Original Article

Methemoglobin Level May Not Predict Organ Failure in Sepsis

Pimwatana Pinsem MD, FRCAT[1], Sahawat Thertchanakun MD, FRCAT[1], Detajin Junhasavasdikul MD[2]

[1] Department of Anesthesiology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2] Department of Internal Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Objective: To evaluate the sensitivity, specificity, and area under receiver operating characteristics (AUROC) of methemoglobin levels to predict worsening sequential organ failure assessment (SOFA) scores at 72 hours in sepsis and septic shock in the intensive care setting.

Materials and Methods: The authors conducted a prospective pilot study in a single university hospital. The present study subjects were adults aged more than 19 years, sepsis and septic shock patients admitted to intensive care units, with an arterial line in place. All patients' arterial blood samples were collected and sent to the central laboratory to analyze for methemoglobin levels at the enrolment time, then 12 and 24 hours later. Patient's characteristics, SOFA scores and other related parameters at enrolment, 12, 24, and 72 hours later were extracted through electronic medical records. Higher SOFA score at 72 hours, comparing to the enrolment value, was considered as a reference standard.

Results: Among 30 patients, seven had worsening SOFA scores. Initial methemoglobin level had an AUROC of 0.5404 (95% confidence interval 0.2603 to 0.8204). Using Youden's index, the determined cut-off value was 0.75%. The sensitivity of this cut-off value was 71.4% (95% confidence interval 29 to 96.3) and the specificity was 52% (95% confidence interval 30.6 to 73.2).

Conclusion: Initial Methemoglobin level had an insufficient AUROC of 0.5404 to predict worsening organ failure in critically ill patients with sepsis. As the present study is a pilot study, a larger scale study may be required.

Keywords: Sepsis; Methemoglobin; Organ Failure; SOFA; Septic shock

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Sepsis, a consequence of severe infection, has a high mortality rate ranging from 23% to 35%[1-3]. An explanation of this high level of mortality rate is the host reaction to microbials leading to the dysfunction of organs such as sepsis-related organ dysfunction[1]. Reactive oxygen species (ROS) and redox reactions are believed to promote this dysfunction[2]. After the infection, human's innate immunity, which consists of neutrophils, macrophages, and endothelial cells, reacts by initiating oxidative burst to produce ROS, to kill the infecting organisms. These ROS also have a negative impact on multiple organ systems by inducing microvascular dysfunction, cellular apoptosis, and endothelial dysfunction[3].

Progressive organ dysfunction after sepsis has no clear definitions. Currently, the tool widely accepted for assessing organ dysfunction in sepsis is sequential organ failure assessment (SOFA)[4]. Predicting an organ failure trend after sepsis is still not established, especially from a redox perspective. A redox marker that was previously studied in sepsis is methemoglobin (MetHb). A single study had found that patients with sepsis had higher MetHb level than the patients without sepsis[5]. MetHb is a result of ROS reducing iron (Fe^{2+}) in hemoglobin to reduced iron (Fe^{3+})[2,6]. In addition, MetHb measurement is readily available by co-oximetry arterial blood gas analyzer. Therefore, MetHb level could theoretically be a parameter to predict sepsis-related organ dysfunction.

The objective of the present study was to evaluate sensitivity, specificity, and area under receiver operating characteristics (AUROC) of MetHb to predict worsening organ failure 72 hours later in sepsis and septic shock patients admitted to intensive care units (ICU). The authors proposed that higher MetHb level could predict worsening organ failure

Correspondence to:

Thertchanakun S.

Department of Anesthesiology, Ramathibodi Hospital, Mahidol University, 270 Rama VI Road, Toong Phayathai, Ratchatewi, Bangkok 10400, Thailand.

Phone: +66-86-9626827, **Fax**: +66-2-2011569

Email: sahawatt@gmail.com

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72 hours later in sepsis and septic shock patients admitted to ICU.

Materials and Methods

The present study was a prospective study, conducted at Ramathibodi Hospital, Thailand. After an approval from the Ethical Committee (MURA2020/573), the authors start enrolling the patients admitted to the ICU. Inclusion criteria were adult patients, older than 19 years old, diagnosed with sepsis or septic shock according to sepsis-3 criteria[1] having an arterial line in place. The patients had received either nitric oxide donor drugs such as isosorbide dinitrate, or nitroglycerin, or drugs known to induce methemoglobinemia (e.g., sulfonamide, chloroquine) before the enrolment and the patients with do-not-resuscitate orders in the medical record were excluded because an enrolment of the former patients would interfere with MetHb level measurement.

Blood samples were collected from each patient's arterial line at the time of enrolment, 12 hours and 24 hours later. Those samples were sent to the hospital laboratory and analyzed for MetHb levels by Meditop Critical Care Xpress (CCX)® co-oximetry arterial blood gas analyzer. Patients' baseline characteristics were collected at the time of enrolment. During the ICU stays, patient cares were decided by the attending intensivists, not involved in the present research. Patient's data including SOFA scores, lactate, fluid intake, vasoactive drugs, hemoglobin, partial pressure of oxygen to fractional inspired oxygen (P/F) ratios at enrolment, 12, 24 and 72 hours after enrolment were extracted through electronic medical record reviews. Moreover, respiratory support status, renal replacement therapies applied, mortality and length of ICU stays, were tracked in a similar fashion.

Since, there was no data available regarding the outcome of interest, the present study was conducted as a pilot study, aiming to collect data from 30 patients. The primary outcome was the receiver operating curve (ROC) analysis of MetHb level to predict worsening of SOFA score at 72 hours later. The definition of worsening SOFA was defined as at least a point increase in the SOFA score assessed at 72 hours after enrolment, comparing with the baseline SOFA score. The single point of difference was deemed clinically significant according to a previous report on organ failure pattern in sepsis and septic shock patients[7,8].

Statistical analyses were performed by Stata, version 14 (StataCorp LP, College Station, TX, USA). Categorical data were presented as n (%) and analyzed by Fisher's exact test. Continuous data were presented as mean ± standard deviation (SD) or median (interquartile range) as appropriate. Continuous data were analyzed by Student's t-test if normal distribution was assumed, otherwise those data were analyzed by Mann-Whitney U test. ROC curve and AUROC were used to assess sensitivity and specificity of MetHb level for predicting worsening SOFA score. Changes of MetHb levels over time were analyzed by Wilcoxon signed-rank test. Statistical significance was determined by p-value less than 0.05.

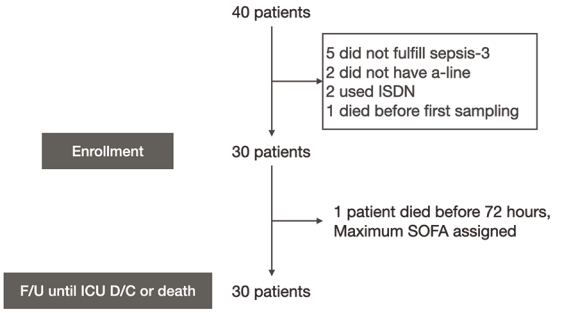

Figure 1. Study flow.

a-line=arterial line; D/C=discharge; F/U=follow up; ICU=intensive care unit; ISDN=isosorbide dinitrate; SOFA=sequential organ failure assessment score

Results

Between July and December 2020, the authors screened 40 patients admitted to the ICU, as shown in Figure 1. Most patients were admitted in medical ICUs, diagnosed with respiratory tract sepsis.

Baseline characteristics were similar both in the worsening SOFA and non-worsening SOFA groups, except that non-worsening SOFA group was older than worsening SOFA group (Table 1). Initial MetHb level did not change significantly over the first and next 12 hours as shown in Table 2. Dividing the value collected into worsening SOFA and non-worsening SOFA group, at initial, 12-, and 24-hour time points, the MetHb levels were also similar with p=0.749, 0.554, and 0.235, respectively (Figure 2). Diagnostic capability of initial MetHb level were assessed using a ROC curve, an AUROC of initial MetHb was 0.5404 (95% confidence interval [CI] 0.2603 to 0.8204). The optimal cutoff was chosen by using Youden index, using a cut point of 0.75, initial MetHb level had 71.4% sensitivity (95% CI 29 to 96.3) and 52% specificity (95% CI 30.6 to 73.2) to detect worsening SOFA (Figure 3). With 23% prevalence, this cutoff point provided a positive predictive value (PPV) of

Table 1. Baseline characteristics of the patients at the enrolment

Characteristics	Overall (n=30)	Non-worsening SOFA (n=23)	Worsening SOFA (n=7)	p-value
Age (years)[a]	68±14	70±15	60±5	0.017
Weight (kg)[a]	60.8±15.5	61.09±16.65	59.86±11.88	0.41
Height (cm)[a]	163.5±6.94	162.78±6.81	165.86±7.36	0.313
Sex[b]				1
Male	19 (63.33)	14 (60.87)	5 (71.43)	
Female	11 (36.67)	9 (39.13)	2 (28.57)	
ICU type[b]				0.29
Medical	24 (80.00)	17 (73.91)	7 (100)	
Surgical	6 (20.00)	6 (26.09)	0 (0.00)	
Time from hemoculture* (hours)[c]	6 (2, 9)	6 (1, 9)	4 (3, 7)	0.639
APACHE II[a]	19±7	18±7	22±8	0.28
SOFA[a]	10±4	10±5	9±3	0.524
Source[b]				0.63
Respiratory system	18 (60.00)	12 (52.17)	6 (85.71)	
Intra-abdominal	10 (33.33)	9 (39.13)	1 (14.29)	
Central nervous system	1 (3.33)	1 (4.35)	0 (0.00)	
Intravenous catheter-related	1 (3.33)	1 (4.35)	0 (0.00)	
Organism identified[b]				0.85
Gram positive cocci	6 (20.00)	4 (17.39)	2 (28.57)	
Gram negative bacilli	8 (26.67)	6 (26.09)	2 (28.57)	
None	16 (53.33)	13 (56.52)	3 (42.86)	
Crystalloid volume (mL)[c]	550 (0, 1,000)	600 (0, 2,100)	500 (0, 800)	0.442
Colloid volume (mL)[c]	0 (0, 250)	0 (0, 250)	0 (0, 250)	0.639
P/F ratio[a]	293.83±116.89	298.96±116.85	277±124.69	0.671
Mean arterial pressure (mmHg)[a]	79±16	78±16	84±18	0.357
Norepinephrine (mcg/kg/minute)[c]	0.07 (0, 0.16)	0.07 (0, 0.17)	0.06 (0, 0.15)	0.729
Hemoglobin (g/dL)[a]	9.6±2.2	9.9±2.2	8.5±2.2	0.17
Lactate (mmol/L)[c]	3.01 (1.8, 5.95)	3 (1.6, 4.5)	5.95 (2.16, 10.6)	0.148

APACHE II=Acute Physiology and Chronic Health Evaluation II score; ICU=intensive care unit; P/F ratio=PaO_2 to FiO_2 ratio; SOFA=sequential organ failure assessment score

[a] Mean ± standard deviation and analyzed by Student's t-test except specified, [b] Number (%) and analyzed by Fisher's exact test, [c] Median (interquartile range) and analyzed by Mann-Whitney U test

* Time from hemoculture is the duration from the hemoculture-taking time to the enrolment time, this parameter represents time elapsed since the onset of sepsis (time zero according to the surviving sepsis campaign guideline[9])

Table 2. Methemoglobin level in the study

	Overall (n=30); median (IQR)	Non-worsening SOFA (n=23); median (IQR)	Worsening SOFA (n=7); median (IQR)	p-value
Initial MetHb	0.8 (0.5, 1.3)	0.7 (0.5, 1.3)	0.9 (0.4, 1.4)	0.749
MetHb at 12 hours	0.8 (0.5, 1.2)	0.8 (0.5, 1.2)	0.6 (0.2, 1.5)	0.554
MetHb at 24 hours	0.7 (0.4, 0.8)	0.8 (0.4, 1)	0.6 (0.3, 0.7)	0.235

IQR=interquartile range; MetHb=methemoglobin level; SOFA=sequential organ failure assessment

Statistical test between groups by Wilcoxon rank-sum test

31.3% (95% CI 11 to 58.7) and a negative predictive value (NPV) of 85.7% (95% CI 57.2 to 98.2).

When compared initial MetHb with other commonly used parameters, the initial lactate level and APACHE II score outperformed initial MetHb in detecting worsening SOFA with an AUROC of 0.6832 (95% CI 0.4905 to 0.957) and 0.6584 (95% CI 0.4356 to 0.8812), respectively (Figure 4).

During the 72-hour period, both worsening SOFA and non-worsening SOFA groups received similar crystalloid intravenous volume, also a similar proportion of patients in both groups received blood

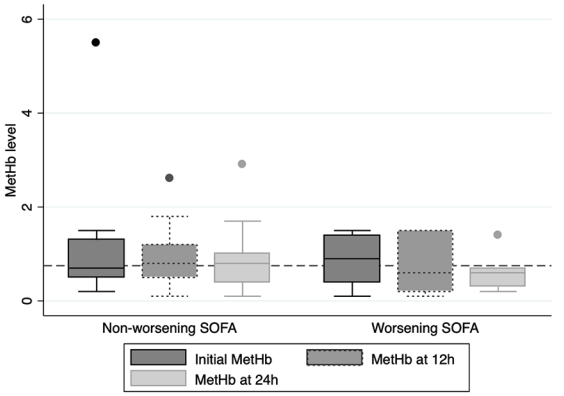

Figure 2. Methemoglobin levels during initial, 12- and 24-hour periods.

Bars and boxes represent median and interquartile ranges, whiskers represent maximum, and minimum values, dots are outlier values. Dotted line is a reference value of 0.75%.

MetHb=methemoglobin level

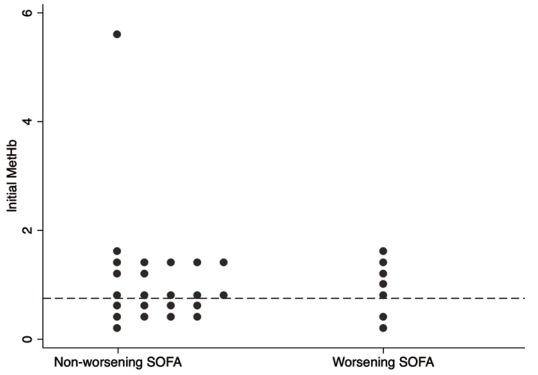

Figure 3. Using a cut point of 0.75% of initial methemoglobin level to detect worsening SOFA.

Each dot represents patient's initial methemoglobin level. Dotted line is a cut point of 0.75.

MetHb=methemoglobin level

components. Worsening SOFA group had been given more colloid intravenous fluid in the 12- and 24-hour period at 750, 970 mL versus 250, 390 mL in non-worsening SOFA group. Moreover, worsening SOFA group were also receiving higher vasoactive drugs at the 24- and 72- hour time points (Table 3).

At ICU discharge, patients in both groups had received equal respiratory supports and renal replacement therapies. They also spent the same amount of time in ICU. However, larger proportions of patients in worsening SOFA group died at 71.43% versus 4.35%, p=0.001 (Table 4).

Discussion

The present study main objective was to assess

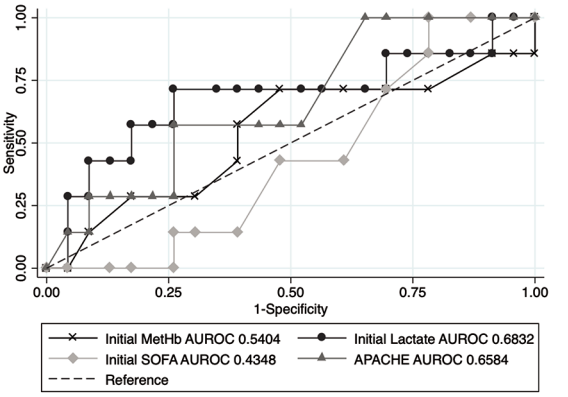

Figure 4. Receiver operating characteristics (ROC) curves of initial methemoglobin, initial lactate, initial SOFA score, and APACHE II score to detect worsening SOFA.

AUROC=area under ROC curve; MetHb=methemoglobin level

Table 3. Fluid intake, vasopressors, and blood components given during the first 72 hours

Fluid or vasopressors	Non-worsening SOFA (n=23)	Worsening SOFA (n=7)	p-value
Crystalloid intake (mL)[a]			
At 12 hours	2,095 (1,110, 3,065)	3,190 (1,258, 6,822)	0.249
At 24 hours	3839 (2,307, 4,763)	5,255 (2,656, 8,076)	0.101
At 72 hoursb	8,024±3,038	9,445±4,501	0.342
Colloid intake (mL)[a]			
At 12 hours	250 (0, 700)	750 (530, 1,100)	0.018
At 24 hours	390 (0, 700)	970 (777, 1,610)	0.025
At 72 hours	500 (0, 1,700)	1,220 (850, 2,170)	0.08
Norepinephrine (mcg/kg/minute)[a]			
At 12 hours	0.06 (0, 0.13)	0.23 (0, 0.98)	0.28
At 24 hours	0 (0, 0.07)	0.17 (0, 1.36)	0.035
At 72 hours	0 (0, 0)	0.07 (0, 1.36)	0.003
Epinephrine (mcg/kg/minute)[a]			
At 12 hours	0 (0, 0)	0 (0, 0)	0.395
At 24 hours	0 (0, 0)	0 (0, 0.41)	0.007
At 72 hours	0 (0, 0)	0 (0, 0.39)	0.001
Receiving blood components[c]			
At 12 hours	9 (39.13)	5 (71.43)	0.204
At 24 hours	11 (47.83)	6 (85.71)	0.104
At 72 hours	12 (52.17)	6 (85.71)	0.193

SOFA=sequential organ failure assessment score

[a] Median (interquartile range) and analyzed by Mann-Whitney U except specified, [b] Mean ± standard deviation and analyzed by Student's t-test, [c] Number (%) and analyzed by Fisher's exact test

the diagnostic ability of MetHb level to predict worsening organ failure at the 72-hour time point. The authors considered SOFA score change as a "gold standard" diagnosis of organ failure in sepsis as this tool has been recognized in sepsis-3 criteria[1]. The time point, 72 hours after ICU admission was

Table 4. Outcomes at ICU discharge

	Non-worsening SOFA (n=24)	Worsening SOFA (n=7)	p-value
Respiratory support status[a]			0.078
None	10 (43.48)	0 (0.00)	
Conventional oxygen therapy	2 (8.70)	0 (0.00)	
High flow nasal cannula	1 (4.35)	0 (0.00)	
Noninvasive ventilation	1 (4.35)	0 (0.00)	
Invasive mechanical ventilation	9 (39.13)	7 (100)	
RRT status[a]			0.245
None	19 (82.61)	5 (71.43)	
Chronic RRT	3 (13.04)	0 (0.00)	
New acute IHD	1 (4.35)	2 (28.57)	
Death at ICU discharge[a]	1 (4.35)	5 (71.43)	0.001
ICU Length of stay (hours)[b]	182 (104, 360)	144 (39, 290)	0.391

SOFA=sequential organ failure assessment score; RRT=renal replacement therapy; IHD=intermittent hemodialysis; ICU=intensive care unit

[a] Number (%) and analyzed by Fisher's exact test except specified,
[b] Median (interquartile range) and analyzed by Mann-Whitney U test

selected according to the ANDROMEDA-SHOCK study[10] to explore the progressing organ dysfunction after sepsis. To the authors' knowledges, MetHb had never been studied in this perspective before, so the authors decided to do the present pilot study. The results were the initial MetHb level of more than 0.75% had 71.4% sensitivity (95% CI 29 to 96.3) and 85.7% NPV (57.2 to 98.2) in the present population groups. The present study finding may indicate that initial MetHb level could be used as a screening tool for predicting organ failure. However, the present study conclusion should be interpreted with caution as the 95% CI was wide, which could be the effect of small sample size.

Andrades et al stated that redox reactions could be a key factor contributing to organ failure in sepsis and septic shock[2]. However, oxidative stress biomarkers and antioxidant levels are not clinically available to use as patient care tools. By using MetHb, a byproduct of redox reactions could be measured in clinical routines. The findings of the present study could be viewed as an indirect measurement of redox reactions to organ failure in sepsis.

Ohashi et al have presented that sepsis and septic shock patients in ICU had MetHb level at about 1%[5]. Median initial MetHb level in the present study was 0.8%, less than the previously reported. This lower value could be interpreted as "normal" in the ICU setting, thus leading to the inability to distinguish worsening from non-worsening SOFA patients.

Previous reports that studied organ dysfunction outcomes in patients with sepsis and septic shock focused on lactate and lactate-derived parameters such as lactate clearance and lactate-albumin ratios. Those findings were in similar directions where lactate, and lactate-derived parameters could be used to predict organ dysfunction in sepsis, which leaded to poor outcomes such as death[11-13]. Those studies used other definitions of organ dysfunction, without prespecified the timing to detect the organ failure outcome. The present study results on lactate, although not in the prespecified plan of analysis, were consistent with those studies, suggesting lactate as a useful parameter to predict organ failure. In the present study, patients with worsening SOFA score received more colloid fluid in the first 12 and 24 hours, and more vasoactive drugs at the 24-, and 72-hour time point. These could be representing an organ failure "symptom" of vessels, which interpreted as systemic vasodilation. Another theory was that human albumin solution could bind MetHb leading to measurement errors, although there are no studies available to support this basis.

As a screening tool, lactate, a marker of tissue hypoxia, can be observed to outperform MetHb, a marker of redox reaction in the present study. This could be explained by hypotheses that redox reaction affects organ functions clinically less than expected or the authors have chosen an inappropriate marker for redox reactions in sepsis. Another hypothesis is that MetHb as a redox marker could be detected in a specific group of sepsis, such as gram-negative bacteria sepsis that could lead to high endotoxin and cytokine levels, then consequentially induce redox reactions.

The present study should be viewed as an introduction of clinical uses of redox biomarkers in sepsis. The authors carefully selected patients so that factors affecting MetHb level measurements are excluded. All patients were assessed at a similar time point after enrolment. By using the present data, the calculated sample size needed to achieve AUROC 0.7 with an α-level of 0.05, and 80% power, would be 69 patients.

The present main study limitation was the small sample size, as the present study is a pilot study, which may be under-powered to detect the actual difference between groups. Another limitation is choosing only a single marker of redox reaction so MetHb may not be an optimal representation of redox reactions in sepsis. Moreover, the optimal time to collect blood samples is not known, as MetHb has short half-lives. The present MetHb result may not reflect the exact MetHb at the onset of sepsis. Finally, the present study patients were

infected with different groups of organisms, which could lead to various levels of cytokine and unequal redox consequences.

In summary, the initial MetHb level showed insufficient AUROC of 0.5404 (95% CI 0.2603 to 0.8204) to diagnose worsening organ failure 72 hours later to sepsis and septic shock patients admitted in ICUs. However, due to wide CIs, MetHb should be further studied.

Conclusion

Initial MetHb level had an insufficient AUROC of 0.5404 to predict worsening organ failure in critically ill patients with sepsis. As the present study is a pilot study, a larger scale study may be required.

What is already known on this topic?

Organ failure progression is a detrimental consequence in sepsis, related to redox reactions. MetHb is a byproduct of redox reactions and has been shown on the previous data to be higher in sepsis patients.

What this study adds?

Initial MetHb level in critically ill patients with sepsis cannot predict worsening organ failure as detected by increasing SOFA.

Conflicts of interest

The authors declare no conflict of interest.

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