

STUDY PROTOCOL

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# Correlation of the oxygen radical activity and antioxidants and severity in critically ill surgical patients – study protocol

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## Abstract

**Background:** Surgical patients who require an emergent operation commonly have severe sepsis or septic shock, followed by high morbidity and mortality rates.

Despite advances in treatment however, no predictable markers are available. In severe sepsis, many pathophysiologic mechanisms are involved in progression to organ failure, and oxygen free radical and antioxidants are known to contribute to this process. Oxygen free radical and antioxidants contribute to progression of organ failure in severe sepsis. In fact, oxygen radical activity has been reported to be correlated with disease severity and prognosis in patients with severe sepsis or septic shock. Accordingly, we aim to assess the usefulness of oxygen free radical and antioxidant concentrations to predict the disease severity and mortality in a cohort of critically ill surgical patients.

**Methods/Design:** This is a prospective observation study including patient demographic characteristics, clinical information, blood sampling/serum oxygen radical activity, serum antioxidant activity, serum antioxidant concentrations (zinc, selenium and glutamate), disease severity scores, outcomes, lengths of stay in intensive care unit, hospital 30-day mortality.

**Keywords:** Oxygen radical activity, Antioxidant activity, Sepsis, Critically ill patient, Zinc, Selenium, Glutamate

## Background

Critically ill surgical patients usually have a septic status combined with severe systemic inflammation and shock. Sepsis is commonly caused by a gastrointestinal tract perforation, bowel ischemia, or postoperative complications, such as, pneumonia, intra-abdominal infection, or anastomotic leakage. Severe systemic inflammation and sepsis can cause organ failure with high risk of mortality (4 ~ 15% vs. 1%). Sepsis affects about 18 million people annually, with a mortality rate of 25% for uncomplicated cases and 80% in patients developing multi-organ failure syndrome [1].

Prognostic markers like natriuretic peptide (NP), B-type natriuretic peptide (BNP), or pro-BNP are used to predict postoperative cardiac complications after cardiac or non-cardiac surgery, while procalcitonin is commonly

used as prognostic marker and indicator of mortality and antibiotics usage in septic patients. In addition, lactate clearance was recently reported to be a useful indicator of resuscitation and prognosis in severe sepsis [2,3]. Furthermore, some scoring systems, such as, the acute physiologic and chronic health evaluation (APACHE) II, the sequential organ failure assessment (SOFA), and multiple organ dysfunction score (MODS) systems, are also used to evaluate critically ill patient's condition. However, no clinically adaptable markers, except lactate clearance and procalcitonin, are available for determining the outcomes of critically ill surgical patients with severe sepsis. Inflammatory processes after infection are known to involve cells, inflammatory mediators, cytokines, pro-inflammatory substances, nitric oxide, arachidonic acid metabolites, and oxygen free radicals. These mediate and induce organ injury leading to organ failure [4-10]. Recently, many reports have been issued on the roles of oxygen free radicals and antioxidants, such as, glutamine, zinc, and selenium, which act as cofactors of

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glutathione peroxidase [11,12]. Oxygen free radicals (OFR) cause oxidative damage in cells, which lead to DNA damage and mitochondrial dysfunction culminates in cell death [13-15]. There is evidence that oxidative stress caused by reactive oxygen species(ROS) in sepsis is characterized by tissue ischemia reperfusion injury and intense systemic inflammatory response [16-19]. Furthermore, oxidative stress and OFR impair the microcirculation, which induce acute renal failure, and have been correlated with sepsis severity and sepsis-induced morbidity. In sepsis, the protective role of antioxidants against oxidative stress has been known for more than 15 years [20-22]. Supplementation with antioxidants, such as, glutamine, zinc, and selenium may decrease oxidative stress and increase antioxidant activity, but apparently, do not affect mortality [23-28]. Early recognition of oxidative damage in sepsis by assessment of oxidative stress biomarkers is an actual topic for future research [29,30].

## Methods

### Aim

The purpose of the study is to assess the usefulness of the concentration of the oxygen free radical and antioxidants to predict the severity and mortality of the critically ill surgical patients.

### Study population

This prospective study will be performed over 2-year periods (May 2012 ~ April 2014) in single institution. About 50 patients having severe sepsis or septic shock requiring emergency operation due to the bowel perforation or strangulation will be included.

### Study design

This study will not change or modify the clinical practices of the participating physicians. Patients are enrolled after acquisition of the informed consent

approved by a Severance hospital institutional review board (Approval No. of IRB: 4-2012-0188).

Blood sample is drawn at 1st day, 3rd day, and 7th day after admitting to intensive care unit (ICU) regardless of the disposition of the patients after discharge from the ICU.

The primary endpoint of this study is to evaluate the correlation of the level of oxygen radical activity and severity of the patients. And secondary endpoints are (1) correlation of the level of oxygen radical activity and outcome, i.e., LOS in ICU and hospital, 30 day mortality, in-hospital mortality; (2) correlation of the level of antioxidant and severity and outcome of the patients; (3) relationship of the level of the oxygen radical activity and antioxidants.

### Data collection

Investigators have collected the data including the followings: (1) patient characteristics, i.e., demographic data, severity of sepsis (severe sepsis or septic shock), presence of shock; (2) severity score for 7 days in ICU, i.e., APACHE II score, SOFA score, MODS; (3) clinical progress, i.e., vital signs, daily intake and output; (4) clinical outcomes, i.e., duration of shock, use of mechanical ventilation (MV), duration of MV, length of stay (LOS) in ICU, LOS in hospital, 30 day mortality, in-hospital mortality, complications. Blood samples are drawn to check the level of oxygen radical activity, antioxidation activity, level of the antioxidant (zinc, selenium, and glutamate) (Table 1).

Oxygen radical activity and antioxidation activity are assessed using CR3000® (Callegari 1930, Italy). Free oxygen radicals test (FORT) kit check the serum H<sub>2</sub>O<sub>2</sub> level directly as oxygen radical. Free oxygen radicals detection (FORD) kit assess the antioxidation activity that check the reactivity with vitamic C, Trolox, albumin, and glutathione to free radical – chromogen.

The levels of the zinc, selenium and glutamate are assessed in the laboratory.

**Table 1 Collection of dataset of the enrolled patients**

Day of ICU <sup>*</sup> admission	APACHE II <sup>**</sup> score	Severity scoring (MODS <sup>†</sup> , SOFA <sup>‡</sup> )	Clinical courses (Vasopressors, Shock, MV <sup>§</sup> , Complications)	Oxygen radical activity and antioxidation activity	Antioxidants (Zn <sup>  </sup> , Se <sup>¶</sup> , Glutamate)
1st day	○	○	○	○	○
2nd day			○		
3rd day		○	○	○	○
4th day			○		
5th day			○		
6th day			○		
7th day		○	○	○	○

<sup>\*</sup>ICU intensive care unit, <sup>\*\*</sup>APACHE II acute physiology and chronic health evaluation II, <sup>†</sup>MODS multi-organ dysfunction score, <sup>‡</sup>SOFA sequential organ failure assessment, <sup>§</sup>MV mechanical ventilation, <sup>||</sup>Zn zinc, <sup>¶</sup>Se selenium.

### Statistical analysis

The results will be expressed as standard statistical metrics: median (range), mean  $\pm$  standard deviation for continuous variables.

The primary endpoint of this study is to evaluate the correlation of the level of oxygen radical activity and severity of the patients.

And secondary endpoints are (1) correlation of the level of oxygen radical activity and outcome, i.e., LOS in ICU and hospital, 30 day mortality, in-hospital mortality; (2) correlation of the level of antioxidant and severity and outcome of the patients; (3) relationship of the level of the oxygen radical activity and antioxidants.

Comparison will be performed using the Student's t-test and chi-test for the relationship of the oxygen radical activity and severity, and Student's t-test and logistic regression test for the relationship of the oxygen radical activity and antioxidants, and outcomes. Statistical significance will be defined as a *p*-value less than 0.05 ( $p < 0.05$ ).

### Inclusion criteria

Patients with severe sepsis or septic shock undergoing emergency surgery due to bowel perforation or strangulation will be screened to enroll the study. And patients requiring ICU care due to postoperative septic complications, such as pneumonia, bacteremia or peritoneal abscess or leakage. After acquisition of the informed consent, they will be assigned as to study.

### Exclusion criteria

The patients are excluded followings; (1) age < 20 years or > 80 years old; (2) other type shock except sepsis; (3) immune compromised patients, i.e., post-transplant status requiring immunosuppressant, patients using steroid due to immune disorders or other disease, patients having chemotherapeutic agents due to advanced malignancy; (4) patients who not agree the informed consent.

### Competing interests

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### Authors' contributions

LJG designed and wrote the manuscript. And SH, JY and LSH will have performed the analyses of antioxidant and oxygen radical activity, and collecting the data. All authors read and approved the final manuscript.

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