Original Article

Balance of the prooxidant and antioxidant system is associated with mortality in critically ill patients

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It is well known that oxidative stress causes certain diseases and organ damage. However, roles of oxidative stress in the acute phase of critical patients remain to be elucidated. This study aimed to investigate the balance of oxidative and antioxidative system and to clarify the association between oxidative stress and mortality in critically ill patients. This cohort study enrolled 247 patients transported to our emergency department by ambulance. Blood was drawn on hospital arrival, and serum derivatives of reactive oxidant metabolites (dROMs, oxidative index) and biological antioxidant potential (BAP, antioxidative index) were measured. Modified ratio (MR) is also calculated as BAP/dROMs/7.51. There were 197 survivors and 50 non-survivors. In the non-survivors, dROMs were significantly lower (274 vs 311, $p<0.01$), BAP was significantly higher (2,853 vs 2,138, $p<0.01$), and MR was significantly higher (1.51 vs 0.92, $p<0.01$) compared to those in the survivors. The AUC of MR was similar to that for the APACHE II score. Contrary to our expectations, higher BAP and lower dROMs were observed on admission in non-survivors. This may suggest that the antioxidative system is more dominant in the acute phase of severe insults and that the balance toward a higher antioxidative system is associated with mortality.

Key Words: **oxidative stress, critically ill patients, biological antioxidant potential, modified ratio, mortality**

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, which are produced during physiologic processes of biological activities.[1,2] ROS are necessary to sustain cellular function, such as bacterial phagocytosis by granulocytes and macrophages as a self-producing weapon.[3] However, the human body possesses an antioxidative system that includes superoxide dismutase and catalase against the oxidative system to maintain balance. This redox reaction directs various important biological processes. Excessive production of ROS or diminished function of the antioxidative system results in "oxidative stress", which can lead to the oxidation of important molecules such as membrane phospholipids, resulting in cellular, tissue, or organ damage. Oxidative stress is well known to have a relationship with the occurrence, progression, and prognosis of many chronic diseases.[4–9]

In critically ill patients, such as those with infectious diseases, cardiopulmonary arrest, trauma, burn, and acute deterioration of chronic diseases, excessive ROS are reported to be produced due to severe inflammation, and the subsequent oxidative stress or antioxidant capacity is associated with disease severity or mortality.[1,2,5,10–16] However, evaluation of the redox capacity in critically ill patients as a simultaneous balance between oxidative and antioxidant capacity has rarely been reported. We hypothesized that critically ill patients would be under oxidative stress and that higher oxidative stress and lower antioxidative potential would be associated with higher mortality. The objective of this research was thus to measure the oxidative and antioxidative capacity simultaneously, to evaluate the balance between them, and to clarify the relationship between the redox system and mortality in critically ill patients in the hyperacute phase.

Materials and Methods

Study design. This study was a single-center retrospective cohort study. The study protocol was approved by the institutional research ethics committee of Nagasaki University Hospital (approval number: 13040152). Patient samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2008.

Patients. Nagasaki University Hospital is a tertiary emergency medical facility that mainly accepts critically ill patients. The patients included in the present study were transported by ambulance to our emergency medical center between May 2012 and August 2013. Among them, the patients who were treated by emergency physicians and who had surplus blood specimens available were selected. Patients 15 years old or younger were excluded from this study.

Blood sampling. Blood samples were obtained concurrently with the primary resuscitation of the patients. The serum was extracted by centrifugation at 3,000 rpm for 15 min and stored at −80°C for the oxidative and antioxidative system assay. The patient characteristics and laboratory data collected included age, sex, vital signs, blood cell counts, results of coagulation tests, blood chemistry, and blood gas analysis, systemic inflammatory response syndrome score, Sequential Organ Failure Assessment score, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and prognosis at hospital discharge.

Oxidative and antioxidative system assay. Serum derivatives of reactive oxidant metabolites (dROMs, an oxidative index) and biological antioxidant potential (BAP, an antioxidative index) were assessed with a Free Radical Elective Evaluator (FREE carpe diem; Wismerll Co. Ltd., Tokyo, Japan), an automated measurement method.

The dROMs test reflects the total amount of organic hydroper-

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oxides, oxidative derivatives of lipids, proteins, and DNA.[7,17–22] When 20 µl of serum was dissolved in an acidic buffer (pH 4.8), hydroperoxides in the samples reacted to generate alkoxyl and peroxyl radicals. By forming radicals, an electron was pulled out from the aromatic amine, and *N*,*N*-diethyl-para-phenylenediamine was oxidized to produce a pink-colored radical cation that was spectrophotometrically quantified at 505 nm. The values were expressed in convenient units called U.CARR (Carratelli units, which were named after the inventor). It was experimentally established that 1 U.CARR equals 0.08 mg/dl of H_2O_2. Although there are many methods to measure hydroperoxides, the dROMs test is quick and easy, with measurement taking 5 min. This method has been reported to favorably compare with electron spin resonance spectroscopy, the gold standard method for oxidative stress assay measurement,[21] and has much a smaller coefficient of variation than other ROS indexes such as isoprostanes.[23] Reference values for healthy subjects are reported to be between 250 and 350 U.CARR.[7,8,20]

The BAP test reflects global antioxidant capacity by measuring iron-reducing ability. In the colored solution containing ferric chloride ($FeCl_3$) and chromogenic substrate (a derivative of thiocyanate), ferric ions (Fe^{3+}) were reduced to the ferrous form (Fe^{2+}) by antioxidants in 10 µl of serum samples, which became decolorized after 5 min at 37°C incubation. Chromatic change was quantified with an automated photometer at 505 nm, as with the dROMs test. As the reduction ability of the sample rises, the value increases. Reference values for healthy subjects are reported to be above 2,200 µmol/L.[8,22,24–26]

The modified ratio (MR) is calculated as BAP/dROMs/7.51, which was adjusted to a level of 1.00 for healthy Japanese adults in previously reported research.[8,26] The value is defined as an index of redox potential, and a value below 1.00 indicates oxidative stress. Blood samples were also obtained from 21 healthy volunteers, and each parameter was measured by the same methods.

Statistical analysis. The primary outcome, survival, was assessed at hospital discharge. Background and clinical features are expressed as median values [interquartile range (IQR)] or the number of patients (percentage). Comparisons between survivors and non-survivors were performed with median test and Pearson's chi-square test. Multiple comparisons between the survivors, non-survivors, and healthy volunteers in dROMs, BAP, or MR were performed with Steel-Dwass test. Analyses for mortality tendency divided by quintiles of dROMs, BAP, or MR were performed using the Cochrane-Armitage trend test. The association between mortality and the status of oxidative stress (dROMs, BAP, MR) was assessed by multivariable logistic regression analysis, respectively. We only included the APACHE II score in the model as an adjusted variable due to the small number of non-surviving patients. To assess performance of model discrimination, receiver-operating characteristic (ROC) curves were calculated and quantified by the integrals of the curve [area under the curve (AUC)].

We considered a value of $p<0.05$ as statistically significant. Missing values were omitted in the analyses. The JMP Pro statistical software package (SAS Institute Inc., Cary, NC) was used for statistical analyses.

Results

This study included 247 patients, whose characteristics are shown in Table 1. The median age of all subjects was 65 (IQR 50–79) years old, and 67.2% of the subjects were male. The numbers of patients treated for the following conditions were as follows: trauma, 106; cardiopulmonary arrest on arrival (CPAOA), 27; infectious disease, 23; intoxication, 23; cardiovas-

Table 1. Patient characteristics

n	Overall 247	Survivors 197	Non-survivors 50	*p* value	Missing data
Age (years)	65 (50–79)	62 (46–77)	77.5 (61–85)	<0.0001	
Sex (male)	67.20%	70.10%	56.00%	0.0588	
Diseases (%, number)					
Trauma	42.9% (106)	47.7% (94)	24.0% (12)	0.0024	
CPAOA	10.9% (27)	1.0% (2)	50.0% (25)	<0.0001	
Infection	9.3% (23)	9.6% (19)	8.0% (4)	1.0000	
Intoxication	9.3% (23)	11.7% (23)	0% (0)	0.0058	
Cardiovascular	8.5% (21)	9.6% (19)	4.0% (2)	0.2643	
GI bleeding	4.4% (11)	5.1% (10)	2.0% (1)	0.6996	
Others	14.6% (36)	15.2% (30)	12.0% (6)	0.6587	
SBP (mmHg)	118 (86–143)	125 (100–148)	20 (0–109.3)	<0.0001	
GCS	14 (7–15)	14 (11.5–15)	3 (3–8)	<0.0001	
WBC (×10²/µl)	101 (75–146)	101 (74–150)	100 (80.5–141)	0.9682	
pH	7.39 (7.28–7.42)	7.40 (7.36–7.43)	7.01 (6.81–7.29)	<0.0001	9
$PaCO_2$ (mmHg)	37.4 (33.2–42.6)	37.0 (33.3–41.0)	44.6 (31.4–68.4)	0.0058	23
Base excess (mM)	−2.0 (−7.6–+0.2)	−1.3 (−4.1–+0.6)	−16.9 (−24.5–−7.5)	<0.0001	
Lactate (mg/dl)	25 (16–54)	22 (15–35.8)	111 (60–172.8)	<0.0001	27
Platelets (/µl)	18.1 (13.5–23.8)	19.2 (15.2–25.0)	14.0 (10.4–17.9)	<0.0001	
D-dimer (µg/ml)	10.4 (2.7–32.7)	7.3 (2.1–21.2)	38.8 (19.7–115.3)	<0.0001	8
SOFA score	5 (3–9)	4 (2.5–6)	12 (10–14)	<0.0001	
APACHE II score	11 (5–20)	8 (5–15)	29 (20.3–35)	<0.0001	

Values are presented with median (IQR) except for sex (male percentage) and diseases (percent and number). *P* values were determined with median test and Pearson's chi-square test. CPAOA, cardiopulmonary arrest on arrival; GI, gastrointestinal; SBP, systolic blood pressure; GCS, Glasgow Coma Scale; WBC, white blood cell; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation.

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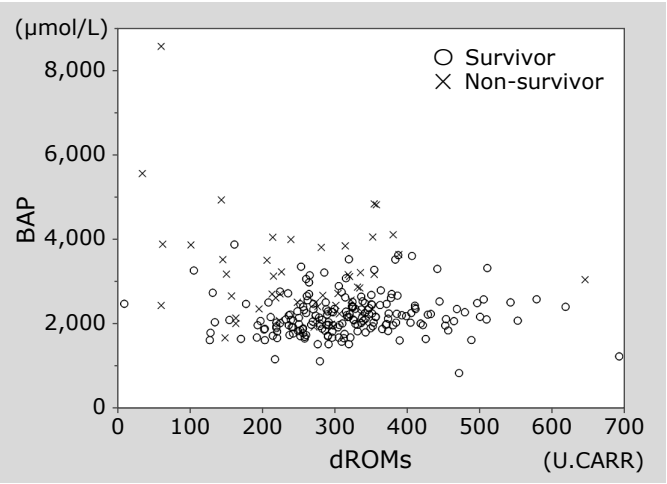


Fig. 1. Relationship between derivatives of reactive oxidant metabolites (dROMs) and biological antioxidant potential (BAP). Each circle indicates a survivor, and a cross indicates a non-survivor. Non-survivors tended to have lower dROMs and higher BAP compared to survivors.

cular disease, 21; gastrointestinal bleeding, 11; and miscellaneous diseases such as heat stroke, drowning, postpartum bleeding, and adrenal crisis, 36. The mortality at hospital discharge was 20.2% ($n = 50$).

Figure 1 shows a scatter plot of dROMs and BAP in the survivors and non-survivors. The survivors were likely to have higher dROMs and lower BAP compared to the non-survivors. Figure 2 compares dROMs, BAP, and MR between the survivors, non-survivors, and healthy volunteers. Although there was no significant difference in dROMs between the healthy volunteers and survivors, that in the non-survivors was significantly lower than that in the survivors. By contrast, BAP in the non-survivors was significantly higher than that in the survivors and healthy volunteers, although there was no difference between the survivors and healthy volunteers. MR showed the same result as BAP. These findings indicate that neither the oxidative nor antioxidative system was activated in the survivors, but the antioxidative system was activated and MR was increased in the non-surviving critically ill patients. Although the values of dROMs, BAP, and MR in the 247 patients were not significantly different from those of the healthy volunteers, there were significant differences between the survivors and non-survivors.

Figure 3 displays stepwise changes in mortality according to quintile divisions of the dROMs, BAP, and MR. As dROMs decreased, BAP and MR increased, and mortality increased in a stepwise manner. An especially clear relationship was observed for MR, suggesting that the balance of the antioxidative and oxidative system is closely related to the outcome of critically ill patients.

Figure 4, ROC and AUC, shows the mortality discrimination ability of each variable. The AUCs for BAP, MR, and APACHE II score were all over 0.80, and that of MR was similar to the APACHE II score. This result, taken together with the results of Fig. 3, indicates that of the three indices, dROMs, BAP, and MR, MR is the most closely related to prognosis.

Table 2 displays the results of the multivariable logistic analysis for mortality adjusted by APACHE II score. Although neither dROMs nor MR was detected as a significant prognostic factor, BAP was found to be a significant prognostic factor even after adjustment with APACHE II score, which indicates that a predictive model combining the APACHE II score and BAP may be useful.

Discussion

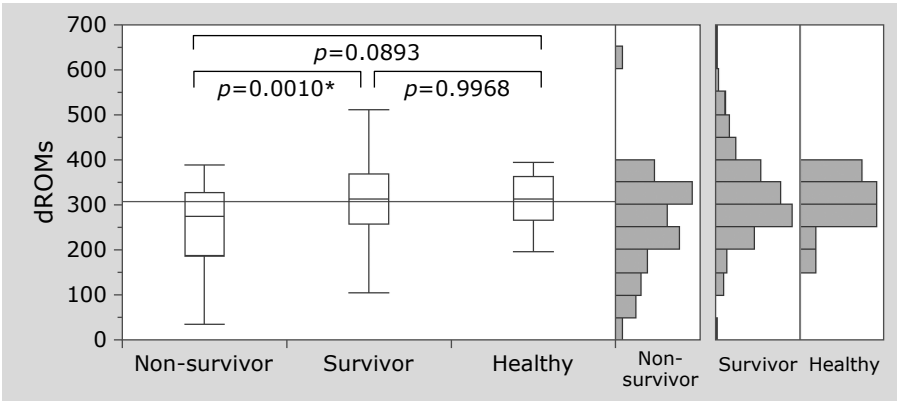
In the present study, we evaluated the oxidative and antioxidative capacity in critically ill patients on hospital arrival and investigated its association with mortality. Contrary to our expectations, the non-survivors showed significantly higher BAP and lower dROMs compared to the survivors. Mortality increased in a stepwise manner as BAP or MR increased and dROMs decreased, and especially, MR showed a significant correlation with mortality. To our knowledge, this is the first report to evaluate the correlation of the ratio of antioxidative to oxidative status with mortality in critically ill patients on hospital arrival.

Major redox reactions involving free radicals *in vivo* are caused by the mitochondrial electron transport system, endoplasmic reticulum, peroxisomes, membrane-bound nicotinamide adenine dinucleotide phosphate oxidase, and nitric oxide synthase. Superoxide anions, hydrogen peroxide, myeloperoxidase, lipid hydroperoxides, malondialdehyde, and 8-hydroxydeoxyguanosine have been measured as indicators of oxidative capacity, and super oxide dismutase, catalase, and plasmatic glutathione peroxidase as indicators of antioxidant capacity.[27] However, it is unclear whether individual marker measurements reflect systemic oxidative or antioxidant capacity in the whole body. The dROMs test does not directly measure ROS and free radicals but captures the metabolite hydroperoxide, which is a metabolite produced when ROS/free radicals oxidize lipids, proteins, amino acids, nucleic acids, and others, and quantifies it as an oxidative stress measurement.[12,16,18,19,22] In the BAP test, instead of measuring individual antioxidants, a sample reflects global antioxidant capacity by measuring the iron-reducing ability.[16,18] The advantages of this measurement system are that it can measure actual systemic functional oxidative and antioxidant capacity, is easily automated, and can be performed in a short time, which is one of the most important requirements for clinical emergency use.[20,25]

In the present study, the more severe cases showed abnormally higher BAP. The research so far has shown that oxidative stress is due to excessive production of ROS or dysfunction of the antioxidant system. Many of the studies showing the presence of oxidative stress in chronic diseases have stated that high dROMs indicate worsening conditions, which is rarely discussed in relation to the antioxidant system.[4–9,24,28] However, some studies in the acute phase showed that antioxidant parameters were higher in the poor prognosis group, including non-survivors, in terms of critical patients such as those with sepsis, traumatic brain injury, and ischemic brain stroke, and postoperative patients.[11–15,29–31] Consistent with the present study is the finding that the groups with a higher antioxidant parameter showed higher mortality. Lorente *et al.*[11,12,14,15] postulated that non-surviving patients with middle cerebral artery infarction displayed higher serum levels of total antioxidant capacity to compensate for the higher lipid peroxidation as assessed by malondialdehyde than surviving patients, as was observed in patients with severe sepsis and those with severe traumatic brain injury. Ishikawa *et al.*[16] reported that BAP could reflect the severity of ischemia-reperfusion injury in the whole body, including the brain. As there are also reports that BAP is increased even in chronic conditions as the disease progresses,[5] BAP could be an indicator of hyperacute physiological conditions. In our study, BAP correlated with the APACHE II score, which indicates its potential as a parameter that can be easily quantified as a physiological indicator in the hyperacute condition.

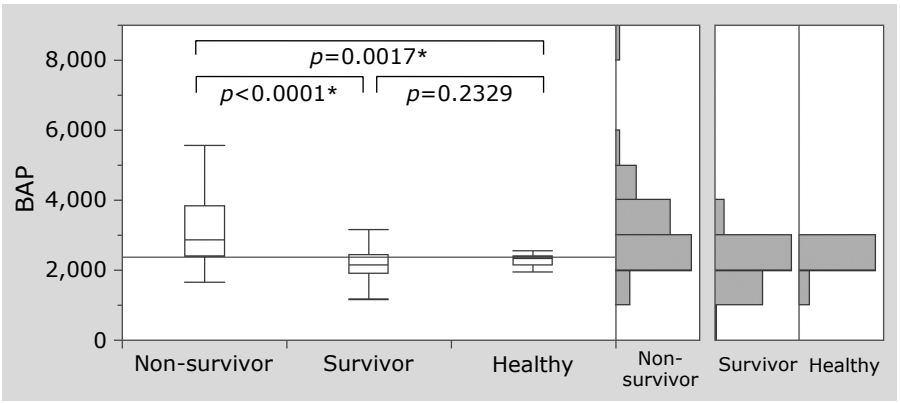
Unlike in previous studies, dROMs as a prooxidant marker did not increase in our study but rather tended to decrease in the non-surviving patients. Regarding the elevation of both oxidant and antioxidant markers in the acute phase, previous studies have speculated that antioxidant markers increased following the

A



	Non-survivor	Survivor	Healthy volunteer
Median	274	311	311
IQR	188–325.25	257–368.5	266.75–360.75

B



	Non-survivor	Survivor	Healthy volunteer
Median	2852.8	2137.8	2320.6
IQR	2,388.95–3,809.95	1,905.65–2,420.3	2,132.05–2,398.08

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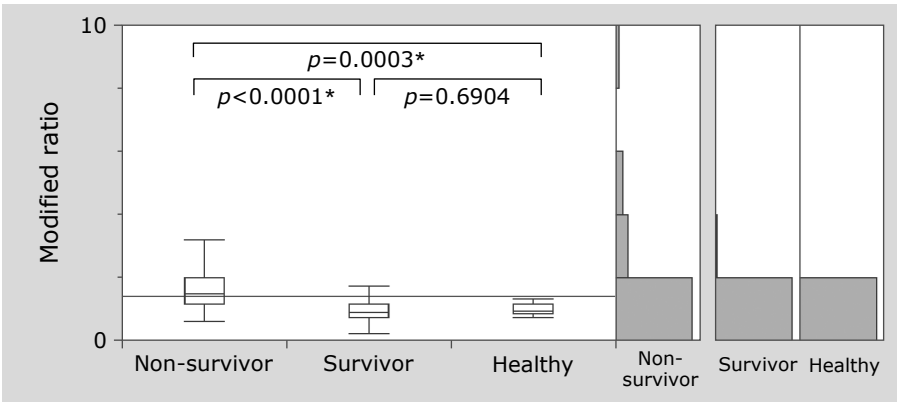

	Non-survivor	Survivor	Healthy volunteer
Median	1.507	0.924	0.943
IQR	1.1525–1.996	0.7505–1.1515	0.8674–1.1468

Fig. 2. Comparison of oxidative stress parameters between survivors, nonsurvivors, and healthy volunteers with box plots, histograms, and median [interquartile range (IQR)]. The horizontal line shows median of all patients. (A) d-ROMs, derivatives of reactive oxidant metabolites, (B) BAP, biological antioxidant potential, and (C) modified ratio. *P* values with * are indicated when the Steel-Dwass multiple comparison test showed significance.

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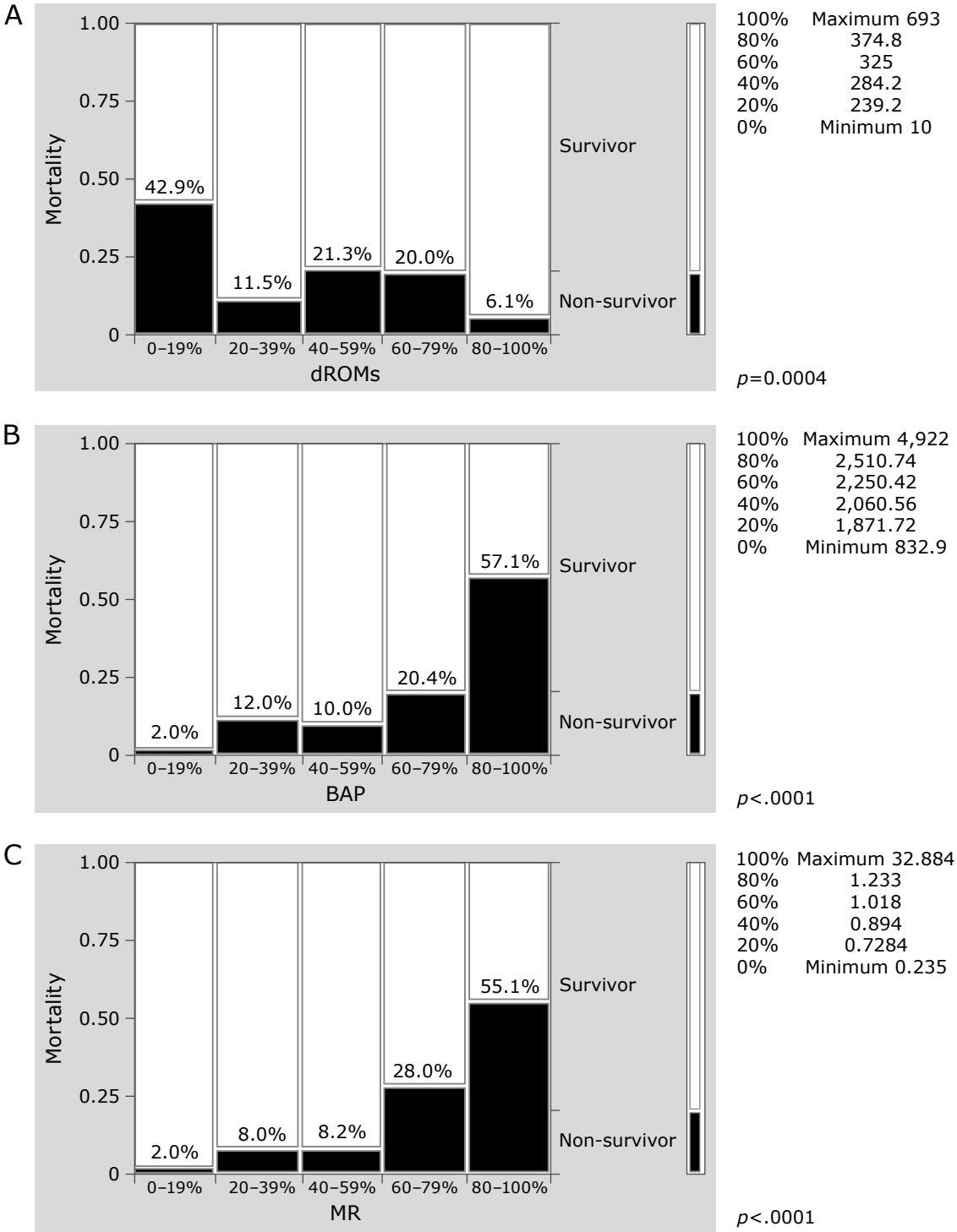

Fig. 3. Stepwise changes in mortality according to quintile divisions of oxidative stress parameters. (A) d-ROMs, derivatives of reactive oxidant metabolites, (B) BAP, biological antioxidant potential, and (C) MR, modified ratio. Percentage on each bar indicates mortality of each group. Trends were evaluated with the Cochrane-Armitage test.

rise in prooxidant markers.[11,12,14–16,22,29–31] However, in some reports, an increase in dROMs was not observed in the acute phase of surgical insults. Kanaoka *et al.*[30] measured serial changes of dROMs and BAP in 15 patients undergoing cardiovascular surgery using extracorporeal circulation. In these patients, dROMs did not increase on day 1 after surgery compared to the values before surgery. However, the mean value of BAP on day 1 was higher than that before surgery. Miyazaki *et al.*[32] evaluated dROMs and BAP in two patients after lung transplantation. In both patients, dROMs 1 day after surgery were less than 300 U.CARR, whereas BAP values were more than 2,000 μmol/L. Cardiovascular surgery using extracorporeal circulation and lung transplantation should have induced significant oxidative stress. Nonetheless, the fact that dROMs did not increase may suggest

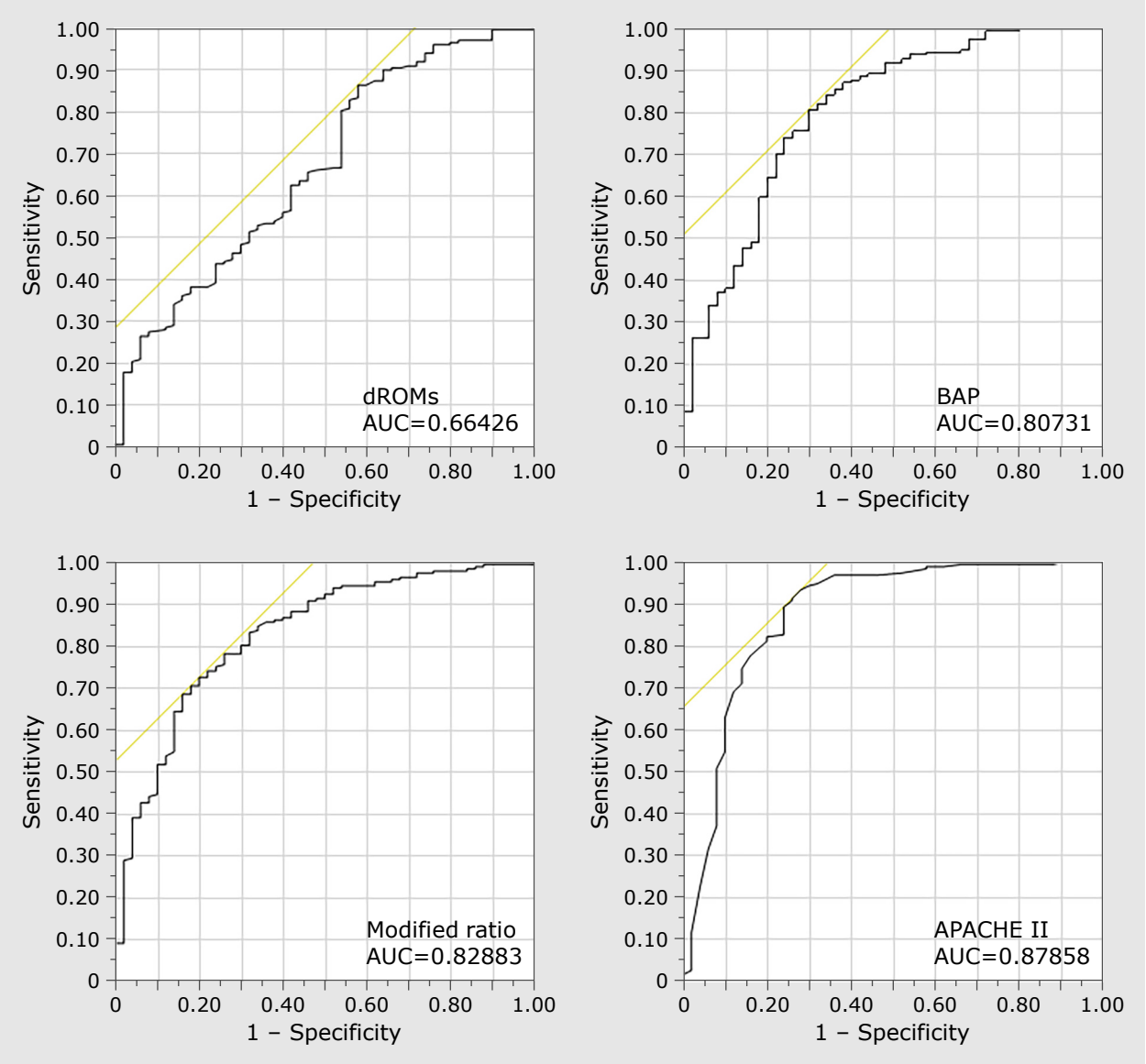

Fig. 4. Receiver-operating characteristic (ROC) analyses for mortality with the area under the curve (AUC) of each parameter. APACHE, Acute Physiology and Chronic Health Evaluation; BAP, biological antioxidant potential; dROMs, derivatives of reactive oxidant metabolites.

Table 2. Multivariable logistic analysis for the association between mortality and oxidative stress parameters

	Estimate	Odds ratio	95% CI	*p* value
dROMs × 1/100	–0.358	0.699	0.452–1.081	0.1077
APACHE II	0.17	1.185	1.133–1.240	<.0001
BAP × 1/1,000	0.723	2.061	1.010–4.205	0.0468
APACHE II	0.147	1.158	1.101–1.218	<.0001
Modified ratio	0.059	1.061	0.938–1.200	0.3488
APACHE II	0.173	1.189	1.137–1.245	<.0001

CI, confidence interval; APACHE, Acute Physiology and Chronic Health Evaluation; dROMs, derivatives of reactive oxygen metabolites; BAP, biological antioxidant potential; MR, modified ratio.

that oxidative stress might have been overwhelmed by antioxidant capacity. Nagashima examined changes dROMs and BAP during endurance exercise with an ergometer and reported that dROMs did not change during and after exercise, but BAP was already significantly higher within 1 h during the exercise and thereafter.[33] She speculated the reason for no increase in dROMs might be that it was suppressed by antioxidant capacity as indicated by the increase in BAP. Finaud *et al.*[34] stated that

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numerous studies, both in animals and humans, have shown that antioxidant enzyme activity increases in blood or in tissues after aerobic exercise. They also stated that this adaptation might occur very quickly (within about 5 min). In the present study, dROMs in the non-survivors decreased compared to those in the survivors, which may suggest that oxidative stress was quickly compensated for and rather overwhelmed by BAP.

Among the dROMs, BAP, and MR, MR was most associated with mortality. Because there is significant variance in the absolute values of dROMs and BAP, it is reasonable to evaluate the ratio of the two values to examine the balance of oxidative and antioxidative status. Tamura *et al.*[35] reported 5 cases of CPAOA with ROSC, and also found the highest BAP/dROMs corresponding to our MR on ICU admission in the deceased cases, consistent with our results in the non-survivor group. MR was proposed by Nagata *et al.*[26] They divided the ratio of BAP to dROMs by 7.51 and set the Japanese mean value to be 1.0. We also adopted this number in the present study. Because MR was no longer associated with mortality if the logistic regression was adjusted by APACHE II score, and MR was significantly corelated with APACHE II score or lactate level on admission (data not shown), MR probably reflects the physiological severity of patients admitted to the emergency department.

This study has some limitations. First, we divided groups by the prognosis at hospital discharge. However, 94% (47/50) of the non-survivors died within 3 hospital days because most of the non-survivors were patients with CPAOA or trauma. Therefore, the data of non-survivors significantly reflects these two etiologies. Also, the great variety of severity and the kinds of diseases cannot be ignored. Second, age is a factor vulnerable to oxidative stress, and the data for the non-survivor group might have been influenced by age. The older a person gets, the higher is their incidence of chronic disease, which may affect mortality and the oxidative or antioxidative capacity. However, Nagata *et al.*[26] reported that neither dROMs nor BAP correlated with age in a survey on lifestyle-related diseases. Third, we measured oxidative and antioxidative status only on arrival and did not check serial changes over time. We may be able to more clearly determine the effects of oxidative and antioxidative balance on the pathophysiology of critically ill patients by performing repeated measurements.

In the present study, non-surviving critically ill patients showed higher BAP, lower dROMs, and higher MR in the hyperacute phase of their condition. MR, which indicates the balance between oxidative and antioxidative markers, was especially correlated with prognosis and considered to represent physiological severity. These results warrant further investigation to clarify the role of oxidative stress.

Author Contributions

Conception and study design: HI and KT; Acquisition of data: HI, GT, and TI; Analysis and interpretation of data: HI, GT, OT, GH, KT, and TM; Drafting of the manuscript: HI, GT, KM, TT, and SS; Critical revision of the manuscript for important intellectual content: OT; Statistical analysis: HI, OT, and SS; Material support: KT; Study supervision: OT and TN.

Abbreviations

APACHE	Acute Physiology and Chronic Health Evaluation
AUC	area under the curve
BAP	biological antioxidant potential
CPAOA	cardiopulmonary arrest on arrival
dROMs	derivatives of reactive oxygen metabolites
IQR	interquartile range
ROC	receiver-operating characteristic
ROS	reactive oxygen species
U.CARR	Carratelli units

Conflict of Interest

No potential conflicts of interest were disclosed.

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