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審査学位論文
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A study on the relationship between early-stage plasma cytokines and neutrophil elastase levels in septic acute respiratory failure, and the prognosis

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Running title: cytokine levels in septic acute respiratory failure

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Abstract

We investigated the plasma levels of cytokines, polymorphonuclear elastase (PMN-E) and endotoxin in 83 patients with septic acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) in the early stage after onset. The results revealed that the plasma levels of tumor necrosis factor α (TNF-α), interleukin 8 (IL-8) and PMN-E were all significantly higher in the ARDS group (n = 48) as compared with those in the ALI group (n = 35). Complications by septic shock occurred in 42.9% of the ALI patients and 85.4% of the ARDS patients. The overall 30-, 60- and 90-day mortality rates from ALI/ARDS were 4.8%, 10.8% and 12.0%, respectively.

The PaO_2/FIO_2 (P/F ratio) and plasma levels of TNF-α, IL-8 or endotoxin in the early stage after the onset of ALI /ARDS did not significantly differ between the survivor and non-survivor groups at 30 days. However, the P/F ratio and plasma levels of TNF-α, IL-8 and PMN-E in the early stage after the onset of ALI /ARDS were significantly higher in the non-survivor groups than in the corresponding survivor groups at 60 and 90 days. No significant differences in the plasma endotoxin levels were noted between the survivor group and the non-survivor group. The present data suggest the possibility that the serum levels of TNF-α, IL-8 and PMN-E measured in the early stage of ALI/ARDS may serve as useful predictors of the long-term prognosis.

Key word: ALI, ARDS, prognosis, TNF-α, IL-8, PMN-E, endotoxin

I. Introduction

Acute respiratory failure in patients with sepsis develops prior to the onset of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). ALI and ARDS are non-specific reactions to diverse local and systemic disorders. Septicemia is one of the most universally known prodromal symptoms of ALI/ARDS [1]. It has been started that the causes of sepsis most profoundly related to the development of ALI/ARDS are pulmonary and intraperitoneal infections [2]. ALI/ARDS has been estimated to occur in 25 to 42% of patients with sepsis, with further increase of the incidence associated with persistence of the shock state [3]. Acute respiratory failure complicating sepsis is often recognized as a systemic process leading to multiple organ failure.

The etiology of ALI/ARDS is complex, and we have reported on several humoral mediators, primarily cytokines, which might possibly be involved in the development of septic ALI/ARDS [4-7]. We have also reported elevations of the plasma levels of many mediators, such as inflammatory cytokines and polymorphonuclear elastase (PMN-E), in patients with septic shock [4, 8, 9].

The reported mortality rate from septic ALI/ARDS is in the range of 30 to 70%, which has essentially remained the same for over 30 years since this disorder was first described [3, 10, 11], although some studies have demonstrated improvement of the survival rate [12]. However, according to a recent report, septic ALI/ARDS still has a poor prognosis [13]. We reported 30-day mortality rates from ALI and ARDS of 11.4% and 20.4%, respectively, in our recent survey of 158 ALI/ARDS patients in the Tohoku district of Japan [14]. In a survey of 79 patients with ALI/ARDS reported by Oda et al., the 28-day mortality rate from ALI/ARDS was 31.6% [15].

In the present study, we investigated the relationships between the serum levels of tumor

necrosis factor α (TNF-α), interleukin 8 (IL-8), PMN-E and endotoxin (ET) measured in the early stage of septic ALI/ARDS, and the survival prognosis.

II. Subjects and Methods

Consent for participation in this study was obtained from the patients or their families. This study was approved by the Ethics Committee of Iwate Medical University.

Sepsis was diagnosed in accordance with the Diagnostic Criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [16].

Diagnoses of ALI/ARDS in this study were made in accordance with the criteria reported by Bernard et al. [17], according to which ALI is diagnosed when the PaO_2/FIO_2 ratio (P/F ratio) is ≥200 but <300, and in this study ARDS is diagnosed when the P/F ratio is <200.

The Acute Physiology and Chronic Health Evaluation score (APACHE II score) [18] and Sequential Organ Failure Assessment score (SOFA score) [19] were employed for grading the severity of the patients' clinical condition. The severity grading was performed through consultation among a plurality of acute medicine specialists qualified as infection control doctors.

The study population comprised 83 tracheally intubated patients with APACHE II scores of ≥15, in whom blood sampling was feasible within about 3 hours of the diagnosis of ALI/ARDS, during the 5-year period from April 2006 to March 2011. There were 35 ALI patients and 48 ARDS patients, with a mean age of 67.7 ± 12.9 years. The underlying disease was panperitonitis in 54 patients, burns in 11 patients, pneumonia in 9 patients, polytrauma in 6 patients, and drug intoxication in 3 patients.

Treatment of sepsis and associated septic shock, disseminated intravascular coagulation (DIC), etc., consisted of generally prescribed therapeutic measures. Treatment of MODS also

consisted of generally prescribed conventional therapeutic measures to combat various pathophysiologic states.

Respiratory management for ALI /ARDS was carried out at a tidal volume of 8 to 10 mL/kg and a positive end-expiratory pressure (PEEP) of 5 to 12 cmH20. Patients received sivelestat sodium hydrate at 0.2 mg/kg/h during tracheal intubation and over 1-2 hours after removal of the endotracheal tube. The maximum duration of administration of sivelestat was 14 days.

Sample collection was performed using a heparinized, endotoxin-free syringe within approximately 3 hours of the diagnosis of ALI/ARDS. Immediately after they were drawn, the samples were centrifuged at 3000 rpm for 40 seconds to separate platelet-rich plasma (PRP), which was immediately assayed for ET. The PRP was stored frozen at -80°C until assay for TNF-α, IL-8 and PMN-E.

TNF-α and IL-8 levels were determined by enzyme-linked immunosorbent assay (ELISA) (TFB, Inc., Tokyo, Japan), their determination limit being set at 3 pg/mL.

PMN-E was also quantitated by ELISA (Merck, Darmstadt, Germany). Its normal range was 21-165 ng/mL.

ET was measured on a Toxinometer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) using Limulus HS-T Single Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan), an ET-specific assay system [20]. The cutoff value for endotoxemia was set at 1.1 pg/mL [21].

The unpaired *t*-test was used to test the significant differences, and Pearson's formula was used to test significant correlations. The χ^2 test was used for comparison of significant difference between the groups, and the log-rank method was used for testing the significance of survival curves. A p value <0.05 was used as the probability value for significant differences in all of the tests.

III. Results

For the 83 cases of ALI/ARDS, the mean APACHE II score was 28.5, and the mean SOFA score was 10.5.

There were 35 ALI patients and 48 ARDS patients (Table 1).

The age and ET levels were somewhat higher in the ARDS group than in the ALI group, although there was no significant intergroup difference in either of the parameters. The APACHE II score, SOFA score, P/F value, TNF-α level, IL-8 level and PMNE level were all significantly higher in the ARDS group than in the ALI group.

Fifty-five patients developed septic shock, while the remaining 28 patients did not (Table 2). Thus, ALI/ARDS was complicated by shock in 66.3% of all cases (55/83), but not in the remaining 33.7% (28/83), the difference being statistically significant ($p < 0.0001$). The shock-complicated group exhibited significantly higher values of all the assessed parameters, except ET, than the non-shock-complicated group. The ET levels tended to be higher in the shock-complicated group as compared with those in the non-shock-complicated group, although the intergroup difference did not reach statistical significance.

For the ALI and ARDS groups combined, the overall 30-, 60- and 90-day mortality rates are shown in Table 3. The overall 30-day mortality rate was 4.8%, 60-day mortality rate as 10.8%, and 90-day mortality rate was 12.0%.

In a comparison of the parameters between the survivor and non-survivor group at 30 days, age was found to be the only parameter that differed significantly between the two groups, being significantly higher in the non-survivor group than that in the survivor group (Table 4).

Comparison of the parameters between the survivor and non-survivor group at 60 days revealed significantly higher values of all of the parameters, except ET, in the non-survivor group as compared with those in the survivor group (Table 5). As for the ET levels, they

tended to be higher in the non-survivor group than those in the survivor group, although the intergroup difference did not reach statistical significance.

Comparison of the parameters between the survivor and non-survivor group at 90 days revealed significantly higher values of all the parameters, except ET, in the non-survivor group as compared with those in the survivor group, similar to the data for day 60.

In the early stage after the onset of ALI/ARDS, 59.0% of the patients (49/83) presented with endotoxemia (ET ≥ 1.1 pg/mL).

There was no significant difference between the ARDS group and the ALI group in overall 90-dau mortality (Fig. 1).

IV. Discussion

Cytokines are secreted from a variety of cells. It has been demonstrated in several experimental models that TNF-α, among others, causes pulmonary disorders, augments the effects of other mediators, and has preclotting activity, which can influence the development of microthrombosis in the lung [22, 23]. Both TNF-α and IL-1β not only increase neutrophil degranulation, reactive oxygen species (ROS) production and lysozyme liberation, but also induce an increase in the synthesis of chemotactic factors for endothelial adhesion factors, neutrophils and monocytes [22].

Several studies have shown that the levels of TNF-α and other cytokines in the BALF are elevated in early-phase and late-phase ARDS patients [24]. The mortality rate is higher in ARDS patients with higher plasma TNF-α levels on the 1st hospital day and persistent elevation of the levels for a long period of time than in those with lower plasma TNF-α levels at the onset and rapid decline of the levels thereafter [24]. It is thus inferred that cytokines adversely affect the endothelium and epidermis to cause an increase in their permeability in

the early stage of ARDS and interact with fibroblasts, which facilitate fibrosis in the late stage of the syndrome [24, 25].

The present data indicate that the more severe the respiratory distress, the higher the plasma TNF-α and IL-8 levels, and suggest the possibility that plasma TNF-α and IL-8 levels in the early stage after the onset of ALI/ARDS may be remotely related to the 30-day survival prognosis, but definitely serve as late-stage prognostic factors for 60-day or 90-day survival.

Inflammatory cytokines are widely recognized to have a profound bearing on the development of septic shock [26-28]. It is also universally recognized that septic shock can be complicated by ALI/ARDS, and the present data indicate a significantly higher incidence rate of ALI/ARDS and elevated plasma TNF-α and IL-8 levels in the shock-complicated group.

PMN-E liberated from neutrophils has become the focus of interest and investigation as an etiologic factor for lung disorders associated with SIRS [29]. Elastase has proteolytic effects on pulmonary connective tissue proteins such as elastin, collagen, fibronectin and proteoglycan, and a vascular permeability-enhancing effect, and also induces the production of leukocyte chemotactic factors (C5a and IL-8). It has been reported that pulmonary disorders are induced via these effects of PMN-E; high elastase levels are detected in the BALF of ARDS patients, and the elastase elevation has been reported to be correlated with depression of pulmonary function. The present authors also reported elevations of the serum IL-8 and PMN-E in patients with ALI/ARDS [4]. There is the possibility that TNF-α, IL-8 and PMN-E are each mutually involved in the production/release of the others.

As is the case for the plasma levels of TNF-α and IL-8, the more severe the respiratory distress, the higher the plasma levels of PMN-E, although its relation with the 30-day prognosis was remote. The results of our study suggest that, on the other hand, it may serve as a 60-day or 90-day late-stage prognostic factor.

Thus, the plasma levels of PMN-E, TNF-α and IL-8 were all significantly higher in the ARDS group than those in the ALI group, although all of these elevations showed only a remote relationship with the 30-day prognosis. This seems to indicate that, while these humoral factors might give rise to tissue damage in the early stage of the disease, various counterbalancing effects probably prevent death in the early stage.

As for ET, our previous report documented that the serum level of ET had no influence upon the risk of development of septic shock [8], and the present data suggest that the serum levels of ET do not reflect the prognosis. When it is considered that 59.0% of the patients presented with endotoxemia, however, it is likely that ET stimulated cytokine production in these cases. The absence of any obvious relation between the serum ET levels and the risk of development of septic shock or ALI/ARDS is considered to be related to the patient sensitivity to ET, and the present data suggest that ET even in trace quantities can function as an immunologically active factor.

In the present series of ALI/ARDS cases, the overall 30-, 60- and 90-day mortality rates were 4.8%, 10.8% and 12.0%, respectively. These rates are remarkably lower than those reported previously [3, 10-15], but are considered to be reproducible data, in that they are essentially comparable to the previously obtained data at our institution [7], despite the varying periods.

There were 472 patients with sepsis treated at this institution during the 5-year period of this survey, of whom 206 patients (43.6%) had ALI/ARDS. The average APACHE II score was 25.9, and the overall mortality rate was 9.2% (unpublished data). The higher overall 28-day mortality rate despite the lower severity than that in the present series seems to be attributable to a noticeable proportion of the study population being studied after a lapse of time following the onset of ALI/ARDS.

We do not employ low tidal volumes [30] at our institution. We make it a rule to perform 48-hour methylprednisolone infusion (41.7 mg/h) at the discretion of the attending physician in patients showing poor improvement of the P/F ratio [31], and to have the patients placed in a prone position for 30 minutes twice daily (in the morning and afternoon).

We make it a rule to administer sivelestat sodium hydrate, a PMN-E inhibitor that is available only in this country, to all patients. Unlike the STRIVE study [32], we believe in the usefulness of sivelestat sodium hydrate [33].

Besides, no particular therapeutic measures other than conventional therapy for sepsis and ALI/ARDS are undertaken at our institution.

The gratifying therapeutic results obtained at our institution, although subject to confirmation in future studies, seem to be directly linked to the feasibility of early initiation of treatment via an integrated start-to-finish process from the initial therapeutic approach to surgery and intensive care management.

Although 10 of the 83 patients died, the cause of death was MODS in 7 cases, complications of heart failure in 3 cases. It would seem important to prevent the onset of MODS as a complication of ALI /ARDS in order to decrease the mortality rate. Consequently, since ALI /ARDS is a pathological condition that has an acute onset and rapidly progresses, it appears necessary to institute multidisciplinary treatment as soon as possible in the early stage.

Many humoral factors are considered to be involved in the development of ALI/ARDS, and we would like to pursue improvement of our therapeutic results by further investigation of the relationships of these factors with the pathophysiological states.

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敗血症性急性呼吸不全発症早期のサイトカイン
および好中球エラスターゼ値と予後との関わりについての検討

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要旨

83 例の septic acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) 発症早期の cytokines 値, Polymorphonuclear elastase (PMN-E)値について検討した. ALI 群(n = 35)に対して ARDS 群(n = 48)では tumor necrosis factor α (TNF-α)値, interleukin 8(IL-8)値および PMN-E 値はいずれも有意に高値を示した. 敗血症性ショックを合併したのは ALI で 42.9%, ARDS で 85.4%であった. ALI/ARDS 症例の overall の 30 日死亡率は 4.8%, 60 日死亡率は 10.8%, 90 日死亡率は 12.0%であった.

30 日目まででは, ALI/ARDS 発症早期の PaO2/FiO2 値(P/F value), TNF-α値, IL-8 値, および endotoxin 値はいずれも生存群と死亡群間で差はみられなかった. ALI/ARDS 発症早期の P/F value, TNF-α値, IL-8 値, および PMN-E 値は 60 日目, 90 日目まででは, 生存群に対して死亡群で高値であった. endotoxin 値は生存群, 死亡群において有意の差はみられなかった. ALI/ARDS 発症早期の TNF-α値, IL-8 値, および PMN-E 値が長期の予後規定因子となる可能性が示唆された.

Key words: ALI, ARDS, TNF-α, IL-8, PMN-E

Legends

Table 1 Comparison of the background characteristics in the early stage between the ALI group and the ARDS group

Table 2 Comparison of the background characteristics in the early stage of ALI/ARDS between the shock-complicated group and the non-shock-complicated group

Table 3 Comparison of the overall 30-, 60- and 90-day mortality rates between the ALI group and the ARDS group

Table 4 Comparison of the background characteristics in the early stage of ALI/ARDS between the survivor and non-survivor groups at 30 days

Table 5 Comparison of the background characteristics in the early stage of ALI/ARDS between the survivor and non-survivor groups at 60 days

Table 6 Comparison of the background characteristics in the early stage of ALI/ARDS between the survivor and non-survivor groups at 90 days

Fig. 1 Comparison of the overall 90-day mortality rates between the ALI group and the ARDS group