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Original Article

Flow Cytometric Measurement of Respiratory Burst Activity and Surface Expression of Neutrophils for Septic Patient Prognosis

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Background: The hyperinflammation that begins with sepsis is essential for eradicating infection but also causes hypoperfusion and organ failure. To understand the innate immune status of septic patients, the functional and phenotypic changes of neutrophils during sepsis and their clinical implication were studied.

Methods: Seventy-four patients who were admitted to intensive care unit due to severe sepsis or septic shock were enrolled. Surface antigens of neutrophils (CD64, CD10, and CD16) were detected by flow cytometry. Respiratory burst activity (RBA) was measured by flow cytometry using 2′,7′-dichlorofluorescein diacetate and phorbol-12-myristate-13-acetate. The parameters were serially examined at Days 1 and 8 in septic shock patients.

Results: High CD64 and low CD10 and CD16 on Day 1 was associated with sepsis severity ($P = 0.003$, 0.017, and 0.007, respectively). On Day 1, RBA and CD64 were higher in survivors than in nonsurvivors of septic shock patients ($P = 0.012$ and 0.027, respectively), and on Day 8, CD10 and CD16 were higher in survivors than in nonsurvivors ($P = 0.019$ and 0.036, respectively). High RBA and high CD64 on Day 1 showed low 28-day mortality in univariate analysis ($P = 0.018$ and 0.034, respectively). In multivariate analysis, RBA maintained statistical significance ($P = 0.042$) but CD64 revealed only a tendency ($P = 0.064$).

Conclusions: Neutrophil surface antigen (CD64, CD10, and CD16) could reflect sepsis severity. High CD64 expression and high RBA at early phase of sepsis might be associated with better prognosis, whereas high expression of CD10 and CD16 at late phase of sepsis might be associated with better prognosis.

Key terms: flow cytometry; sepsis; neutrophil; respiratory burst; CD64; CD10; CD16

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Although sepsis is a major cause of morbidity and mortality in the intensive care unit (ICU), its pathogenesis is poorly defined and hematologic markers are limited. The number of circulating neutrophils increases massively during sepsis and is a key marker of activation of innate immunity. Neutrophils are primed at the beginning of sepsis, acquiring direct bactericidal activity through large intracellular stores of proteolytic enzymes and rapid production of reactive oxygen species (ROS) (1–3). After migration to the infection site, primed neutrophils are activated by additional signals and eliminate invading bacteria (4).

Primed neutrophils show alterations in surface antigen expression through translocation of these proteins from intracellular pools to the plasma membrane, and vice versa (5). Some surface antigens, particularly neutral endopeptidase (CD10), high-affinity immunoglobulin-Fc fragment receptor I (CD64), and low-affinity immunoglobulin-Fc fragment receptor III (CD16), have been associated with the status of sepsis. Decreased expression of CD10 and CD16 of peripheral neutrophils reflects release of immature granulocytes from bone marrow (6,7). Expression of CD10 on neutrophils decreases in patients with septic shock (8) and after *in vivo* inflammatory challenge (2). CD16 is generally expressed on the surface of mature neutrophils, and its expression is decreased in severe systemic inflammatory conditions (9). In contrast to these markers, expression of CD64 increases in the early phase of sepsis (10). However, studies investigating the prognostic implication of surface antigen expression changes during sepsis are sparse or controversial. High CD64 expression showed conflicting prognostic impact (3,11), and one study reported increased proportions of granulocyte with CD16 dim positive and CD10 negative showed unfavorable prognosis in sepsis patients (7). The surface antigen expression of neutrophils changes early phase of sepsis, and it reflects the total effects of multiple inflammatory mediators during sepsis. Thus, surface antigen expression could be a good marker for estimating the disease state of patients, and more clinical and experimental evidence is required to confirm this.

Neutrophils function through three processes: adhesion, phagocytosis, and respiratory burst activity (RBA). Among the functional roles of neutrophils, cellular damage during sepsis is directly associated with RBA (4,9). RBA can be measured by neutrophil activity to produce ROS using flow cytometric techniques using 2′,7′-dichlorofluorescein-diacetate (DCF-DA) (12). DCF-DA is a small nonpolar nonfluorescent molecule that diffuses into cells, where it is enzymatically deacetylated by intracellular esterase to the polar nonfluorescent compound 2′,7′-dichlorofluorescein (DCF) (12). DCF becomes trapped within neutrophils and, during the neutrophil respiratory burst, is rapidly oxidized to highly fluorescent DCF by H_2O_2 in the presence of peroxidase. RBA is used as a screening marker for abnormalities of neutrophil function in chronic granulomatous disease (13) and is also associated with other acquired immunocompromised conditions such as liver cirrhosis (14). However, clinical implication of RBA has not been well investigated in sepsis.

As mentioned earlier, the phenotypes and functions of neutrophils directly change in response to inflammatory signals. We studied changes in RBA and the expression levels of CD64, CD10, and CD16 in neutrophils during sepsis and evaluated the prognostic implications of RBA, CD64, CD10, and CD16 in patients with septic shock.

MATERIAL AND METHODS

Study Design and Approval

This single-center prospective study was performed in the 28-bed medical ICU of the Asan Medical Center, a 2,800-bed tertiary referral hospital in Seoul, Republic of Korea. Patients with a diagnosis of severe sepsis or septic shock who received ICU care from January 2011 to November 2012 were enrolled in this study. Informed consent was obtained from participating patients (or their relatives) and from age-matched healthy volunteers upon enrollment. Blood was collected from patients at the time of study entry (Day 1) and on Day 8, and simultaneously from healthy controls. After determining routine blood counts, the RBA and expression levels of CD64, CD10, and CD16 in neutrophils were measured for each blood sample. The Institutional Review Board for Human Research of Asan Medical Center approved this study (2011-0001).

Patient Selection

The study population consisted of adult patients ($\geq$20 years of age) who had clinical and laboratory findings that fulfilled the criteria of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) consensus definitions for sepsis, severe sepsis, and septic shock (15) as follows: (1) evidence of infection; (2) evidence of a systemic response to infection; (3) systolic blood pressure $<$ 90 mmHg despite adequate fluid resuscitation; and (4) hypoperfusion or organ dysfunction attributable to sepsis.

All patients received prompt treatment with antibiotics, adequate fluid resuscitation, and support for damaged organ function, including mechanical ventilation and continuous renal replacement therapy. Exclusion criteria included patients with a do-not-resuscitate order and those with a terminal illness before the onset of severe sepsis or septic shock. Patients who presented with positivity for human immunodeficiency virus, neutropenia, or end-stage liver disease and those who had received immunosuppressive agents were also excluded.

Measurement of Neutrophil RBA

The RBA of neutrophils was determined using ethylenediaminetetraacetic acid (EDTA)-treated blood samples within 2 h after the sample was drawn. Samples from patients and age-matched healthy controls were simultaneously tested using DCF-DA, and the RBA of baseline and phorbol-12-myristate-13-acetate (PMA)-stimulated

state was measured at the same time. EDTA whole blood (350 μL) was incubated with 7 μL of 100 mM DCF-DA at 37°C on a shaking incubator for 15 min. Subsequently, 5 μL of 500 mM sodium azide was added to prevent the breakdown of H_2O_2 into other ROS. Aliquots (100 μL) of this mixture were transferred to two tubes and incubated for 5 min at 37°C. To determine the baseline RBA of neutrophils, the cells in one tube were treated with 10 μL phosphate-buffered saline (PBS) and 5 μL 100 nM EDTA. The sample in the other tube was stimulated with 10 μL of 100 ng/mL PMA and 5 μL of 100 nM EDTA (PMA-stimulated state). Both tubes were incubated for 15 min at 37°C. After incubation, the specimens were mixed with 2 mL of cold lysis solution (BD Bioscience, San Jose, CA) for 10 min and centrifuged to remove the supernatant. Samples were washed three times with 4°C PBS. The final cell pellets were resuspended in PBS (750 μL) and 4% paraformaldehyde (250 μL) and stored on ice until flow cytometric analysis. A FACSCanto™ II flow cytometry system (BD Bioscience) with FACSDiva software (BD Bioscience) was used. After acquiring 10,000 cells for each sample, neutrophils were selectively gated by a forward scatter versus side scatter dot plot, and the measured RBA of neutrophils was expressed as the mean fluorescence intensity (MFI) using mean channel fluorescence of FL1. To adjust for variation in functional study, the RBA index was calculated for PMA-stimulated states by the following equation: RBA index = MFI of patient's neutrophils of PMA stimulation/MFI of healthy control's neutrophils of PMA stimulation.

Surface Immunophenotyping

EDTA whole blood samples were used for three-color direct immunofluorescence flow cytometry. The same flow cytometry system and software were used for RBA testing and immunophenotyping. The samples were incubated with the following specific antibodies: a fluorescein isothiocyanate-conjugated anti-CD64 antibody, a phycoerythrin-conjugated anti-CD10 antibody, and a peridinin-chlorophyll protein-conjugated anti-CD16 antibody. After erythrocyte lysis and a wash step, a total of 30,000 leukocytes were gated from each sample, and MFI was measured using mean channel fluorescence.

Clinical Data Collection

Data on patient death, laboratory results, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and Sequential Organ Failure Assessment (SOFA) score were collected. The APACHE II and SOFA scores of all patients were measured when patients were screened or detected. The disease status and treatment response were assessed from electronic medical records.

Statistical Analysis

Data are summarized as mean ± standard deviation or median and range. A Student's *t*-test or Mann–Whitney *U*-test was used for the comparison of differences between two groups. A correlation study was performed with Spearman's rank correlation coefficient. Binary logistic regression analysis was used to evaluate associations of risk factors with hospital mortality. Variables with *P*-values < 0.05 by univariate analysis were selected for multivariate analysis. SPSS version 11 (SPSS, Chicago, IL) was used for statistical analysis, and a two-tailed *P*-value < 0.05 was considered significant.

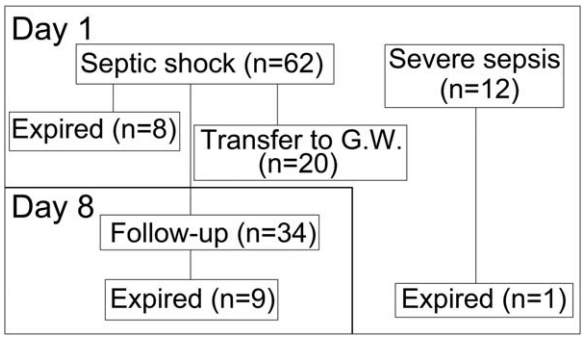

FIG. 1. Flow diagram of the clinical course and results obtained in 74 patients with severe sepsis or septic shock. Initial testing on Day 1 was performed in 74 patients. The follow-up test at Day 8 was performed in only 34 patients because eight patients died before Day 8, and 20 were transferred to the general ward.

RESULTS

Patient Characteristics

A total of 74 patients were treated in the ICU for severe sepsis or septic shock during the study period. The origin of infection was the lungs ($n = 43$), gastrointestinal tract ($n = 15$), urinary tract ($n = 7$), soft tissue or bone ($n = 6$), murine or scrub typhus ($n = 2$), or the central nervous system ($n = 1$). The study included 12 patients with severe sepsis and 62 patients with septic shock (Fig. 1). The overall 28-day mortality was 24.3%. We were able to analyze survival and follow-up in the patients with septic shock, and 17 patients with septic shock died within 28 days from study entry. Twenty patients were transferred to the general ward, and eight patients died before Day 8; therefore, follow-up samples on Day 8 were obtained from 34 patients.

There were no significant differences between two sepsis groups in age, sex, APACH II score, SOFA score, WBC count, including neutrophil, lymphocyte, and monocyte counts, serum procalcitonin level, and serum C-reactive protein (CRP) level (Table 1). However, patients with septic shock showed higher serum lactate and CD64 MFI than patients with severe sepsis ($P = 0.045$ and 0.003, respectively) and they showed lower CD10 and CD16 MFI ($P = 0.017$ and 0.007, respectively, Table 1).

Functional and Phenotypic Changes of Neutrophils During Sepsis

To determine whether the functional activity (RBA) or phenotype (surface antigen expression) of neutrophils changes during sepsis, the subjects were divided into four groups: normal control, severe sepsis, septic shock

Table 1
Baseline Characteristics of Patients on the Day of Diagnosis of Severe Sepsis or Septic Shock

Variable	Severe sepsis ($n = 12$)	Septic shock ($n = 62$)	*P*-value
Age (years)	64.1 ± 14.2	64.3 ± 13.5	0.918
Sex, M:F	5:1	2.6:1	0.438
APACHE II score	20.9 ± 4.1	22.2 ± 7.3	0.430
SOFA score	8.7 ± 3.4	10.3 ± 3.3	0.189
WBC count (10^3 μL^{-1})	14.0 ± 6.4	15.4 ± 10.0	0.820
Neutrophil count (10^3 μL^{-1})	11.5 ± 6.5	13.4 ± 9.4	0.587
Lymphocyte count (10^3 μL^{-1})	1.1 ± 1.1	1.1 ± 1.1	0.839
Monocyte count (10^3 μL^{-1})	0.6 ± 0.4	0.7 ± 0.5	0.822
Procalcitonin (ng mL^{-1})	28.0 ± 51.2	24.9 ± 38.7	0.144
C-reactive protein (mg dL^{-1})	15.0 ± 9.2	20.4 ± 11.1	0.129
Lactate (mmol L^{-1})	2.4 ± 3.0	3.4 ± 3.1	0.045
Neutrophil RBA index	1.3 ± 0.6	1.2 ± 0.4	0.671
Neutrophil surface antigen expression (10^3, MFI)			
CD64	1.1 ± 0.6	2.1 ± 1.4	0.003
CD10	1.4 ± 1.0	0.9 ± 0.9	0.017
CD16	1.6 ± 0.6	1.0 ± 0.7	0.007

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell; RBA, respiratory burst activity; MFI, mean fluorescence intensity.

on Day 1, and septic shock on Day 8. Baseline MFI of RBA was significantly higher in sepsis patients than in healthy controls ($P < 0.001$); in particular, patients with septic shock showed a higher baseline RBA than patients with severe sepsis due to already activated neutrophils in sepsis ($P = 0.036$; Fig. 2). The baseline RBA in patients with septic shock decreased from Day 1 to Day 8 ($P < 0.001$).

Surface antigen expression of neutrophils differed according to the disease state and measurement time point (Fig. 3). Compared with healthy individuals, expression of CD64 on neutrophils was high in patients with severe sepsis ($P < 0.001$), and the expression was higher in patients with septic shock than in those with severe sepsis ($P = 0.003$) on Day 1, and decreased by Day 8 ($P = 0.001$). In contrast, CD10 and CD16 expression decreased with severity of sepsis on Day 1 (control vs. sepsis, $P < 0.001$; severe sepsis vs. septic shock, $P = 0.017$ and 0.007,

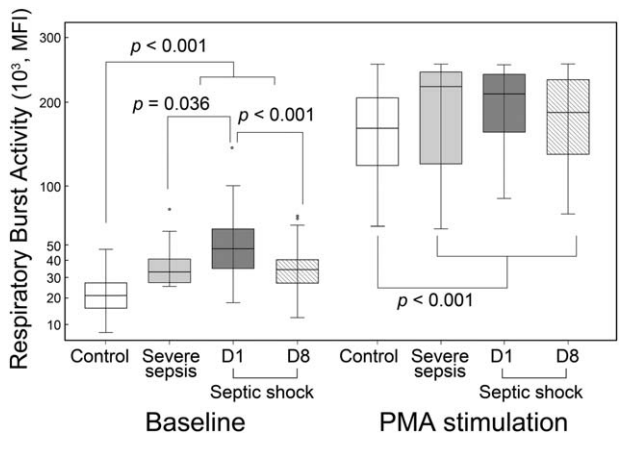

FIG. 2. Comparison of the mean fluorescence intensity (MFI) of neutrophil respiratory burst activity (RBA) in baseline and PMA-stimulated states. The box plots indicate the RBA results of four groups, in the following order: healthy controls ($n = 117$), severe sepsis ($n = 12$), septic shock patients on Day 1 ($n = 62$), and septic shock patients on Day 8 ($n = 34$).

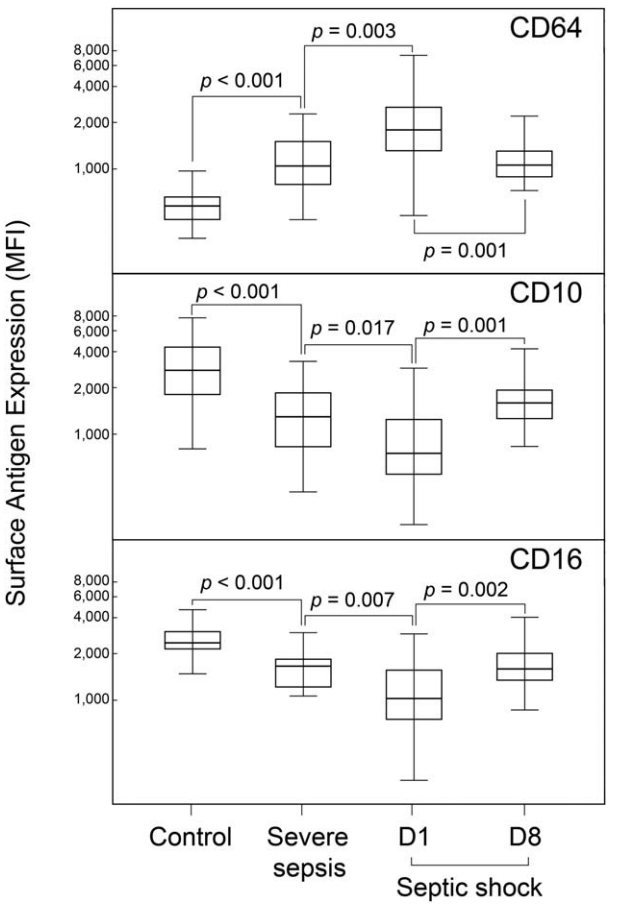

FIG. 3. Comparison of surface antigen expression of neutrophils. Data are shown for controls ($n = 117$), severe sepsis patients ($n = 12$), septic shock patients on Day 1 (D1, $n = 62$), and septic shock patients on Day 8 (D8, $n = 34$).

Table 2
Baseline Characteristics on the Day of Diagnosis of Septic Shock in Survivor And Nonsurvivor Groups

Variable	Survivors ($n = 45$)	Nonsurvivors ($n = 17$)	*P*-value
Age (years)	62.7 ± 13.3	68.5 ± 13.6	0.123
Sex, M:F	2.8:1	2.4:1	0.832
APACHE II score	20.5 ± 6.9	27.3 ± 5.9	0.001
SOFA score	10.3 ± 3.4	10.4 ± 3.2	0.886
WBC count (10^3 μL^{-1})	14.4 ± 10.1	18.3 ± 9.5	0.167
Neutrophil count (10^3 μL^{-1})	12.7 ± 9.4	15.3 ± 9.2	0.324
Lymphocyte count (10^3 μL^{-1})	0.9 ± 0.8	1.6 ± 1.6	0.078
Monocyte count (10^3 μL^{-1})	0.6 ± 0.5	0.9 ± 0.5	0.034
Procalcitonin (ng mL^{-1})	24.9 ± 34.0	25.1 ± 50.2	0.986
C-reactive protein (mg dL^{-1})	18.7 ± 11.1	24.7 ± 9.9	0.055
Lactate (mmol L^{-1})	2.9 ± 2.3	4.7 ± 4.5	0.039
Neutrophil RBA index	1.3 ± 0.4	1.0 ± 0.2	0.012
Neutrophil surface antigen expression (10^3, MFI)			
CD64	2.4 ± 1.5	1.5 ± 1.0	0.027
CD10	0.9 ± 1.0	0.9 ± 0.6	0.999
CD16	1.0 ± 0.7	1.1 ± 0.7	0.645

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell; RBA, respiratory burst activity; MFI, mean fluorescence intensity.

respectively), and increased on Day 8 ($P = 0.001$ and 0.002, respectively).

RBA Index and Surface Antigen Expression on Day 1 and Day 8 in Patients with Septic Shock

Clinical and laboratory characteristics of survivor and nonsurvivor groups among the 62 patients with septic shock are described in Table 2. Age and sex were not different between the two groups. The APACHE II score, a marker for illness severity, on the day of admission to the ICU was significantly lower in the survivor group than in the nonsurvivor group ($P = 0.001$), but the SOFA score was similar ($P = 0.886$). WBC count and procalcitonin level were not significantly different between groups ($P = 0.167$ and 0.986, respectively), but the monocyte count and lactate level were significantly elevated in the nonsurvivor group ($P = 0.034$ and 0.039, respectively), and C-reactive protein showed a tendency to be increased in the nonsurvivor group ($P = 0.055$). The RBA index and CD64 expression were significantly higher in the survivor group ($P = 0.012$ and 0.027, respectively).

Follow-up specimens were obtained at 8 days from 34 patients with septic shock. Of these, nine patients died within 28 days from the study entry (Day 1). The RBA index and CD64 expression did not significantly differ between survivor and nonsurvivor ($P = 0.669$ and 0.591, respectively, Table 3). However, the expression of CD10 and CD16 on Day 8 were higher in survivors than in nonsurvivors ($P = 0.019$ and 0.036, respectively).

RBA Index and Surface Antigen Expression as Predictors of 28-Day Mortality in Patients with Septic Shock

Univariate logistic regression analysis showed significant associations between 28-day mortality and the APACHE II score, RBA index, and CD64 expression ($P = 0.004$, 0.018, and 0.034, respectively) (Table 4). An increase in the RBA index, increased CD64 expression, and a low APACHE II score correlated with low 28-day mortality. Multivariate analysis of the RBA index and CD64 expression was performed with the APACHE II score as a covariate. The RBA index retained prognostic significance in multivariable analysis ($P = 0.042$). High CD64 expression of neutrophils tended to indicate improved survival, but without statistical significance ($P = 0.064$).

Table 3
Day 8 Laboratory Values of Patients with Septic Shock in Survivor and Nonsurvivor Groups

Variable	Survivors ($n = 25$)	Nonsurvivors ($n = 9$)	*P*-value
WBC count (10^3 μL^{-1})	16.2 ± 8.3	12.9 ± 10.1	0.378
Neutrophil count (10^3 μL^{-1})	13.8 ± 7.8	1.1 ± 1.0	0.338
Lymphocyte count (10^3 μL^{-1})	1.4 ± 0.8	1.4 ± 0.6	0.867
Monocyte count (10^3 μL^{-1})	0.7 ± 0.4	0.8 ± 0.7	0.544
Neutrophil RBA index	1.2 ± 0.4	1.2 ± 0.3	0.669
Neutrophil surface antigen expression (10^3, MFI)			
CD64	1.2 ± 0.7	1.2 ± 0.8	0.591
CD10	1.7 ± 1.0	1.0 ± 0.9	0.019
CD16	1.9 ± 1.0	1.1 ± 0.6	0.036

WBC, white blood cell; RBA, respiratory burst activity; MFI, mean fluorescence intensity.

Table 4
Association Between 28-Day Mortality and Clinical and Laboratory Findings at Diagnosis and Day 8 in Septic Shock Patients

	Univariate			Multivariate[a]		
Prognostic marker	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Day 1						
Age	1.035	0.989–1.083	0.135	–	–	–
APACHE II score	1.186	1.055–1.334	0.004	1.185	1.045–1.343	0.008
Neutrophil RBA index	0.064	0.007–0.626	0.018	0.079	0.007–0.909	0.042
Neutrophil CD64	0.508	0.271–0.951	0.034	0.458	0.200–1.046	0.064
Neutrophil CD10	1.004	0.985–1.023	0.680	–	–	–
Neutrophil CD16	0.991	0.975–1.007	0.259	–	–	–
WBC count	1.038	0.983–1.096	0.178	–	–	–
Neutrophil count	1.029	0.972–1.089	0.328	–	–	–
Lymphocyte count	1.033	0.962–1.108	0.372	–	–	–
Monocyte count	3.621	0.892–14.702	0.072	–	–	–
C–reactive protein	1.053	0.998–1.111	0.061	–	–	–
Lactate	1.191	0.994–1.426	0.058	–	–	–
Day 8						
Neutrophil RBA index	0.993	0.097–10.163	0.995	–	–	–
Neutrophil CD64	1.000	0.999–1.001	0.975	–	–	–
Neutrophil CD10	0.999	0.998–1.000	0.087	–	–	–
Neutrophil CD16	0.998	0.997–1.000	0.060	–	–	–

OR, odds ratio; CI, confidence interval; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell; RBA, respiratory burst activity; MFI, mean fluorescence intensity.
[a]The respiratory burst activity (RBA) index and CD64 were analyzed with the APACHE II score in separate multivariate analyses. The multivariate result shown for APACHE II is an estimation based on analysis with the RBA.

DISCUSSION

The massive hyperinflammatory response of the innate immune system begins promptly after sepsis initiation and is critical for eradication of pathogens. However, it can cause hypoperfusion and multiple organ failure, and is thought to be responsible for many early deaths in patients with sepsis (16). Several attempts have been made to reduce the mortality of sepsis, but not all were effective. Early aggressive supportive therapy performed before the critical time point improved patient outcome (17), and immune stimulators such as granulocyte-macrophage colony stimulating factor were effective in patients with multiple organ dysfunction syndrome (18) and sepsis-associated immunosuppression (19). However, the mortality rate of sepsis is still high.

In this study, RBA of neutrophils at early phase of sepsis was associated with mortality (Tables 2 and 4), and it provided evidence that the overwhelming nonspecific hyperinflammatory state of innate immune system would contribute to hypoperfusion and tissue injury in the early phase of sepsis. Because functional tests are likely to be influenced by experimental conditions, the RBA of the patient was adjusted by the RBA of an age-matched control that was tested simultaneously; this adjusted value was termed the RBA index. In a 28-day mortality study among 62 patients with septic shock, a high RBA index predicted superior outcomes ($P = 0.042$, Table 4). Our results are in accordance with the notion that an initial intense inflammatory response is essential for resolving infection and critical for the survival of patients with septic shock (20). Because physiologic compensatory mechanisms become dominant within 24 h to prevent immoderate systemic inflammation (21), the immune reactivity in the first day of sepsis determines the resolution of infection.

CD64 is considered a marker of neutrophil activation (22) and infection (23). Many studies have reported that CD64 expression of neutrophils is a sensitive and specific marker for systemic infection and sepsis in adults (10) and children (24). Nonetheless, other studies have reported contradictory results regarding the prognostic impact of CD64 expression. In one study with 47 patients, increased CD64 expression of neutrophils was associated with sepsis severity and poor 28-day mortality (3), whereas another study with 41 patients showed that increased expression of CD64 on neutrophils and monocytes correlated with a favorable prognosis (11). In this study, high expression of CD64 on neutrophils was associated with sepsis severity ($P = 0.003$, Table 1 and Fig. 3), and it showed superior outcome in patients with septic shock ($P = 0.027$, Table 2), but showed only tendency without statistical significance when analyzed with APACHE II score as a covariate ($P = 0.064$, Table 4). APACHE II score is the most powerful prognostic indicator in the ICU setting, but has high complexity because it uses 12 measurements. Thus, measurement of CD64 expression has the advantage of being relatively easy, while showing considerable prognostic value.

CD10 is a neutral endopeptidase that degrades bioactive peptides released in response to inflammatory challenges (25), and its expression as well as CD16 expression is associated with the maturity of neutrophils (6,26,27). Immature neutrophils with a $CD10^{-}/CD16^{dim}$ phenotype provide a quantitative index of the active release of neutrophils from the bone marrow into circulation in response to acute inflammatory stimuli (28),

and another study indicated that immature neutrophils with low CD16 expression are capable of mediating crucial innate immune function during severe sepsis (9). Recent study reported high proportions of neutrophils with $CD10^{dim}$ and/or $CD16^{dim}$ predicted sepsis deterioration at 48 h and high mortality of Day 30 in patients with sepsis or severe sepsis or septic shock (7). In our study, low MFI of CD10 and CD16 was associated with a more severe septic condition (Table 1) and lower CD10 correlated with higher RBA index, indicating a hyperinflammatory state (data not shown). The expression levels of CD10 and CD16 at diagnosis were not different between survivor and nonsurvivor groups among septic shock patients. However, expressions of these surface markers increased at later stage of sepsis (on Day 8) in survivor group in contrast to nonsurvivor group, which showed no change between Day 1 and Day 8 (Table 3). It suggests that a sustained hyperinflammatory state or uncontrolled ongoing infection in the later stage of sepsis could exacerbate organ dysfunction. The inflammatory responses of septic patients change during the disease course according to the pathophysiology of sepsis (29), but there is a lack of useful markers that can be used to determine the host immune status in a timely manner. This study suggests that neutrophil CD10 and CD16 could be a good marker for monitoring the patients with septic shock in later stage of sepsis.

Considering the limitation of small sample size in this study, especially 34 septic shock patients (25 survivors and nine nonsurvivors) at Day 8, a multicenter study including more patients is needed to consolidate the results.

CONCLUSIONS

Neutrophil surface antigen (CD64, CD10, and CD16) could reflect sepsis severity. High CD64 expression and high RBA at early phase of sepsis might be associated with better prognosis, whereas high expression of CD10 and CD16 at late phase of sepsis might be associated with better prognosis.

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C.-J.P. and S.-B.H. designed and conducted the study. E.-J.C., B.-R.L., Y.J.K., Sa.H.P., Y.-U.C., S.J., and D.-G.S. performed experiments and collected data. S.-B.H. and So.H.P. managed patients. M.H.B. and So.H.P. analyzed and interpreted data and prepared the manuscript. All authors read and approved the manuscript. C.-J.P. and S.-B.H. contributed equally to this study, having full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. M.H.B. and So.H.P. contributed equally to this work as co-first authors. The authors have no conflicts of interest to report.

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