Original Article

Morphological changes in white blood cells in systemic inflammatory response syndrome (SIRS) with and without sepsis: An observational study

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Abstract

Background: This is an observational study which aims to research morphological changes of white blood cells in patients with Systemic Inflammatory Response Syndrome (SIRS) with and without sepsis and evaluate morphological changes in white blood cells as predictors of sepsis. **Methods:** Patients aged 18 years or more with SIRS with sepsis and SIRS without sepsis were included and those with haematological disorders or pregnant patients were excluded. A total of 52 patients with SIRS with sepsis and 32 patients of SIRS without sepsis were included. Peripheral blood smear was prepared from the venous blood sample drawn. The presence of toxic granules, cytoplasmic vacuoles, and Dohle bodies in both cases of SIRS with sepsis without sepsis were assessed and it was compared with culture-positive sepsis and shock. **Results:** The difference in the presence of toxic granules (55.8% vs. 12.5%; p <0.001), cytoplasmic vacuoles (30.8% vs. 6.3%; p -0.012), and Dohle bodies (17.3% vs. 0%; p = 0.012) was significantly higher in the SIRS with sepsis group, compared to the SIRS without sepsis group. In the subgroup analysis of patients in the sepsis group, it was observed that patients with positive blood culture (9%) had a significantly higher proportion of toxic granules (100% vs. 51.1%; p=0.059), cytoplasmic vacuoles (40% vs. 29.8%; p=0.637) and Dohle bodies (40% vs. 14.9%; p=0.202). However, these differences were not statistically significant. **Conclusion:** Toxic granules and cytoplasmic vacuoles in the neutrophils of patients with SIRS with sepsis were found more frequently, compared to patients of SIRS without sepsis. Dohle bodies were found only in patients with sepsis and not in those with SIRS without sepsis.

Keywords: Dohle bodies, sepsis, SIRS, WBC morphology

Introduction

Sepsis is a major cause of morbidity and mortality worldwide despite immense advancement in its diagnosis and management. It accounts for 28.3% to 41.1% of in-hospital mortality in developed nations and 63.6% in India.[1,2]

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Received: 29-12-2022 **Revised:** 13-02-2023
Accepted: 24-02-2023 **Published:** 30-06-2023

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_2512_22

Blood culture is the gold standard test for diagnosis of sepsis; however, it takes at least 48 to 72 h for its results to be available and requires an appropriate laboratory facility.[3] Early diagnosis and rapid intervention are essential to improve outcomes, which inspired the concept of the Golden hour during which correction of shock and organ dysfunction can improve patient outcomes.[1] Every hour delay in starting therapy results in a 7–10% increase in sepsis-related mortality.[4]

In India, around 70% of the population resides in rural areas.[5] Healthcare in our country is primarily assessed in rural areas

How to cite this article: Sharma S, Pratima K, Ambedkar SN, Kumar R, Kundan M. Morphological changes in white blood cells in systemic inflammatory response syndrome (SIRS) with and without sepsis: An observational study. J Family Med Prim Care 2023;12:1179-84.

through primary healthcare centers (PHCs). It is the first point of contact between the healthcare facility and rural people. According to the Indian Public Health standard, PHC should have laboratories for hemoglobin estimation and rapid diagnostic tests; however, facilities for culture are lacking.[6]

A study conducted by IIT Bombay in 42 PHCs of Osmanabad in Maharashtra found that only 38.72% PHCs had overall preparedness according to IPHS criteria and had a reduced workforce.[7] In another study by the same author, they found staff workload (73.17%), physicians' needs (51.22%), and organization structure (36.59%) as the most prevalent challenges across PHCs.[8]

The rural areas in India report more death (22.9%) than urban areas (18.6%) due to communicable diseases as well as records for maternal, perinatal, and nutritional conditions.[9] Therefore, it becomes necessary that a low-cost screening test is easily available to identify sepsis. As peripheral smear examination is available at PHC and it can be used as an inexpensive and cost-effective modality to screen for sepsis.

The complete blood count and leukocyte morphology have played an integral part in diagnosing sepsis. In a study by Harada *et al.*,[10] the incidence of bandemia (band cell >10%) was 15.6% and the incidence of bacteremia was significantly higher in cases with bandemia (52.3% vs. 13.3%; odds ratio [OR] = 7.15; 95% confidence interval [CI] 4.91–10.5).

The total count and morphology of white blood cells (WBCs) in bacteria and sepsis have been studied in many studies but none of them have compared the morphology of leucocytes in systemic inflammatory response syndrome (SIRS) with sepsis and without sepsis besides the changes in the number of granulocytes.[11-13] Newman *et al.*,[14] in a large study on neonates, found that ratio of immature to total neutrophil count was able to discriminate newborns with pathogenic bacteremia from other newborns.

Increased levels of circulating band cells are found more frequently in patients with sepsis. Further, increased counts of myelocytes and metamyelocytes have prognostic significance in sepsis.[15] The morphological changes in leucocytes are an attractive, easily available, and cheap method to differentiate between SIRS with and without sepsis.

Though WBC morphology is an age-old screening test for sepsis, its usefulness is again re-emphasized in this study as an early screening modality for sepsis in a resource-limited setting, especially in rural areas, which lacks advanced laboratory facilities. Although there are many studies on the subject worldwide, particularly in children and neonates, there are very few studies in India particularly in the adult population.

Hence, this study was planned to compare the morphological changes of WBCs in systemic inflammatory response syndrome (SIRS) with and without sepsis and evaluate morphological changes in WBCs as predictors of sepsis.

Materials and Methods

Study setting-Observational study in the department of Medicine at a tertiary-level hospital in New Delhi.

Study Duration-2017 to 2019.

Study Design-Cross-sectional study.

Sample size calculation

Sample size calculation was based on the percentage of band cells in a peripheral blood smear for the detection of definite sepsis. According to the American College of Chest Physicians/Society of Critical Care Medicine, a band cell count of greater than 10% is used as a criterion for the diagnosis of sepsis. The sensitivity of this test in previous studies ranges between 80% and 90% in detecting sepsis. Therefore, assuming (P) = 84% as the sensitivity of band cells for the detection of definite sepsis, with an optimum cut-off point of 8.5 with a 10% margin of error, the minimum required sample size at a 5% level of significance is 52 patients.[15]

Methodology

Patients aged 18 years or more who were diagnosed with SIRS with sepsis and SIRS without sepsis were included in the study. Pregnant women and those with any hematological disorders were excluded from the study.

A detailed history, physical examination, and investigations were carried out. A total of 52 patients of SIRS with sepsis and 32 of SIRS without sepsis were included in the study.

Definitions

Systemic Inflammatory Response Syndrome (SIRS): According to the American College of Chest Physicians/Society of Critical Care of Medicine Consensus Conference Committee, it is defined as the presence of two or more of the following four criteria:

(1) A body temperature greater than 38°C or less than 36°C
(2) A heart rate greater than 90 beats per min
(3) Tachypnea manifested by a respiratory rate greater than 20 breaths per min or hyperventilation as indicated by a $PaCO_2$ of less than 32 mm Hg
(4) WBC count greater than 12000/mm^3 or less than 4000/mm^3, or the presence of more than 10% immature neutrophils.

Sepsis was defined as patients with SIRS and any suspected or proven signs of infection, as provided in Annexure 1.

Patients who had SIRS but no evidence of any infection were taken as SIRS without sepsis.

A peripheral blood smear was prepared from the venous blood sample drawn by taking aseptic precautions from enrolled

patients within 6 h of admission and examined through a binocular light microscope under oil immersion at a magnification of 10 × 100. A total of 500 WBCs were counted for calculating the percentage of individual white cells for differential leukocyte counts. All observations made were entered into the datasheet for statistical analysis.

The m**ain outcomes** were the presence of toxic granules, cytoplasmic vacuoles, and Dohle bodies in patients of SIRS with and without sepsis and its comparison among blood culture-positive patients in SIRS with sepsis.

Data collection

Data were collected in a predesigned proforma and were coded and entered into an Excel sheet.

Data analysis

Statistical test was conducted with the statistical package for the social science system version SPSS 17.0. Continuous variables are presented as mean ± standard deviation (SD) and categorical variables as absolute numbers and percentages. The comparison of normally distributed continuous variables between the groups was performed using the Student *t*-test. Nominal categorical data between the groups were compared using the Chi-squared test or Fisher's exact test, as appropriate. $P < 0.05$ was considered statistically significant.

Ethics

The Ethics Committee of the Institute had approved the study (s. no. IEC/VMMC/Thesis/October/2017-098), and informed and written consent was taken from the enrolled patients.

Strengthening of reporting of observational study in epidemiology (STROBE) checklist was used for reporting the study.

Results

In our study, patients in two groups showed no statistical significance in the distribution of age and sex, indicating the comparability of the subjects. Table 1 shows the demographic data and vitals of the patients in both groups. The heart rate and respiratory rate were significantly higher in the SIRS with sepsis group ($P < 0.001$); however, the difference in temperature between the two groups was not statistically significant.

The mean total leukocyte count (TLC) count was significantly higher in the SIRS with sepsis group compared to the SIRS without sepsis group and the difference was statistically significant [Figure 1].

Table 1: Demographic profile and vitals of the study subjects in both groups

Characteristics	SIRS with sepsis	SIRS without sepsis	*P*
Age (mean±SD)	46.21±14.13	41.78±12.15	0.145
Male (frequency with %)	22 (42.3%)	8 (25.0%)	0.108
Female (frequency with %)	30 (57.7%)	24 (75.0%)	0.108
Mean heart rate (SD)	107.65 (11.77)	96.25 (10.08)	<0.001
Mean respiratory rate (SD)	21.79 (2.27)	19.75 (2.38)	<0.001
Mean temperature (SD)	101.97 (1.06)	101.42 (0.80)	0.014
Total (*n*)	52	32	

SIRS=Systemic inflammatory response syndrome

Table 2: Comparison of toxic granules, cytoplasmic vacuole and Dohle bodies in SIRS with sepsis and SIRS without sepsis

	SIRS with sepsis		SIRS without sepsis		*P*
	Frequency	%	Frequency	%	
Toxic granules					
Absent	23	44.2%	28	87.5%	<0.001
Present	29	55.8%	4	12.5%	
Cytoplasmic vacuoles					
Absent	36	69.2%	30	93.8%	0.012
Present	16	30.8%	2	6.3%	
Dohle bodies					
Absent	43	82.7%	32	100.0%	0.012
Present	9	17.3%	0	0.0%	

SIRS=Systemic inflammatory response syndrome

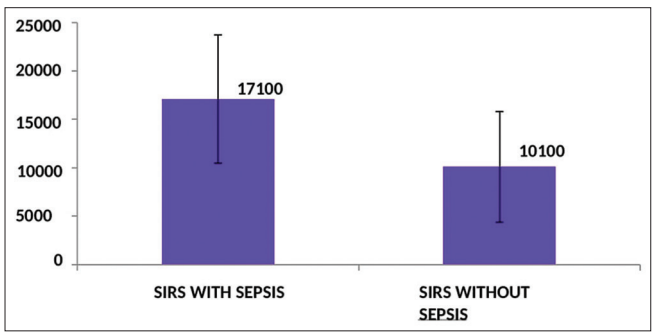

Figure 1: Total leukocyte count in SIRS with and without sepsis

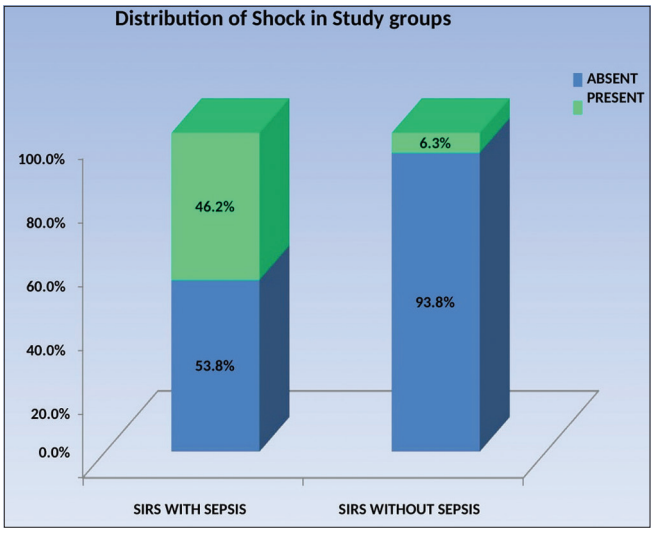

Figure 2: Distribution of shock in SIRS with sepsis and SIRS without sepsis

The presence of toxic granules and cytoplasmic vacuoles in the neutrophils of patients with SIRS with sepsis was significantly more common, compared to patients with SIRS without sepsis. In our study, Dohle bodies were found only in patients with SIRS with sepsis. No Dohle bodies were found in patients of SIRS without sepsis [Table 2].

The shock was present in 24 (46.2%) patients in the sepsis group and 2 (5.3%) in the SIRS without sepsis group, with a statistically significant and higher prevalence of shock in the SIRS with sepsis group [Figure 2].

Blood culture was positive in five (9.6%) patients in the SIRS with sepsis group. The shock was present in all five (100%) cases who had positive blood cultures and in 19 (40.4%) cases with negative blood cultures. The difference was statistically significant [Figure 3].

Of the five patients with positive blood cultures, all had toxic granules (100%), 2 (40%) had cytoplasmic vacuoles, and 2 (40%) had Dohle bodies. However, in the culture-negative SIRS with sepsis group, toxic granules were present in 24 (51.1%) cases, cytoplasmic vacuoles in 14 (29.8%) cases, and Dohle bodies in 7 (14.9%) cases though the difference was not statistically significant [Table 3].

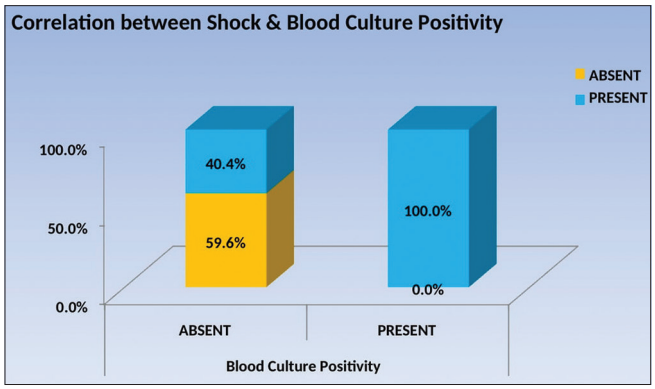

Figure 3: Correlation between shock and blood culture positivity

Table 3: Comparison of toxic granules, cytoplasmic vacuole and Dohle bodies among blood culture-positive cases in SIRS with sepsis

	Blood culture positivity				*P*
	%	Frequency	%	Frequency	
Toxic granules					
Absent	23	48.9%	0	0.0%	0.059
Present	24	51.1%	5	100.0%	
Cytoplasmic vacuoles					
Absent	33	70.2%	3	60.0%	0.637
Present	14	29.8%	2	40.0%	
Dohle bodies					
Absent	40	85.1%	3	60.0%	0.202
Present	7	14.9%	2	40.0%	

SIRS=Systemic inflammatory response syndrome

Discussion

In our study, patients in two groups showed no statistical significance in the distribution of age and sex, indicating their comparability. The subjects with SIRS with sepsis, compared to those with SIRS without sepsis, showed a significant difference in TLC, heart rate, and respiratory rate but not in temperature, possibly indicating the greater specificity of these three criteria for sepsis.

The TLC was higher in the sepsis group than in the SIRS without sepsis group and the difference was statistically significant. We could not find any study comparing TLC in patients with sepsis with SIRS and SIRS without sepsis. Gwaiz *et al.*[16] studied morphological changes of neutrophils in 105 patients with culture-proved bacterial infection and observed that absolute neutrophil count was the best predictor of bacterial infection, followed by toxic granules, band cells and Dohle bodies, neutrophil vacuoles were the least sensitive.

The difference in the presence of toxic granules in the two groups was significantly higher in the SIRS with sepsis than in SIRS without sepsis group (55.8% vs. 12.5%; $P < 0.001$). Similarly, cytoplasmic vacuoles were observed in significantly higher proportions in patients with SIRS with sepsis than in the SIRS without sepsis (30.8% vs. 6.3%; $P = 0.012$). Dohle bodies were observed only in patients with sepsis and not in any patient of SIRS without sepsis and the difference was statistically significant (17.3% vs. 0%; $P = 0.012$). We could not find any study comparing toxic granules, cytoplasmic vacuoles, and Dohle bodies in patients with SIRS with sepsis and those with SIRS without sepsis. However, Mare *et al.* found a higher percentage of band cells in patients with SIRS with sepsis than in patients with non-infectious SIRS and healthy controls ($P < 0.001$).[15] Malcolm *et al.*[17] also found cytoplasmic vacuolization to be more in bacteremia patients, which is in accordance with our study. Our finding indicates that there is a difference in the morphology of neutrophils in patients of SIRS with sepsis and SIRS without sepsis though further studies are required to substantiate.

A significant difference was observed in the presence of shock between the two groups, shock being higher in patients of SIRS with sepsis than in the group with SIRS without sepsis (46.2% vs. 5.3%; $P < .001$). Campos *et al.*[18] had also observed that the average number of vacuoles was significantly higher in the shock group than in the non-shock group.[18]

In the subgroup analysis of patients in the sepsis group, it was observed that patients with positive blood culture (9%) had a significantly higher proportion of toxic granules (100% vs. 51.1%), cytoplasmic vacuoles (40% vs. 29.8%) and Dohle bodies (40% vs. 14.9%). However, these differences were not statistically significant. In another study by Jafri and Kass[19] a high correlation between vacuolated neutrophils and culture-positive infection was found.

Early identification and prompt treatment remain the cornerstone of sepsis management and reducing its morbidity and mortality,

thereby decreasing the overall burden of sepsis-related hospitalization. Peripheral smear is a simple and easily available investigation that can help in the early recognition of sepsis. As neutrophils are the key cells acting against invading pathogens, morphological changes in them can aid in diagnosing sepsis.

Limitations of the study

Our study had a small number of study subjects and our institution, being a tertiary care facility, receives many patients who have already been treated before coming to our institute.

Conclusion

Our study indicates that morphological changes in neutrophils can help differentiate patients with SIRS with sepsis from SIRS without sepsis. If the results are supported by more studies, this can be a useful tool in the early management of septic patients while awaiting the results of culture reports, especially in a rural and resource-limited setting.

Key Points

- WBC morphology changes are an early marker of sepsis
- Leukocyte morphological changes are significantly associated with sepsis with SIRS
- It is an easily available and cost-effective method, which can be used in a rural setting to screen for sepsis.

Take home message

Leukocyte morphology on peripheral smear can be used for screening sepsis in a resource-poor setting.

Acknowledgement

To all the patients who participated in this study, and all the staff of our concern departments who sincerely served in the interest of patient care and management.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Urrechaga E. Reviewing the value of leukocytes cell population data (CPD) in the management of sepsis. Ann Transl Med 2020;8:953.
2. Chatterjee S, Bhattacharya M, Todi SK. Epidemiology of adult-population Sepsis in India: A single center 5 year experience. Indian J Crit Care Med 2017;21:573-7.
3. Sampath S, Baby J, Krishna B, Dendukuri N, Thomas T. Blood cultures and molecular diagnostics in intensive care units to diagnose sepsis: A Bayesian latent class model analysis. Indian J Crit Care Med 2021;25:1402-7.
4. Shashikumar SP, Stanley MD, Sadiq I, Li Q, Holder A, Clifford GD, Nemati S. Early sepsis detection in critical care patients using multiscale blood pressure and heart rate dynamics. J Electrocardiol 2017;50:739-43.
5. Office of Registrar General and Census Commissioner. Census 2011: Population Enumeration Data. Ministry of Home Affairs (India), New Delhi. 2011. Available from: https://censusindia.gov.in/census.website/. [Last Accessed on 2023 Feb 06].
6. Ministry of Health and Family Welfare.Indian Public Health Standards (IPHS) for primary health centres revised guidelines 2012.Available from: https://nhm.gov.in>iphs-revised-guidelines-2012. [Last accessed on 2012 Feb12].
7. Jain R, Rao B. Assessment of Primary Laboratory Facilities for Rural Health Care Preparedness in Osmanabad District, India. SocArXiv 2019.Available from: https://osf.io>xapzj. [Last accessed on 2023 Feb 12].
8. Jain R, Rao B. Role of laboratory services in primary health center (PHC) outpatient department performance: An Indian case study. Prim Health Care Res Dev 2019;20:e112.
9. Office of Registrar General and Census Commissioner. Sample Registration system statistical report 2018. New Delhi (India): MHA; 2018. Available from: https://censusindia.gov.in/census.website/data/SRSSTAT. [Last accessed on 2023 Feb 06].
10. Harada T, Harada Y, Morinaga K, Hirosawa T, Shimizu T. Bandemia as an early predictive marker of bacteremia: A retrospective cohort study. Int J Environ Res Public Health 2022;19:2275.
11. Sahu P, Bhake A. Morphological abnormalities of leucocytes and leucocytic indices in patient of clinical septicemia. Ann RSCB 2021;25:3880-8.
12. Nierhaus A, Klatte S, Linssen J, Eismann NM, Wichmann D, Hedke J, *et al.* Revisiting the white blood cell count: Immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis--a prospective, observational study. BMC Immunol 2013;14:8.
13. Agnello L, Giglio RV, Bivona G, Scazzone C, GambinoCM, Iacona A, *et al.* The value of a complete blood count (CBC) for sepsis diagnosis and prognosis. Diagnostics 2021;11:1881.
14. Newman TB, Draper D, Puopolo KM, Wi S, Escobar GJ. Combining immature and total neutrophil counts to predict early onset sepsis in term and late preterm newborns: Use of the I/T2. Pediatr Infect Dis J 2014;33:798-802.
15. Mare TA, Treacher DF, Shankar-Hari M, Beale R, Lewis SM, Chambers DJ, *et al.* The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. Crit Care 2015;19:57.
16. Al-Gwaiz LA, Babay HH. The diagnostic value of absolute neutrophil count, band count and morphologic changes of neutrophils in predicting bacterial infections. Med Princ Pract 2007;16:344-7.
17. Malcolm ID, Flegel KM, Katz M. Vacuolization of the neutrophil in bacteremia. Arch Intern Med 1979;139:675-6.
18. Campos JR, Parreirra JG, Rezende-Neto JB, de Abreu EMS, Thais A, *et al.* Neutrophil vacuolization in peripheral blood smear assessed with maygrunwald-giemsa stain has direct correlation with the severity of hemorrhagic shock and serum lactate in trauma patients. Int J Med Sci 2017;9:92-9.
19. Jafri AK, Kass L. Vacuolated neutrophils can predict serious infection. Lab Med 1998;29:633-36.

Annexure 1

Diagnostic Criteria for Sepsis

Infection, documented or suspected, and some of the following:

General variables
- Fever (>38.3°C)
- Hypothermia (core temperature <36°C)
- Heart rate >90/min–1 or more than 2 SD above the normal value forage
- Tachypnea
- Altered mental status
- Significant edema or positive fluid balance (>20 mL/kg over 24 h)
- Hyperglycemia (plasma glucose >140 mg/dL or 7.7 mmol/L) in the absence of diabetes.

Inflammatory variables
- Leukocytosis (WBC count >12,000 μL^{-1})
- Leukopenia (WBC count <4,000 μL^{-1})
- Normal WBC count with greater than 10% immature forms
- Plasma C-reactive protein more than 2 SD above the normal value
- Plasma procalcitonin more than 2 SD above the normal value.

Hemodynamic variables
- Arterial hypotension (SBP <90 mm Hg, MAP <70 mm Hg, or an SBP decrease >40 mm Hg in adults or less than two sd below normal forage).

Organ dysfunction variables
- Arterial hypoxemia (PaO_2/FiO_2 <300)
- Acute oliguria (urine output <0.5 mL/kg/h for at least 2 h despite adequate fluid resuscitation)
- Creatinine increase >0.5 mg/dL or 44.2 μmol/L
- Coagulation abnormalities (INR > 1.5 or aPTT >60 s)
- Ileus (absent bowel sounds)
- Thrombocytopenia (platelet count <100,000 μL^{-1}).

Hyperbilirubinemia (plasma total bilirubin >4 mg/dL or 70 μmol/L) tissue perfusion variables
- Hyperlactatemia (>1 mmol/L)
- Decreased capillary refill or mottling.

WBC = white blood cell; SBP = systolic blood pressure; MAP = mean arterial pressure; INR = international normalized ratio; aPTT = activated partial thromboplastin time.

Severe sepsis definition = sepsis-induced tissue hypoperfusion or organ dysfunction (any of the following thought to be due to the infection)

Sepsis-induced hypotension
Lactate above upper limits laboratory normal
Urine output <0.5 mL/kg/h for more than 2 h despite adequate fluid resuscitation
Acute lung injury with PaO_2/FiO_2 <250 in the absence of pneumonia as an infection source
Acute lung injury with PaO_2/FiO_2 <200 in the presence of pneumonia as an infection source
Creatinine >2.0 mg/dL (176.8 μmol/L).
Bilirubin > mg/dL (34.2 μmol/L)
Platelet count <100,000/μL
Coagulopathy (international normalized ratio > 1.5).