Usefulness of a selective neutrophil elastase inhibitor, sivelestat, in acute lung injury patients with sepsis

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\textbf{Background:} Neutrophil elastase plays a crucial role in the development of acute lung injury (ALI) in patients with systemic inflammatory response syndrome (SIRS). The clinical efficacy of the neutrophil elastase inhibitor, sivelestat, for patients with ALI associated with SIRS has not been convincingly demonstrated. The aim of this study was to determine if there are clinical features of patients with this condition that affect the efficacy of sivelestat.

\textbf{Methods:} This was a retrospective study of 110 ALI patients with SIRS. Clinical information, including the etiology of ALI, the number of organs failing, scoring systems for assessing the severity of illness, and laboratory data, was collected at the time of diagnosis. Information on the number of ventilator-free days (VFDs) and changes in $\text{PaO}_2/\text{FiO}_2$ ($\Delta \text{P/F}$) before and 7 days after the time of ALI diagnosis was also collected. The effect of sivelestat on ALI patients was also examined based on whether they had sepsis and whether their initial serum procalcitonin level was $\geq 0.5 \text{ ng/mL}$.

\textbf{Results:} There were 70 patients who were treated with sivelestat and 40 control patients. VFDs and $\Delta \text{P/F}$ were significantly higher in the treated patients than in the control patients. However, there was no significant difference in the patient survival rate between the two groups. Sivelestat was more effective in ALI patients with a $\text{PaO}_2/\text{FiO}_2$ ratio $\geq 140 \text{ mmHg}$ or sepsis. Sivelestat significantly prolonged survival and led to higher VFDs and increased $\Delta \text{P/F}$ in septic patients and patients with initial serum procalcitonin levels $\geq 0.5 \text{ ng/mL}$.

\textbf{Conclusion:} The results may facilitate a future randomized controlled trial to determine whether sivelestat is beneficial for ALI patients with sepsis.

\textbf{Keywords:} systemic inflammatory response syndrome, procalcitonin, ventilator-free days, neutrophil elastase

\section*{Introduction}

Acute lung injury (ALI), with acute onset, bilateral pulmonary infiltrates, and hypoxemia, is a complex disorder characterized by pulmonary inflammation and increased pulmonary vascular permeability.\textsuperscript{1} ALI is caused by an excessive inflammatory response to various assaults on the body, such as pneumonia, sepsis, trauma, and surgery. The pathogenesis of ALI involves inflammatory reactions associated with the accumulation of neutrophils in the lungs.\textsuperscript{2–6} In particular, elastase, which is released from activated neutrophils, has attracted attention as a factor that causes lung injury in patients with systemic inflammatory response syndrome (SIRS).\textsuperscript{7}

Systemic inflammation induces vascular endothelial injury and results in organ dysfunction.\textsuperscript{8,9} Leukocyte–endothelial cell interaction resulting from systemic inflammation plays an important role in the pathogenesis of vascular endothelial injury.\textsuperscript{8,9}
Neutrophil elastase, located downstream in the humoral mediator network, contributes to the development of vascular endothelial injury in concert with other mediators, which leads to increased permeability, vasodilation, and activation of the coagulation cascade.\textsuperscript{10}

Sivelestat (Ono Pharmaceutical, Osaka, Japan) is a selective neutrophil elastase inhibitor\textsuperscript{11} that has been reported to be effective for endotoxin-induced lung injury in hamsters, guinea pigs, and sheep.\textsuperscript{11,12} However, the clinical efficacy of sivelestat in patients with SIRS and ALI remains controversial. Two clinical studies have shown that sivelestat reduced the duration of mechanical ventilation, shortened stays in the intensive care unit (ICU), and prolonged survival in patients with ALI,\textsuperscript{1,13} whereas the Sivelestat Trial in ALI Patients Requiring Mechanical Ventilation (STRIVE) study failed to demonstrate its efficacy.\textsuperscript{14} These discrepant results may be due to differences in patient characteristics such as age, baseline respiratory condition, and the number of non-pulmonary failed organs.\textsuperscript{1,14} In addition, there have been a few reports indicating that sivelestat is effective in patients with ALI or acute respiratory distress syndrome (ARDS) and sepsis.\textsuperscript{15,16} However, the clinical characteristics associated with the efficacy of sivelestat in patients with ALI associated with SIRS have not been convincingly elucidated.

The four aims of this study were to: (1) evaluate the clinical efficacy of sivelestat in ALI patients with SIRS, (2) determine if there are clinical features of patients with this condition that affect the efficacy of sivelestat, (3) assess the efficacy of sivelestat in ALI patients based on whether they have sepsis, and (4) assess the efficacy of sivelestat based on the initial serum procalcitonin (PCT) level.

**Materials and methods**

**Study population**

This was a retrospective study of ALI patients with SIRS who were admitted to Ehime University Hospital, Sumitomo Besshi Hospital, Ehime Prefectural Central Hospital, or Matsuyama Red Cross Hospital during the period 2009–2011.

Patients were excluded if they were aged <20 years, if they had a neuromuscular disease that impaired spontaneous ventilation, severe chronic pulmonary disease, severe central nervous system disease, uncontrolled malignancy, or severe chronic liver disease. The ethical committee of each hospital approved the study protocol, and informed consent was waived because of the retrospective design.

**Diagnosis of SIRS, ALI, disseminated intravascular coagulation (DIC), and sepsis**

The diagnosis of SIRS was confirmed by the presence of at least two of the following (originally proposed by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference\textsuperscript{17}): body temperature $<$36°C or $>$38°C; heart rate $>$ 90 bpm; respiratory rate $>$ 20 breaths/minute or PaCO\textsubscript{2} $<$ 32 mmHg; white blood cell (WBC) count $>$ 12,000 cells/µL or $<$ 4000 cells/µL, or $>$10% immature (band) cells. The three criteria for the diagnosis of ALI, which were based on the definition from the American-European Consensus Conference,\textsuperscript{18} were: (1) PaO\textsubscript{2}/F\textsubscript{O\textsubscript{2}} (P/F) $<$ 300 mmHg, (2) bilateral pulmonary infiltrates on chest X-ray, and (3) pulmonary edema of non-cardiogenic origin (pulmonary capillary wedge pressure $<$ 18 mmHg or, in the absence of pulmonary capillary wedge pressure measurement, no clinical evidence of elevated left arterial pressure). The scoring system of the Japanese Association for Acute Medicine\textsuperscript{19} was used for the diagnosis of DIC (Table 1). The Japanese Association for Acute Medicine DIC score has been shown to identify most of the patients diagnosed by the overt International Society of Thrombosis and Hemostasis criteria.\textsuperscript{20}

Sepsis was diagnosed based on the criteria proposed by the Society of Critical Care Medicine/European Society of Intensive Care Medicine/American College of Chest Physicians/American Thoracic Society/Surgical Infection Society International Sepsis Definition Conference:\textsuperscript{21}

**Table 1 New scoring system for disseminated intravascular coagulation (DIC) by the Japanese Association for Acute Medicine\textsuperscript{19}**

| Criterion | Score |
|----------|-------|
| Systemic inflammatory response syndrome criteria | |
| $\geq 3$ | 1 |
| 0–2 | 0 |
| Platelet count ($\times$ 10\textsuperscript{9}/L) | |
| $< 80$ or $> 50\%$ decrease within 24 hours | 3 |
| $\geq 80$ and $< 120$ or $> 30\%$ decrease within 24 hours | 1 |
| $\geq 120$ | 0 |
| Prothrombin time (value of patient/normal value) | |
| $\geq 1.2$ | 1 |
| $< 1.2$ | 0 |
| Fibrin/fibrinogen degradation products (mg/L) | |
| $\geq 25$ | 3 |
| $\geq 10$ and $< 25$ | 1 |
| $< 10$ | 0 |

Note: A score of four points or more is considered to indicate DIC.
confirmed source of infection and fulfillment of SIRS criteria. If these criteria were not fulfilled, patients were diagnosed as non-sepsis.

**Intervention and treatment**

All patients received pressure-controlled and pressure-supported mechanical ventilation with a positive end-expiratory pressure. At the time of ALI diagnosis, sivelestat was administered intravenously at a rate of 0.2 mg/kg/h continuously for a maximum of 14 days.

**Data collection**

Baseline data were collected from patient records. Clinical data, including the etiology of ALI, the number of failed organs, and the values of assessment systems (which included the sequential organ failure assessment [SOFA] score, the gas exchange, organ failure, cause, associated disease [GOCA] score, the SIRS score, and the DIC score), and the types of infections and causative pathogens, were collected at the time ALI was diagnosed. SOFA comprises separate scores for the respiratory, cardiovascular, renal, central nervous systems and coagulation and hepatic failure and each organ system may be awarded 0–4 points. The SOFA score can help assess organ dysfunction or failure over time and is useful to evaluate morbidity.\(^\text{22}\) The GOCA score is the sum of four variables: (1) the severity of gas exchange (0–3), (2) the number of failed organs (0–3), (3) the cause of lung injury (0: lung only, 1: direct lung injury, 2: indirect injury), and (4) associated diseases (0: no associated disease that will cause death within 5 years, 1: coexisting diseases that will cause death within 5 years but not within 6 months, 2: coexisting diseases that will cause death within 6 months). It was reported that the GOCA score might prove more convenient to use than the Acute Physiology and Chronic Health Evaluation (APACHE) II and the Simplified Acute Physiology Score (SAPS) II because it requires fewer variables but provides the same predictive power.\(^\text{23}\)

Laboratory data, including WBC count, C-reactive protein level, and PCT level, were collected at the time ALI was diagnosed. For PCT measurements, we used a solid-phase, semi-quantitative immunoassay BRAHMS PCT-Q (Thermo Fisher Scientific Clinical Diagnostics BRAHMS GmbH, Hennigsdorf, Germany).

Sivelestat efficacy was evaluated based on survival rate, the number of ventilator-free days (VFDs) and change in PaO\(_2\)/FiO\(_2\) ratio (AP/F) between the P/F values determined before and 7 days after diagnosis of ALI. “VFDs” were defined as the number of days (from day 1 to day 28) that a patient breathed without assistance.\(^\text{24}\)

**Statistical analysis**

Results are expressed as median values, with interquartile ranges in parentheses. The Mann–Whitney \(U\) test or the chi-square test was used to compare groups. To assess the clinical efficacy of sivelestat, survival was analyzed using the Cox proportional hazards model with sex, age, P/F at the time of ALI diagnosis, the number of failed organs, septic status, and ALI etiology as covariates.

Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off values for the P/F at the time of diagnosis that discriminated between survivors and non-survivors. A cut-off value for P/F that provided the highest sensitivity and specificity was chosen.

In this study, we used a solid-phase, semi-quantitative immunoassay for PCT measurements. This test kit categorized the PCT levels into four grades: (1) <0.5 ng/mL; (2) 0.5 to <2.0 ng/mL; (3) 2.0 to <10.0 ng/mL; and (4) \(\geq\)10.0 ng/mL. When the cut-off level was set as 0.5, 2.0, or 10.0 ng/mL, the sensitivity of PCT for sepsis was 95.9%, 59.5%, and 35.1%, the specificity was 86.1%, 91.7%, and 97.2%, and the diagnostic accuracy was 92.7%, 70.0%, and 55.5%, respectively. The PCT level at 0.5 ng/mL was the best cut-off value for PCT that could discriminate between septic and non-septic patients. In addition, previous reports demonstrated that the cut-off value for PCT was 0.5 ng/mL in differentiating between bacterial infection and other kinds of inflammatory processes.\(^\text{25}\) Therefore, in this study, we set the cut-off value for PCT at 0.5 ng/mL, and PCT levels \(\geq\)0.5 ng/mL were considered PCT positive.

When patients were divided into two groups – those with and those without sepsis, and those who were PCT positive and those who were PCT negative – a univariate analysis using the Cox proportional hazards model was used to assess the relationship between mortality and the following variables: sex; age; ALI etiology; the number of failed organs; administration of sivelestat; administration of steroids; SOFA, GOCA, SIRS, and DIC scores; P/F; WBC count; and C-reactive protein serum level at the time of ALI diagnosis. Parameters found to be significant by univariate analysis were taken as potential predictors of mortality and used as covariates in multivariate analysis to identify independent predictors of mortality. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for the variables. The Kaplan–Meier method was used to estimate survival rates, and comparisons were made using the log-rank test. All tests
were two-tailed, and \( P \) values < 0.05 were considered statistically significant. Statistical analysis was performed using SPSS for Windows version 19 (IBM, Armonk, NY, USA).

**Results**

**Patient characteristics**

A total of 537 ALI patients were enrolled in this study. Of these, we excluded 39 patients who were aged < 20 years, 147 patients with uncontrolled malignancy, 35 patients with severe chronic pulmonary diseases, 48 patients with severe chronic liver disease, three patients with neuromuscular disease that impaired spontaneous ventilation, 31 patients with severe central nervous system disease, 68 patients who did not receive mechanical ventilation, and 56 patients with inadequate data. This left 110 patients (71 men and 39 women) who were included in the study. The characteristics of the patients with SIRS at the time of ALI diagnosis are presented in Table 2. At baseline, there were no statistically significant differences between the patients treated with sivelestat \((n = 70)\) and the control patients \((n = 40)\).

The types of infections and causative pathogens in ALI patients with sepsis are shown in Table 3.

**Sivelestat efficacy in ALI patients with SIRS**

The survival rate of the sivelestat patients showed a tendency to be higher than that of the control patients, although the difference was not significant \((P = 0.064)\) (Figure 1A). The VFDs and \( \Delta P/F \) of the sivelestat patients were significantly higher than those of the control patients \((P = 0.031 \text{ and } 0.018, \text{ respectively})\) (Figure 1B and C).

**Patient clinical factors favorable to sivelestat administration**

Sivelestat administration was significantly effective in ALI patients with a P/F ratio \( \geq 140 \text{ mmHg} \) and sepsis \((P = 0.004 \text{ and } 0.011, \text{ respectively})\) (Figure 2). Although statistical significance was not obtained, sivelestat administration was also associated with a trend toward prolonged survival of other subgroups, except for patients with P/F ratios < 140 mmHg, more than three failed organs, or without sepsis.

**Efficacy of sivelestat in septic and non-septic ALI patients**

The characteristics of septic and non-septic patients at the time of ALI diagnosis are shown in Table 4. There were 74 patients diagnosed with sepsis and 36 non-septic patients. Among the septic patients, the P/F of the control group was significantly higher than that of the sivelestat group. In non-septic patients, there were no significant differences for any of the variables of the treated and control patients.

The number of VFDs and \( \Delta P/F \) of the septic patients receiving sivelestat were significantly higher than those of the septic control patients \((P = 0.035 \text{ and } 0.008, \text{ respectively})\).
Table 3 Types of infections and causative pathogens in acute lung injury patients with sepsis

| Parameter                          | N (%) |
|-----------------------------------|-------|
| Infectious types                  |       |
| Pneumonia                         | 33 (44.6) |
| Abdominal infection               | 14 (18.9) |
| Urinary tract infection           | 12 (16.2) |
| Bacterial pleuritis               | 8 (10.8) |
| Others (neck abscess, gas gangrene, etc) | 7 (9.5) |
| Causative pathogens               |       |
| *Pseudomonas aeruginosa*          | 15 (20.3) |
| *Staphylococcus aureus*           | 13 (17.6) |
| *Escherichia coli*                | 9 (12.2) |
| *Enterococcus*                    | 9 (12.2) |
| *Streptococcus pneumonia*         | 7 (9.5) |
| *Klebsiella pneumonia*            | 5 (6.8) |
| *Prevotella*                      | 4 (5.4) |
| *Corynebacterium*                 | 4 (5.4) |
| *Stenotrophomonas maltophilia*    | 3 (4.1) |
| *Staphylococcus epidermidis*      | 2 (2.7) |
| *Haemophilus influenzae*          | 1 (1.4) |
| *Chryseobacterium meningosepticum* | 1 (1.4) |
| *Acinetobacter*                   | 1 (1.4) |

Figure 1 Clinical efficacy of sivelestat for acute lung injury patients with systemic inflammatory response syndrome. (A) Kaplan–Meier curves for acute lung injury patients with systemic inflammatory response syndrome who did or did not receive sivelestat. Statistical analysis was performed using the log-rank test. Solid line, sivelestat patients; dashed line, control patients. (B and C) Clinical efficacy of sivelestat based on ventilator-free days (VFDs) and changes in \( \text{PaO}_2/\text{FiO}_2 \) (\( \Delta \text{P/F} \)) before and 7 days after diagnosis of acute lung injury.

Note: Statistical analysis was performed using the Mann–Whitney U test.

Analysis of predictive parameters of mortality in patients with or without sepsis

Univariate analysis showed that the number of failed organs; sivelestat administration; and the SOFA, GOCA, and DIC scores at diagnosis were significantly correlated with mortality in septic patients (all \( P < 0.05 \)) (Table 5). Multivariate analysis demonstrated that sivelestat administration and the GOCA score were predictors of mortality in septic patients (all \( P < 0.05 \)) (Table 5). Kaplan–Meier curves revealed significantly prolonged survival in the sivelestat group compared with the control group in septic patients (\( P = 0.008 \)) (Figure 4A). In non-septic patients, the SOFA and GOCA scores at diagnosis, but not sivelestat administration, were
significantly correlated with mortality (Table 6). In addition, there were no significant differences in survival rate between the control and sivelestat groups (Figure 4B).

Efficacy of sivelestat in PCT-positive and PCT-negative patients

Patients were stratified according to whether they were PCT positive or negative, and the efficacy of sivelestat was evaluated for each group. Patient characteristics are shown in Table 7. In both PCT-positive and -negative patients, there were no significant differences in any clinical variable between the control and the sivelestat groups.

Among PCT-positive patients, the number of VFDs and ∆P/F of the sivelestat group were significantly higher than those of the control group ($P = 0.001$ and 0.009, respectively) (Figure 3C and D). Among PCT-negative patients, there were no significant differences in these values between the control and sivelestat patients (Figure 3C and D).

Analysis of predictive parameters of mortality in PCT-positive and PCT-negative patients

Univariate analysis showed that the number of failed organs, sivelestat administration; and the SOFA, GOCA, and DIC

Table 4 Clinical characteristics of non-septic patients and septic patients

|                | Non-septic |          | Sivelestat |          | P   |          | Sivelestat |          | P   |
|----------------|------------|----------|------------|----------|------|----------|------------|----------|------|
| Patients       | 13         | 23       |            |          |      |          |            |          |      |
| Sex (male/female) | 7/6        | 16/7     | 0.346      |          |      |          |            |          |      |
| Age (yrs)      | 73 (62–76) | 75 (64–81)| 0.365      |          |      |          |            |          |      |
| Etiology of ALI (direct/indirect) | 6/7        | 8/15     | 0.501      |          |      |          |            |          |      |
| Number of failed organs | 2 (1–3)   | 2 (1–2)  | 0.805      |          |      |          |            |          |      |
| Administration of steroid (yes/no) | 5/8        | 13/10    | 0.298      |          |      |          |            |          |      |
| SOFA score     | 9 (5–13)   | 8 (6–10) | 0.542      |          | 10/7 | 9 (6–11) | 0.259      |          |      |
| GOCA score     | 5 (4–7)    | 6 (4–6)  | 0.730      |          | 6    | 5 (4–7)  | 0.213      |          |      |
| SIRS score     | 3 (3–4)    | 3 (2–3)  | 0.753      |          | 3    | 3 (2–3)  | 0.312      |          |      |
| DIC score      | 5 (3–5)    | 3 (2–4)  | 0.096      |          | 4    | 2 (1–5)  | 0.556      |          |      |
| PaO$_2$/FIO$_2$ (mmHg) | 168.3 (115.9–178.8) | 135.2 (103.7–222.2) | 0.693 | 195.7 (138.5–250.5) | 140.0 (120.8–197.8) | 0.046 |
| WBC (×10$^3$/µL) | 12.70 (3.30–14.60) | 11.44 (9.06–14.41) | 0.521 | 12.80 (10.08–19.15) | 12.20 (6.68–17.68) | 0.419 |
| CRP (mg/dL)    | 6.88 (3.28–16.01)| 17.48 (4.81–20.29)| 0.134 | 13.59 (7.16–24.98) | 15.29 (6.91–21.62) | 0.978 |

Note: Results are median values (interquartile ranges in parentheses).

Abbreviations: ALI, acute lung injury; SOFA score, sequential organ failure assessment score; GOCA score, gas exchange, organ failure, cause, associated disease score; SIRS, systemic inflammatory response syndrome; DIC, disseminated intravascular coagulation; WBC, white blood cell; CRP, C-reactive protein.

Figure 2 Hazard ratios and 95% confidence intervals for survival based on the Cox proportional hazards model for assessing acute lung injury patients with systemic inflammatory response syndrome.
scores at diagnosis were significantly correlated with mortality in PCT-positive patients (all \( P < 0.05 \)) (Table 8). Multivariate analysis showed that sivelestat administration was a predictor of mortality only in PCT-positive patients (\( P = 0.030 \)) (Table 8). Kaplan–Meier curves showed that sivelestat administration significantly prolonged survival in comparison to the control group in PCT-positive patients (\( P = 0.015 \)) (Figure 4C). Although the data are not shown, the SOFA (HR for death, 1.176; 95% CI, 1.057–1.308; \( P = 0.003 \)), GOCA (HR for death, 1.472; 95% CI, 1.185–1.830; \( P < 0.001 \)), and DIC scores (HR for death, 1.344; 95% CI, 1.089–1.659; \( P = 0.006 \)) at diagnosis, but not sivelestat administration (HR for survival, 0.992; 95% CI, 0.400–2.462; \( P = 0.987 \)), were significantly correlated with survival in PCT-negative patients. In addition, there were no significant differences in the survival rate between the control and the sivelestat groups (Figure 4D).

**Discussion**

Our study showed that treatment with sivelestat improved the respiratory status of ALI patients with SIRS. When survival was evaluated as a function of sivelestat efficacy, drug administration was significantly more effective for patients with better respiratory function at diagnosis or with sepsis than for patients without these features. In addition, sivelestat significantly prolonged survival, led to a greater...
Table 5  The hazard ratios and 95% confidence intervals (CIs) for mortality based on univariate and multivariate Cox analysis in septic acute lung injury patients

| Parameter                      | Hazard ratio | 95% CI Low | 95% CI High | P     |
|-------------------------------|--------------|------------|-------------|-------|
| Univariate                    |              |            |             |       |
| Male                          | 1.260        | 0.607      | 2.617       | 0.535 |
| Age                           | 1.023        | 0.994      | 1.052       | 0.124 |
| Indirect lung injury          | 1.364        | 0.662      | 2.809       | 0.400 |
| Number of failed organs       | 2.704        | 1.699      | 4.305       | <0.001|
| Administration of sivelestat  | 0.393        | 0.191      | 0.808       | 0.011 |
| Administration of steroid     | 0.909        | 0.441      | 1.871       | 0.795 |
| SOFA score                    | 1.185        | 1.106      | 1.268       | <0.001|
| GOCA score                    | 1.553        | 1.303      | 1.851       | <0.001|
| SIRS score                    | 1.321        | 0.785      | 2.224       | 0.294 |
| DIC score                     | 1.317        | 1.136      | 1.526       | 0.003 |
| PaO₂/FIO₂ ratio               | 1.004        | 0.998      | 1.011       | 0.145 |
| WBC                           | 1.000        | 1.000      | 1.000       | 0.550 |
| CRP                           | 0.969        | 0.936      | 1.005       | 0.087 |
| Multivariate                  |              |            |             |       |
| Number of organ failures      | 1.185        | 0.604      | 2.323       | 0.621 |
| Administration of sivelestat  | 0.366        | 0.171      | 0.783       | 0.010 |
| SOFA score                    | 1.000        | 0.876      | 1.143       | 0.995 |
| GOCA score                    | 1.448        | 1.042      | 2.012       | 0.028 |
| DIC score                     | 1.106        | 0.929      | 1.317       | 0.257 |

Notes: SOFA score, sequential organ failure assessment score; GOCA score, gas exchange, organ failure, cause, associated disease score; SIRS, systemic inflammatory response syndrome; DIC, disseminated intravascular coagulation; WBC, white blood cell; CRP, C-reactive protein.

Sivelestat improved the respiratory status of patients with ALI associated with SIRS in our study. Others have also reported that sivelestat improved the lung injury score, reduced the duration of mechanical ventilation, and shortened the time in the ICU of ALI patients with SIRS. In addition, Aikawa et al reported that sivelestat for ALI patients with SIRS contributed to early weaning from mechanical ventilation. We have also shown that the number of VFDs and ∆P/F were significantly higher in the sivelestat group than in the control group. The survival rate tended to be higher in patients treated with sivelestat group than in the control patients, although statistical significance was not achieved.

The results of STRIVE, a prospective, randomized, double-blind, placebo-controlled, multicenter trial, indicated that sivelestat probably did not have beneficial effects on the respiratory function and survival of ALI/ARDS patients, whereas Phase III and IV Japanese studies showed that sivelestat was beneficial. This discrepancy might be due to the different study designs and patient populations.
Table 6 The hazard ratios and 95% confidence intervals for mortality based on univariate and multivariate Cox analysis in non-septic acute lung injury patients

| Parameter                        | Hazard ratio | 95% CI Low | 95% CI High | P   |
|----------------------------------|--------------|------------|-------------|-----|
| **Univariate**                   |              |            |             |     |
| Male                             | 0.824        | 0.313      | 2.170       | 0.695|
| Age                              | 1.023        | 0.984      | 1.064       | 0.250|
| Indirect lung injury             | 0.905        | 0.364      | 2.522       | 0.831|
| Number of failed organs          | 1.058        | 0.555      | 2.015       | 0.864|
| Administration of sivelestat     | 1.206        | 0.458      | 3.175       | 0.705|
| Administration of steroid        | 2.136        | 0.840      | 5.433       | 0.111|
| SOFA score                       | 1.137        | 1.003      | 1.288       | 0.044|
| GOCA score                       | 1.429        | 1.105      | 1.848       | 0.007|
| SIRS score                       | 0.902        | 0.494      | 1.647       | 0.738|
| DIC score                        | 1.184        | 0.965      | 1.453       | 0.106|
| PaO2/FIO2 ratio                  | 0.991        | 0.982      | 1.001       | 0.070|
| WBC                              | 1.000        | 1.000      | 1.000       | 0.391|
| CRP                              | 0.986        | 0.935      | 1.041       | 0.618|
| **Multivariate**                 |              |            |             |     |
| SOFA score                       | 0.958        | 0.787      | 1.167       | 0.670|
| GOCA score                       | 1.530        | 1.023      | 2.288       | 0.038|

Abbreviations: ALI, acute lung injury; SOFA score, sequential organ failure assessment score; GOCA score, gas exchange, organ failure, cause, associated disease score; SIRS, systemic inflammatory response syndrome; DIC, disseminated intravascular coagulation; WBC, white blood cell; CRP, C-reactive protein.

Table 7 Clinical characteristics of procalcitonin (PCT)-negative patients and PCT-positive patients

| Parameter                        | Control | Sivelestat | P   |
|----------------------------------|---------|------------|-----|
| PCT negative                     |         |            |     |
| Patients                         | 12      | 22         |     |
| Sex (male/female)                | 5/7     | 16/6       | 0.075|
| Age (yrs)                        | 71 (62–76)| 74 (69–81) | 0.387|
| Etiology of ALI (direct/indirect)| 5/7     | 9/13       | 0.966|
| Number of failed organs          | 2 (1–3) | 2 (1–2)    | 0.843|
| Administration of steroid (yes/no)| 4/8     | 14/8       | 0.091|
| SOFA score                       | 10 (5–14)| 8 (6–10)   | 0.601|
| GOCA score                       | 6 (4–8) | 6 (4–8)    | 0.732|
| SIRS score                       | 4 (3–4) | 3 (2–3)    | 0.056|
| DIC score                        | 4 (3–5) | 3 (2–5)    | 0.221|
| PaO2/FIO2 ratio (mmHg)            | 170.8 (107.8–187.5)| 130.4 (102.6–200.0) | 0.829|
| WBC (<10^10/μL)                  | 9.15 (3.20–14.90)| 11.92 (9.90–14.50) | 0.428|
| CRP (mg/dL)                      | 5.94 (2.56–11.82)| 16.88 (4.32–18.74) | 0.066|

| PCT positive                     |         |            |     |
| Patients                         | 28      | 48         |     |
| Sex (male/female)                | 18/10   | 32/16      | 0.833|
| Age (yrs)                        | 70 (57–79)| 73 (61–77) | 0.576|
| Etiology of ALI (direct/indirect)| 14/14   | 22/26      | 0.726|
| Number of failed organs          | 2 (1–3) | 2 (1–2)    | 0.096|
| Administration of steroid (yes/no)| 11/17   | 22/26      | 0.578|
| SOFA score                       | 10 (7–13)| 9 (6–10)   | 0.232|
| GOCA score                       | 6 (5–8) | 5 (4–7)    | 0.202|
| SIRS score                       | 3 (3–4) | 3 (2–3)    | 0.369|
| DIC score                        | 4 (2–5) | 2 (1–5)    | 0.383|
| PaO2/FIO2 ratio (mmHg)            | 184.6 (141.1–245)| 150.1 (127.0–205.5) | 0.090|
| WBC (<10^10/μL)                  | 13.30 (10.25–17.25)| 12.15 (6.75–17.60) | 0.383|
| CRP (mg/dL)                      | 15.22 (6.50–22.78)| 15.22 (6.50–22.78) | 0.667|

Note: Results are median values (interquartile ranges in parentheses).

Abbreviations: ALI, acute lung injury; SOFA score, sequential organ failure assessment score; GOCA score, gas exchange, organ failure, cause, associated disease score; SIRS, systemic inflammatory response syndrome; DIC, disseminated intravascular coagulation; WBC, white blood cell; PLT, platelet; CRP, C-reactive protein.
with SIRS. Differentiating sepsis from noninfectious SIRS is difficult, because sepsis is a complex, heterogeneous disorder. Previous reports have shown that PCT was a useful diagnostic marker for sepsis, and that PCT levels are closely correlated with the severity of sepsis. Therefore, we evaluated whether the initial serum PCT level was a useful indicator for the administration of sivelestat. In PCT-positive patients, the survival rate, number of VFDs, and ΔP/F of the sivelestat group were significantly higher than in the control group (P = 0.015, 0.001, and 0.009, respectively), whereas sivelestat efficacy was not demonstrated for PCT-negative patients. In addition, multivariate analysis showed that sivelestat administration was a predictor of mortality only in PCT-positive patients. These results suggest that the initial serum PCT level may be an indicator for whether to administer sivelestat to ALI patients with SIRS.

The relationship between PCT and sivelestat remains unclear. It was reported that interleukin (IL)-1β and tumor necrosis factor-α, which have been ascribed significant roles in the cytokine mediation of sepsis and septic shock, acted as potent stimulators of calcitonin messenger RNA expression and procalcitonin synthesis. Further, Suda et al reported that treatment with sivelestat suppressed the serum concentrations of IL-1β and tumor necrosis factor-α in septic animals. However, further studies are needed to clarify the relationship between procalcitonin and the neutrophil elastase inhibitor.

Sivelestat may be useful for patients with ALI and sepsis, as it has been reported that sivelestat might attenuate the vicious inflammatory cycle; reduce the sequestration, infiltration and activation of inflammatory cells; and suppress IL-8 and high-mobility group box chromosomal protein 1 (HMGB1) protein expression. Activated alveolar macrophages in sepsis produce various mediators such as IL-8 and HMGB1 that attract neutrophils to the lungs. Neutrophil elastase released from activated neutrophils stimulates protease-activated receptor 2 and induces production of proinflammatory cytokines such as IL-8, which leads to amplified sequestration, activation of neutrophils, and exacerbation of inflammation. Suda et al demonstrated that sivelestat significantly decreased the number of HMGB1- and IL-8-positive cells in the lungs of rats with sepsis. It was also reported that sivelestat administration decreased IL-8 serum levels in septic ALI patients.

## Limitations

Our study was a retrospective analysis, and the number of patients was small. There were no restrictions on the use of other drugs in addition to sivelestat, and we did not evaluate other therapies except for the use of steroids. We did not evaluate the effect of steroid administration on P/F or other parameters. Although there was no significant difference in the number of patients receiving steroid therapy in the control and sivelestat groups, additional studies examining the effects of steroid and sivelestat coadministration to ALI patients are needed. We did not evaluate any other inflammatory mediators. Finally, the PCT measurements were performed using semi-quantitative instead of quantitative analysis.

## Conclusion

Our study demonstrated that sivelestat might improve the ALI and survival of septic patients. The initial serum PCT level may be useful for indicating whether to use sivelestat for ALI patients with SIRS. However, a large prospective study is needed to clarify the usefulness of sivelestat for ALI patients with sepsis.

## Disclosure

The work reported here was undertaken at: Department of Integrated Medicine and Informatics, Ehime University Graduate School of Medicine; Department of Respiratory

### Table 8 The hazard ratios and 95% confidence intervals (CIs) for mortality based on univariate and multivariate Cox analysis in procalcitonin-positive patients

| Parameter                  | Hazard ratio | 95% Cl Low | 95% Cl High | P     |
|----------------------------|--------------|------------|-------------|-------|
| Univariate                 |              |            |             |       |
| Male                       | 1.246        | 0.583      | 2.682       | 0.570 |
| Age                        | 1.030        | 0.999      | 1.062       | 0.056 |
| Indirect lung injury       | 1.289        | 0.610      | 2.725       | 0.506 |
| Number of organ failure    | 2.616        | 1.616      | 4.234       | <0.001|
| Administration of sivelestat| 0.409        | 0.194      | 0.862       | 0.019 |
| Administration of steroid  | 0.942        | 0.446      | 1.992       | 0.876 |
| SOFA score                 | 1.173        | 1.091      | 1.261       | <0.001|
| GOCA score                 | 1.511        | 1.253      | 1.822       | <0.001|
| SIRS score                 | 1.451        | 0.841      | 2.504       | 0.181 |
| DIC score                  | 1.269        | 1.092      | 1.475       | 0.002 |
| PaO$_2$/FiO$_2$ ratio      | 1.003        | 0.997      | 1.010       | 0.323 |
| WBC                        | 1.000        | 1.000      | 1.000       | 0.582 |
| CRP                        | 0.981        | 0.947      | 1.017       | 0.295 |
| Multivariate               |              |            |             |       |
| Number of organ failure    | 1.275        | 0.635      | 2.561       | 0.494 |
| Administration of sivelestat| 0.422        | 0.194      | 0.919       | 0.030 |
| SOFA score                 | 1.001        | 0.868      | 1.154       | 0.992 |
| GOCA score                 | 1.365        | 0.959      | 1.941       | 0.084 |
| DIC score                  | 1.096        | 0.917      | 1.310       | 0.315 |

Abbreviations: ALI, acute lung injury; SOFA score, sequential organ failure assessment score; GOCA score, gas exchange organ failure, cause, associated disease score; SIRS, systemic inflammatory response syndrome; DIC, disseminated intravascular coagulation; WBC, white blood cell; CRP, C-reactive protein.
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The authors declare no conflicts of interest in this work.

References

1. Aikawa N, Ishizaka A, Hirasawa H, et al. Reevaluation of the efficacy and safety of the neutrophil elastase inhibitor, Sivelestat, for the treatment of acute lung injury associated with systemic inflammatory response syndrome; a phase IV study. *Pulm Pharmacol Ther*. 2011;24(5):549–554.

2. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000;342(18):1334–1349.

3. Janoff A, White R, Carp H, Harel S, Dearing R, Lee D. Lung injury induced by leukocytic proteases. *Am J Pathol*. 1979;97(1):111–136.

4. Itell S, Kucich U, Fein A, et al. Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. *Am Rev Respir Dis*. 1986;133(2):218–225.

5. Ueno H, Hirasawa H, Oda S, Shiga H, Nakanishi K, Matsuda K. Plasma elastase levels and inflammation and sepsis. *Jpn J Acad Sci*. 1991;62:267–277.

6. Delévaux I, André M, Colombier M, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? *Ann Rheum Dis*. 2003;62(4):337–340.

7. Luzzani A, Polati E, Dorizzi R, Rungatscher A, Pavan R, Merlini A. Diagnosis and treatment of sepsis and septic shock. *Am J Emerg Med*. 2000;18(5):499–508.

8. Wang H, Yang H, Tracey KJ. Extracellular role of HMGB1 in inflammation and sepsis. *Clin Sci (Lond)*. 2003;6(5):526–531.

9. Suda K, Takeuchi H, Hagiwara T, et al. Neutrophil elastase inhibitor improves survival of rats with clinically relevant sepsis. *Shock*. 2010;33(5):526–531.

10. Endo S, Sato N, Yasgami Y, et al. Sivelestat sodium hydrate improves septic acute lung injury by reducing alveolar dysfunction. *Res Commun Mol Pathol Pharmacol*. 2006;119(1–6):53–65.

11. Tsubokyo Y, Takeda S, Mitani S, et al. Clinical evaluation of sivelestat for acute lung injury in sepsis and acute respiratory distress syndrome following surgery for abdominal sepsis. *Drug Des Devel Ther*. 2012;6:273–278.

12. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20(6):864–874.
