.932	0.210	4.128
7.92%-33.99%	-0.071	0.76	0.009	1	0.926	0.821	0.673	6.152
>33.99%	0 ^b			0				

* $p < 0.05$ significant. 0^b, this parameter is redundant, so set it to zero.

Table 5. Comparison between survival versus dead patients with sepsis.

	Survival group N = 20	Death group N = 9	p
Age, years	54.5 (50-62.2)	56 (50-67)	0.602
INR	1.3 (1.2-1.4)	1.3 (1.2-1.4)	0.235
Blood glucose on admission, mmol/L	6.6 (5.7-8.9)	8.7 (5.6-12.4)	0.335
Admission to sepsis time, days	11 (8.3-18.6)	16 (6-25)	0.038*
Length of stay, days	37 (27-67)	30 (21-41)	0.011*
GCS score on admission	8 (6-8)	7.5 (5.5-8)	0.879
APACHE-II score on admission	17 (15-19)	20 (18-22)	0.049*
Δ APACHE-II score	9 (7-11)	10 (7-12)	0.734
Δ SOFA score	5 (3.8-6)	4 (4-7)	0.028*

Values are reported as median (interquartile range).

GCS: Glasgow Coma Scale; APACHE-II: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; Δ APACHE-II score, value-added of APACHE-II score after sepsis; Δ SOFA: score, value-added of SOFA score after sepsis.

* $p < 0.05$ significant.

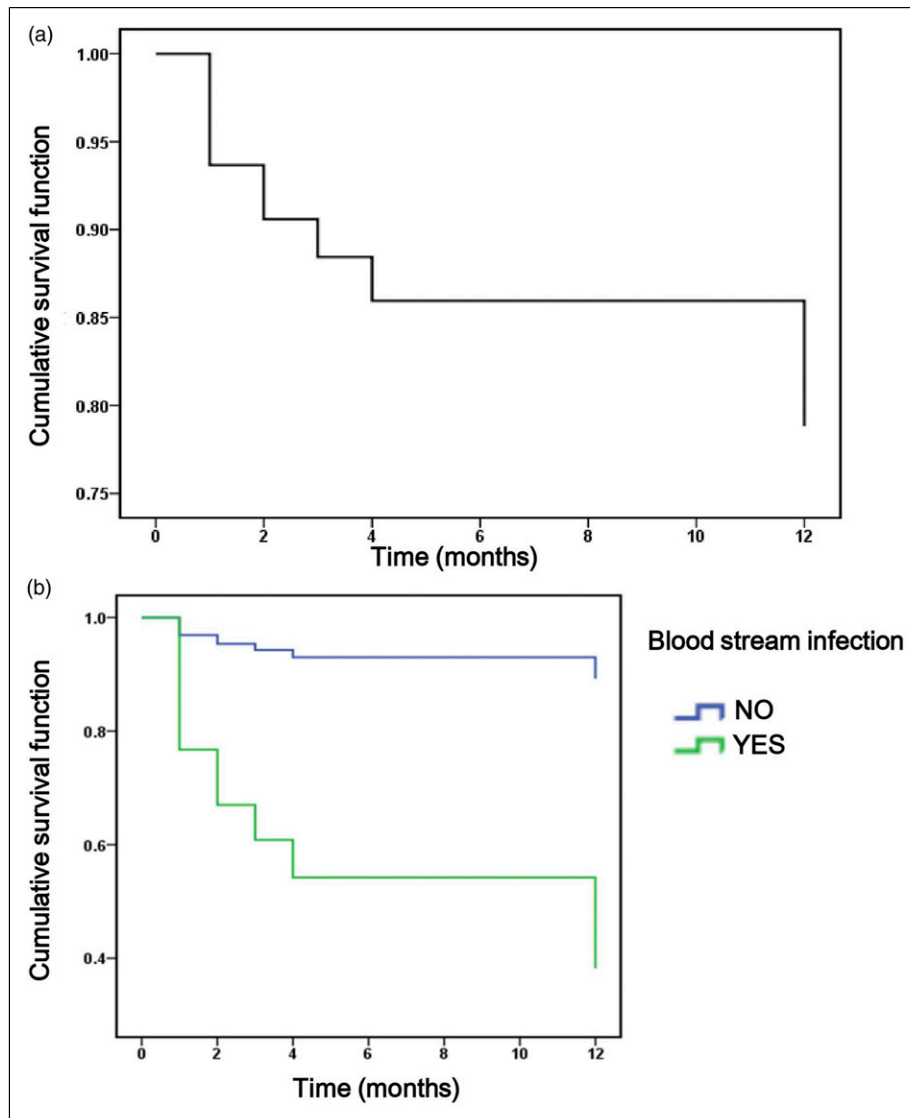


Figure 3. Multivariate Cox survival curves of septic patients with critical ICH. (a) Overall survival status of septic ICH patients. (b) Bloodstream infection was independently associated with the survival status of septic patients. Septic ICH patients without bloodstream infection survived significantly longer than bloodstream infection patients. Time to death or last follow-up in months.

neutrophils, and conventional T lymphocytes.¹⁹ Regarding adaptive immunity, sepsis-induced lymphopenia involves all types of T cells (CD4, CD8, and Natural Killer), thus favoring immunosuppression. Dr. Veltkamp R found that patients with ICH had shrinkage spleen volumes and significant deficiencies of T/NK cells, which were associated with increased infection risk.¹⁶ Dr. Antonella Frattari found that higher CD19 B-cells were significantly associated with ensuing sepsis and lower CD19 B-cells and higher CD8⁺ T-cells proportions might be somehow protected from ensuing sepsis comprehensive ICU.²⁰ Our study showed CD4/CD8 ratio was elevated, and adaptive immune system was activated after ICH. After sepsis, decreased CD4⁺T and NK cells and increased CD8⁺T cells followed by a decreased CD4/CD8 ratio

suggested a severe impaired immune function in those critical ICH patients.

NK cells represent a small proportion (4–15%) of lymphocytes that are classically referred to as the early soldiers of the innate immunity.¹⁹ During the early stage of sepsis, NK cells may play a key role in the promotion of the systemic inflammation by producing INF- γ and IL-10 and inhibits bacterial growth.²¹ At a later stage, NK cells-acquired dysfunction could favor nosocomial infections and mortality.²² Dr. Taisheng Li reported CD8⁺ T increased, and the percentage of NK cells and CD19⁺B cells maintained stable with aging in healthy individuals.²³ The middle aged and the elderly patients account for a large proportion with median age 57 years/o in our study. NK

Table 6. Comparisons of PBLs in different groups.

A. PBLs between septic and nonseptic patients on admission

	Nonseptic group (N = 83)	Septic group (N = 29)	p
CD3+T Abs	693 (458.5–885)	571 (390.5–1020)	0.703
CD3+T lym %	66.1 (57.9–75.7)	67.0 (60.2–72.5)	0.806
CD 3+CD4+T abs	376 (284.5–597.5)	363 (282.5–713)	0.448
CD 3+CD4+T %	44.3 (37.9–50.3)	48.4 (44.0–51.6)	0.157
CD 3+CD8+T abs	207 (135–277.5)	165 (102.5–257)	0.75
CD 3+CD8+T %	21.1 (15.5–23.9)	17.0 (14.0–19.6)	0.021*
CD4/CD8 ratio	2.3 (1.5–3.5)	2.7 (2.4–3.2)	0.153
CD19+ B abs	161 (115.5–245)	230 (125.5–349.5)	0.115
CD19+ B lym %	18.6 (11.4–24.0)	19.2 (16.2–31.4)	0.042*
CD16+CD56+ NK abs	112 (65.5–146)	69 (53.5–141.5)	0.19
CD16+CD56+ NK %	10.9 (7.6–15.7)	8.4 (5.2–11.2)	0.017*

B. PBLs on admission and after sepsis in septic patients

	On admission	After sepsis	p
CD3+T abs	571 (390.5–1020)	488 (389–826.5)	0.674
CD3+T lym %	67.0 (60.2–72.5)	70.0 (63.6–77.3)	0.344
CD 3+CD4+T abs	363 (282.5–713)	314 (206.5–476.5)	0.105
CD 3+CD4+T %	48.4 (44.0–51.6)	41.1 (36.6–45.9)	<0.001*
CD 3+CD8+T abs	165 (102.5–257)	189 (136–346.5)	0.338
CD 3+CD8+T %	17.0 (14.0–19.6)	26.6 (21.2–29.9)	<0.001*
CD4/CD8 ratio	2.7 (2.4–3.2)	1.5 (1.3–1.9)	<0.001*
CD19 ⁺ B abs	230 (125.5–349.5)	145 (94–214)	0.372
CD19 ⁺ B lym %	19.2 (16.2–31.4)	17.6 (12.5–23.9)	0.164
CD16 ⁺ CD56 ⁺ NK abs	69 (53.5–141.5)	63 (39–119)	0.920
CD16 ⁺ CD56 ⁺ NK %	8.4 (5.2–11.2)	8.2 (5.4–11.5)	0.415

C. PBLs after sepsis between survival and dead groups in septic patients

	Survival group (N=20)	Dead group (N=9)	p
CD3+T abs	472 (372–842)	659 (472–659)	0.872
CD3+T lym %	69.6 (63.4–76.9)	75.9 (65.7–78.1)	0.901
CD 3+CD4+T abs	301 (173–484)	397 (287–496)	0.982
CD 3+CD4+T %	40.1 (35.9–44.2)	44.3 (38.7–46.1)	0.481
CD 3+CD8+T abs	181.5 (136.3–323.8)	241 (189–347)	0.941
CD 3+CD8+T %	27.3 (20.9–30.3)	26.6 (25.9–29.8)	0.571
CD4/CD8 ratio	1.45 (1.26–1.73)	1.4 (1.38–1.53)	0.901
CD19 ⁺ B abs	170 (96–238.3)	145 (66–156)	0.342
CD19 ⁺ B lym %	18.5 (12.9–25.3)	13.3 (12.1–16.6)	0.567
CD16 ⁺ CD56 ⁺ NK abs	57 (36–131.5)	66 (63–110)	0.540
CD16 ⁺ CD56 ⁺ NK %	6.9 (5.2–9.6)	9.4 (8.6–13.0)	0.417

Values are reported as median (IQR). Abs, absolute counts; Lym, lymphocytes; NK, natural killer; PBLs, Peripheral blood lymphocytes subsets.

*p<0.05 significant.

cells deficiency increased the susceptibility to infections in those elderly ICH people. In patients with severe Gram-negative sepsis, an increased percentage of blood NK cells could be an improved survival strategy in the patients with high NK counts.²⁴ In our study, significant decreased

percentages of CD3⁺CD8⁺ T cells, B cells, and NK cells with normal absolute counts were observed in septic patients on admission. After sepsis, only the decline of T cells and CD4/CD8 ratio persisted, indicating severe sepsis-induced T cells immunoparalysis. It is reported that the

changes of NK cells mediated early course of sepsis with underlying infection,²⁵ whereas T cells play important roles in the development of cellular and humoral immune responses in the following infection.^{26,27} Recent results of 50 patients with severe sepsis admitted in the ICU showed that NK cell count on day 1 but not on days 3 and 10 post-ICU admission were associated with final outcome.²⁸ In fact, patients who survive from sepsis display long-term immunity impairments with reductions of cell counts and functions. This chronic immunoparalysis status renders sepsis survivors susceptible to infection with new infections.²⁹

Some limitations should be acknowledged in our study. Our results derived from a single center and small sample size might leave us with minimal power to determine the associations between lymphocyte subsets, sepsis, and prognosis. In addition, dynamic changes of lymphocyte subsets were not available in our dataset, and we were not able to reflect the change process of immune function completely for ICH. Therefore, a long recruitment period should continue to expand the sample size and improve the accuracy.

Conclusions

Our data provide preliminary results that adaptive immune system was activated after ICH. NK cells deficiency favored nosocomial threatening sepsis with suppressed immunity. Though it is underpowered to determine a cause-effect relationship, it still provides evidence that improved diagnosis and optimal management of sepsis in ICH patients may have a significant impact on outcomes. Further polycentric evaluations are encouraged to confirm our results and investigate the precise mechanisms of immunosuppression after ICH.

Author Contributions

XHW carried out the concepts, designed, and collected important background information. QW and YF had an equal contribution in that they mainly assisted with data acquisition, data analysis, and manuscript drafting. TBZ carried out the literature search, and JCC completed the interpretation of the data. All authors read and approved the final manuscript.

Declaration of conflicting interests

The authors have no conflicts of interest to declare.

Funding

Funding support was provided by a grant from the National Natural Science Foundation of China (No. 81771280).

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