Damage-associated molecular patterns in intensive care unit patients with acute liver injuries
A prospective cohort study

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Abstract

Acute liver injury (ALI) is frequently detected in an intensive care unit (ICU) and reportedly affects prognosis. Experimental animal studies suggested that increased extracellular histone and high morbidity group box-1 (HMGB1) levels might contribute to ALI development. Whether these damage-associated molecular patterns (DAMPs) play a crucial role in ALI remains unclear in the human clinical setting.

We consecutively enrolled the patients admitted to our ICU. The patients with ALI were included in the analysis together with those without ALI by using frequency matching. Extracellular histone, HMGB1, soluble thrombomodulin (sTM), and interleukin-6 (IL-6) levels were measured in plasma collected at ICU admission. ALI was defined as an acute elevation in serum aminotransferase levels to >200 IU/L.

A total of 805 patients were enrolled. Twenty ALI and forty non-ALI patients were analyzed. Plasma histone levels were significantly higher in the ALI group than in the non-ALI group, whereas HMGB1 levels were significantly lower in the ALI group. Furthermore, sTM was significantly increased in the ALI patients, whereas IL-6 levels were comparable between the groups. Multivariate logistic regression analysis demonstrated that histones were independently associated with ALI. There was no significant impact of ALI on in-hospital mortality.

Extracellular histones showed an independent association with ALI. Histone elevation might be one of the possible pathogenic mechanisms in the development of ALI of ICU patients.

Abbreviations: AKI = acute kidney injury, ALF = acute liver failure, ALI = acute liver injury, ALT = alanine aminotransferase, APTT = activated partial thromboplastin time, ARDS = acute respiratory distress syndrome, AST = aspartate aminotransferase, BMI = body mass index, CKD = chronic kidney disease, COPD = chronic obstructive pulmonary disease, DAMPs = damage-associated molecular patterns, DIC = disseminated intravascular coagulation, DM = diabetes mellitus, ELISA = enzyme-linked immunosorbent assay, FFP = fresh frozen plasma, FiO_2 = fraction of inspired oxygen, GCS = Glasgow Coma Scale, HMGB1 = high morbidity group box-1, ICU = intensive care unit, IL-6 = interleukin-6, IP = interstitial pneumonia, JMHW = Japanese Ministry of Health and Welfare, LDH = lactate dehydrogenase, NET = neutrophil extracellular trap, PaO_2 = partial pressure of oxygen, PC = platelet concentration, PRBC = packed red blood cells, PT-INR = prothrombin time-international normalized ratio, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SOFA = sequential organ failure assessment, sTM = soluble thrombomodulin, KDIGO = Kidney Disease Improving Global Outcomes.

Keywords: acute liver injury, high morbidity group box-1, histone, Interleukin-6, mortality, soluble thrombomodulin

1. Introduction

Acute liver injury (ALI) is frequently encountered in an intensive care unit (ICU), where liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were routinely measured with biochemistry tests.[1,2] Critically ill patients can inevitably be exposed to hypoxic, toxic, and inflammatory hepatic insults because of high incidence of shock or sepsis, a large number of pharmacological drugs used, and potential interaction of them.[3] At ICU admission, the liver enzyme tests have been reported to be abnormal in 61% of patients, impacting on 30-day mortality.[3,4]

Recent studies in animal ALI models revealed that damaged hepatocytes released damage-associated molecular patterns (DAMPs) including extracellular histones and high morbidity group box-1 (HMGB1) protein, which exacerbated liver injury by activating neutrophils to release proinflammatory cytokines and

chemokines.[5,6] Moreover, DAMPs were shown to promote the formation of neutrophil extracellular traps (NETs), which are increasingly recognized as an emerging mechanism of host defense against various pathogens.[7] Released NET webs produce more histones and HMGB1 to drive an auto-amplification loop of NET formation, leading to additional liver injury.[8] Similarly, several clinical studies demonstrated increased levels of extracellular histones and HMGB1 in patients with acute liver failure (ALF) compared to outpatients with chronic liver disease.[9,10]

Only supportive care still remains the main treatment option for ALI patients. Recently, DAMPs have been suggested as a promising target for the treatment of ALI.[8] Therefore, elucidating contribution of DAMPs to ALI development in the clinical is necessary. Nonetheless, thus far, the role of these DAMPs in ALI remains to be fully explored in critically ill patients. In addition, there are increasing number of diseases and treatments found to be associated with elevated levels of histones and HMGB1.[11,12] Because those conditions are frequently observed with ALI in the ICU setting, the association of the DAMPs with ALI needs to be evaluated with controlling the multiple confounders.

We conducted a prospective cohort study including patients in our ICU, to evaluate the association of extracellular histones and HMGB1 with ALI. We further evaluated soluble thrombomodulin (sTM), a factor which binds HMGB1 via an N-terminal lectin-like domain and was reported to reflect histone-induced endothelial damage, and interleukin 6 (IL-6), one of the inflammatory mediators induced by histones and HMGB1.[5,8,13,14] We also evaluated the impact of ALI on mortality and morbidity of ICU patients.

2. Materials and methods

2.1. Patient population and study design

The study protocol adhered to the Declaration of Helsinki and was approved by the institutional review board of the University of Tokyo. Informed consent was obtained from all participants or their legal representatives at study inclusion. In this prospective cohort study, we consecutively enrolled patients above 18 years of age who were admitted to the mixed medical-surgical ICU of the University of Tokyo Hospital between April and October 2015. Patients who received anticoagulant therapy at the time of ICU admission, who died within 24 hours following admission, and those who did not consent to participate were excluded. Among the enrolled patients, the patients with ALI were included in the analysis. For each ALI patient, 2 non-ALI patients were selected using frequency matching on sex, age (within 5 years), and the sequential organ failure assessment (SOFA) score (within 2 points) at admission. All matched patients were followed until hospital discharge or death.

2.2. Definitions

Reportedly, the most sensitive and specific aminotransferase cut-off levels to discriminate ALI lied within the moderate range of increase (ie, 5–10 times the upper reference limit).[2] To avoid the gray zone in which ALI and other causes overlap, ALI was defined as acute increase in a serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level to above 200 IU/L, whereas non-ALI was defined as both serum AST and ALT levels of less than 100 IU/L. The causes of ALI were diagnosed based on clinical histories, serological or radiological tests. Diagnosis of hypoxic hepatitis was done using the criteria

proposed by Henrion.[15] Chronic liver disease was defined as clinically stable liver disease lasting for more than six months.[9,10] Sepsis diagnosis was based on the definition of the American College of Chest Physicians/Society for Critical Care Medicine.[16] Acute respiratory distress syndrome (ARDS) was diagnosed based on the criteria of the Berlin definition.[17] Acute kidney injury (AKI) was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines for AKI.[18] Disseminated intravascular coagulation (DIC) definition was based on the Japanese Ministry of Health and Welfare (JMHW) DIC diagnostic criteria which was applicable to coagulopathy with various underlying diseases including liver and hematopoietic diseases.[19] In the current study, shock status was defined as the combination of hypotension (systolic blood pressure <90 mmHg or mean arterial pressure <60 mmHg) and signs of tissue hypoperfusion (≥2-point decrease in the Glasgow Coma Scale score, mottled skin, urinary output <25 mL/h, or capillary refilling time ≥3 seconds; and arterial lactate levels >2 mmol/L).[20]

2.3. Data collection and laboratory analysis

The following clinical information were collected from the medical records: history of chronic diseases such as chronic liver disease, chronic obstructive pulmonary disorder (COPD), diabetes mellitus (DM), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA); diagnosis for admission; presence of shock during admission; implementation of hemodialysis and mechanical ventilation; administration of platelet concentration (PC), fresh frozen plasma (FFP), and packed red blood cells (PRBC). We also documented the following prognostic data: length of ICU stay, 28-day mortality, and in-hospital mortality. Arterial blood samples were analyzed to determine the ratio of arterial oxygen partial pressure to the fraction of oxygen in inspired air (PaO_2/FiO_2 ratio) as a marker of oxygenation, and standard hematological, biochemical, and coagulation parameters were analyzed at ICU admission. Serum aminotransferase levels were followed until discharge or death of the matched patients.

For laboratory analysis, blood samples of enrolled patients were prospectively collected into potassium ethylenediaminetetraacetic acid-containing plastic tubes at ICU admission and centrifuged at 1500g for 10 min at 4°C immediately after collection. The plasma samples were stored at −80°C until analysis. Extracellular histone levels in the plasma of the ALI and the matched non-ALI patients were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cell Death Detection ELISA[plus]; Sigma Aldrich, St. Louis, MO). This assay uses monoclonal mouse antibodies against single-strand and double-strand DNA and histones (H1, H2A, H2B, H3, and H4) to determine histone-associated DNA fragments. Plasma HMGB1 levels were analyzed by an established ELISA kit (Fuso Pharmaceutical, Osaka, Japan), according to the manufacturer's protocol. Plasma concentrations of soluble thrombomodulin (sTM) and interleukin-6 (IL-6) were determined using commercially available ELISA kits against human thrombomodulin and human IL-6, respectively (Quantikine; R&D Systems, Minneapolis, MN).[13,14]

2.4. Sample size calculation

The primary variable of our study was the plasma level of extracellular histones. In our small pilot study conducted before

the current study, we measured plasma histone levels of 5 ALI and 5 non-ALI patients and estimated difference of mean and standard deviation would be 0.9 and 1.0, respectively in log-transformed. Based on this observation, the sample size was calculated as being 20 ALI patients and 40 non-ALI patients, assuming a type I error rate of 0.05, a power of 0.9, an anticipated effect size d = difference of means/standard deviation = 0.9, and 1:2 in number of ALI and non-ALI patients.

2.5. Statistical analysis

Continuous variables were presented as means ± standard deviation or medians with interquartile ranges, and categorical variables were presented as percentages. Categorical data were compared by the χ^2 or Fisher exact test, while continuous data were compared by student's t test or Wilcoxon rank-sum test. Correlations between variables were analyzed using Spearman rank correlation. To evaluate whether DAMPs were independently associated with ALI, the following confounding factors for DAMPs and ALI were prespecified among disorders or conditions that were previously shown to be associated with extracellular histones or HMGB1: age, sex, SOFA score, chronic conditions (chronic liver disease, hematopoietic disease, cancer, COPD/IP, and SLE/RA), stroke, sepsis, ARDS, AKI, DIC, presence of shock status at admission, plasma sTM levels, and blood transfusion.[11,12,21–27] Univariate and multivariate logistic regression analyses were conducted with ALI diagnosis as a dependent variable. Only parameters that were significantly associated with ALI in univariate logistic analysis were included in a multivariate logistic regression model. Finally, the amount of multicollinearity between the covariates was assessed using the variance inflation factor (VIF). Goodness-of-fit was evaluated using the log-likelihood and chi square statistic. All continuous parameters with skewed distribution were entered into these models as log-transformed variables using natural logarithm to the base e.

All statistical analyses and calculations were conducted by the JMP Pro software (version 11.0.0; SAS Institute, Cary, NC). A 2-tailed $P < .05$ was considered statistically significant for all tests.

3. Results

3.1. Patient characteristics

Eight hundred and eighty-seven patients were admitted to the ICU during the study period. Eighty-two patients were excluded; 81 patients were treated with anticoagulants and 1 patient declined the participation in our study at ICU admission. Finally, 805 patients were enrolled. A total of 60 patients consisting of 20 ALI patients and 40 non-ALI patients were selected by frequency-matching and included in the analysis (Fig. 1).

Tables 1 and 2 show the baseline characteristics, laboratory variables at admission, and treatment-related factors of the study population. The sex ratio, age, and SOFA score were comparable between the ALI and non-ALI groups, whereas the ALI group had significantly higher levels of lactate dehydrogenase and bilirubin and lower platelet counts. As shown in Figure 2, serum aminotransferase levels in the patients with ALI declined to baseline levels within 7 to 10 days after ICU admission. The proportion of the coexisting disorders and treatments reportedly associated with the levels of extracellular histones and HMGB1 were comparable between the 2 groups with 2 exceptions: the history of chronic liver disease and FFP administration were more frequent in the ALI group.

Table 3 shows the etiologies of ALI in this study. Half of the ALI cases were attributed to the surgical procedure, whereas the remaining ALI cases were mainly due to hypoxic hepatitis from sepsis, hemorrhage, or pulmonary thromboembolism.

3.2. Association of extracellular histones, HMGB1, sTM, and IL-6 with ALI

At ICU admission, extracellular histone levels were significantly elevated in the ALI group (Fig. 3A), whereas HMGB1 levels were significantly lower in the ALI group than in the non-ALI group (Fig. 3B). Table 4 shows the correlations between DAMPs (ie, histone and HMGB1) and serum aminotransferase levels. Both histone and HMGB1 levels were significantly correlated with serum AST and ALT levels. In addition, histone and HMGB1 levels were inversely correlated in the study population (ρ, −0.449; $P < .001$).

To investigate the mechanism underlying the changes of histones and HMGB1 in ALI, we compared the levels of sTM, an anti-inflammatory protein blocking HMGB1, at ICU admission between the ALI and non-ALI groups. Figure 3C shows that sTM levels in the ALI group were significantly higher than those in the non-ALI group. There were no significant differences between the groups in serum creatinine levels or the proportion of patients with DM, SLE/RA, DIC, or ARDS, which were previously reported to impact sTM levels [19,22,28–31]. The levels of sTM were significantly correlated with the histone levels in the study population (ρ, 0.26; $P = .048$). However, no difference in the levels of IL-6, an inflammatory cytokine induced by DAMPs, was observed between the 2 groups (Fig. 2D).

Furthermore, we constructed logistic regression models to evaluate associations of histones and HMGB1 with ALI, with a priori defined confounding factors. First, we investigated each factor individually in a univariate logistic analysis and included all significant factors (histones, HMGB1, sTM, history of chronic liver disease, and administration of FFP) in a multivariate logistic regression model, with the diagnosis of ALI as the dependent variable. Table 5 shows that histones were independently associated with ALI. However, HMGB1 failed to show an independent relationship with the presence of ALI. The VIFs of histones, HMGB1, sTM, history of chronic liver disease, and administration of FFP were 1.77, 1.42, 1.21, 1.10, and 1.41, respectively, showing no multicollinearity among the covariates. A goodness-of-fit test ($P = .95$) suggested that the regression model was appropriate for our data.

3.3. Impact of ALI on the prognosis of ICU patients

The diagnosis of ALI did not have a significant effect on 28-day and in-hospital mortality rates (Table 6). Similarly, the length of ICU stay did not differ between the ALI and the non-ALI groups.

4. Discussion

This study demonstrated the difference in associations between extracellular histone and HMGB1 levels and ALI in the ICU setting. Significantly higher levels of histones and lower levels of HMGB1 were observed in the ALI patients compared with the non-ALI patients. We also demonstrated that histones were independently associated with the presence of ALI, although HMGB1 was not, after adjustment for numerous confounding factors. However, the 28-day and in-hospital mortality rates were not significantly higher in the ALI patients in the current study.
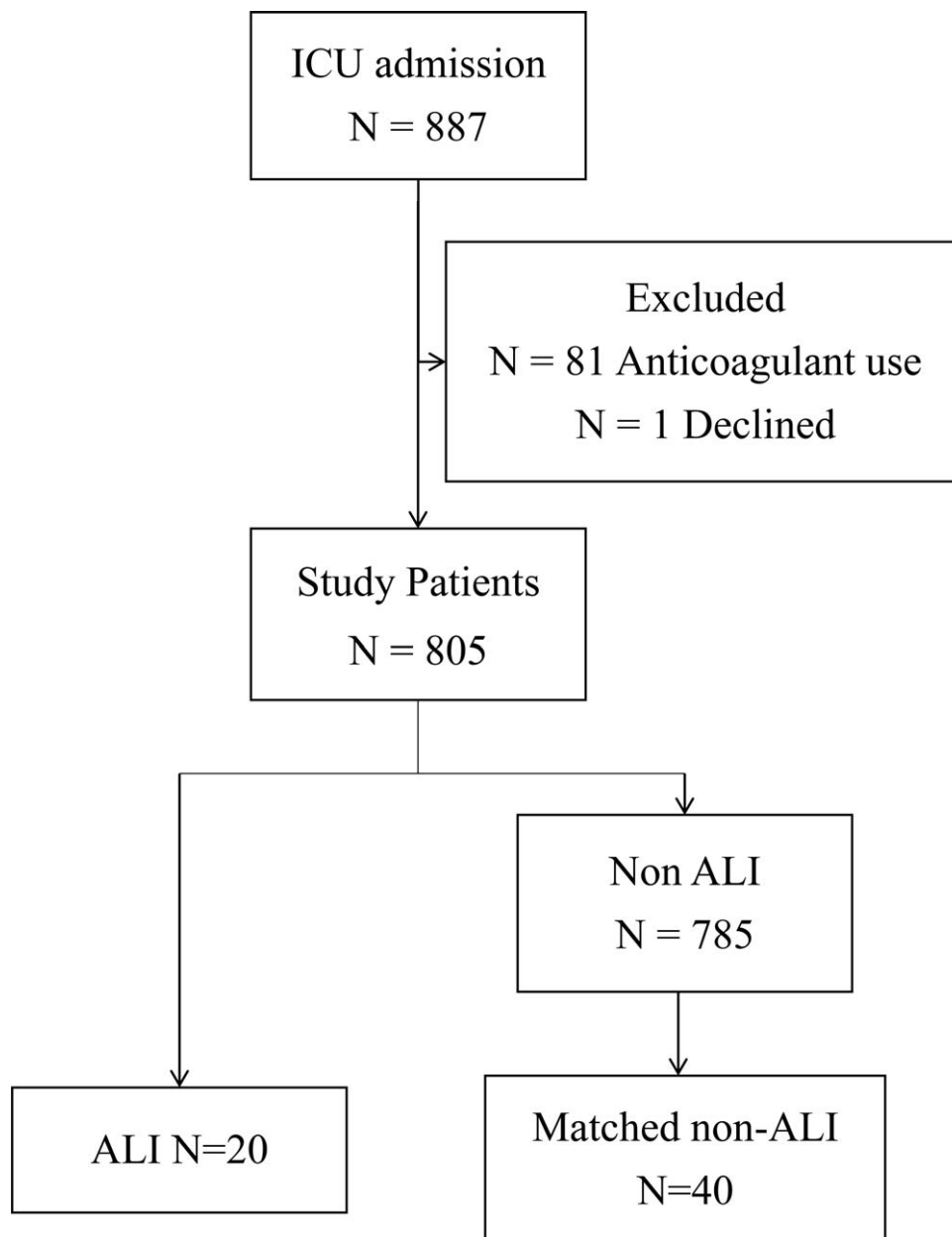
Figure 1. Study flow showing sample selection. ALI, acute liver injury; ICU, intensive care unit.

Extracellular histones and HMGB1 have recently been recognized as DAMPs or alarmins, which play a crucial role as harmful mediators of organ damage in various inflammatory conditions such as sepsis, ARDS, stroke, AKI, trauma, cancer, and autoimmune disorders.[11,14,24,32] The basic studies in murine ischemia reperfusion-induced ALI models demonstrated that histones and HMGB1 released from damaged hepatocytes activated neutrophils to form NETs through the activation of toll-like receptors (TLR4 or TLR9).[5,6] Moreover, histones and HMGB1 in the NET fibers were considered to promote self-amplification of NET formation and production of proinflammatory cytokines and chemokines, thereby exacerbating liver injury.[8] Other ALI models induced by Concanavalin A or acetaminophen also showed the involvement of extracellular histones in their pathogenesis. [10,33]

This is the first study to assess DAMPs in ALI with a wide range of severity in the ICU, taking into account co-existing diseases and treatments related to DAMPs. We herein demonstrated that extracellular histone levels were significantly increased in ALI patients, in accordance with the findings of previous studies. We also demonstrated that histone levels were independently associated with the diagnosis of ALI after adjusting for confounding factors that were shown to be associated with changes in histone levels. The prominent increase in plasma histone levels might reflect the increase in NET formation that in turn induces the release of extracellular histones in damaged liver. In our study, the majority of ALI were caused by liver surgery and hypoxic hepatitis. A recent translational study reported that high levels of circulating histones were detected in patients with ALF due to viral hepatitis, drug-induced hepatitis, and autoimmune

Table 1

Demographic characteristics and chronic conditions of the study patients.

	ALI (N=20)	Non-ALI (N=40)	P
Age, y	63.0±4.3	62.4±3.1	.90
Sex (male), n (%)	9 (45)	21 (53)	.78
SOFA score	6.5 (2–11)	5 (2–8)	.26
BMI, kg/m²	22.7±4.4	22.6±4.0	.98
Reason for admission to ICU, n (%)			
Medical	8 (40)	20 (50)	.59
Elective surgery	10 (50)	17 (44)	.78
Emergency surgery	2 (10)	3 (8)	>.99
Chronic condition, n (%)			
Chronic liver disease	6 (30)	2 (5)	.013
Hepatitis B	0 (0.0)	1 (2.5)	
Hepatitis C	3 (15)	0 (0.0)	
Hepatocellular carcinoma	2 (10)	0 (0.0)	
Metastatic liver tumor	4 (20)	0 (0.0)	
Alcoholic liver cirrhosis	2 (10)	0 (0.0)	
Biliary atresia	1 (5.0)	0 (0.0)	
Primary biliary cirrhosis	0 (0.0)	1 (2.5)	
Hematopoietic disease	2 (10)	2 (5)	.60
CKD	2 (10)	9 (23)	.31
DM	7 (35)	8 (20)	.22
COPD or IP	2 (10)	3 (7.5)	>.99
SLE or RA	1 (5.0)	3 (7.5)	>.99
Cancer	6 (30)	7 (18)	.33

ALI = acute liver injury, BMI = body mass index, CKD = chronic kidney disease, COPD = chronic obstructive pulmonary disease, DM = diabetes mellitus, ICU = intensive care unit, IP = interstitial pneumonia, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SOFA = sequential organ failure assessment.
Continuous data are presented as means ± standard deviation or medians (interquartile range), and categorical data are presented as n (%).

Table 2

Clinical characteristics of the study patients.

	ALI (N=20)	Non-ALI (N=40)	P
Physiological and laboratory variables			
GCS	15 (14–15)	15 (14–15)	.36
PaO₂/FiO₂	335 (206–478)	263 (184–415)	.24
AST, IU/L	375 (253–1476)	23 (17–35)	<.001
ALT, IU/L	254 (174–922)	14 (10–18)	<.001
LDH, IU/L	487 (404–3285)	256 (201–295)	<.001
Serum creatinine, mg/dL	0.89 (0.68–1.51)	0.78 (0.62–1.37)	.60
Serum bilirubin, mg/dL	1.6 (1.1–2.3)	0.6 (0.4–1.2)	<.001
Platelet count, 10⁴/dL	9.1 (5.8–15.2)	18.6 (11.8–24.1)	<.001
PT-INR	1.21 (1.09–1.61)	1.03 (0.95–1.12)	<.001
APTT, sec	31 (26–38)	30 (26–34)	.22
Organ failure, n (%)			
Presence of shock	10 (50)	10 (25)	.081
Sepsis	4 (20)	14 (35)	.37
ARDS	6 (30)	15 (38)	.58
AKI	10 (50)	12 (30)	.16
Stroke	1 (5.0)	7 (18)	.25
DIC	8 (40)	7 (18)	.11
Treatment factors, n (%)			
Mechanical ventilation	9 (45)	22 (55)	.59
Hemodialysis	4 (20)	7 (18)	>.99
Transfusion			
PRBC	10 (50)	10 (25)	.081
FFP	9 (45)	3 (7.5)	.001
PC	6 (30)	6 (15)	.19

AKI = acute kidney injury, ALI = acute liver injury, ALT = alanine aminotransferase, APTT = activated partial thromboplastin time, ARDS = acute respiratory distress syndrome, AST = aspartate aminotransferase, DIC = disseminated intravascular coagulation, FFP = fresh frozen plasma, FiO₂ = fraction of inspired oxygen, GCS = Glasgow coma scale, LDH = lactate dehydrogenase, PaO₂ = partial pressure of oxygen, PC = platelet concentration, PRBC = packed red blood cells, PT-INR = prothrombin time-international normalized ratio.
Continuous data are presented as medians (interquartile range), and categorical data are presented as n (%).
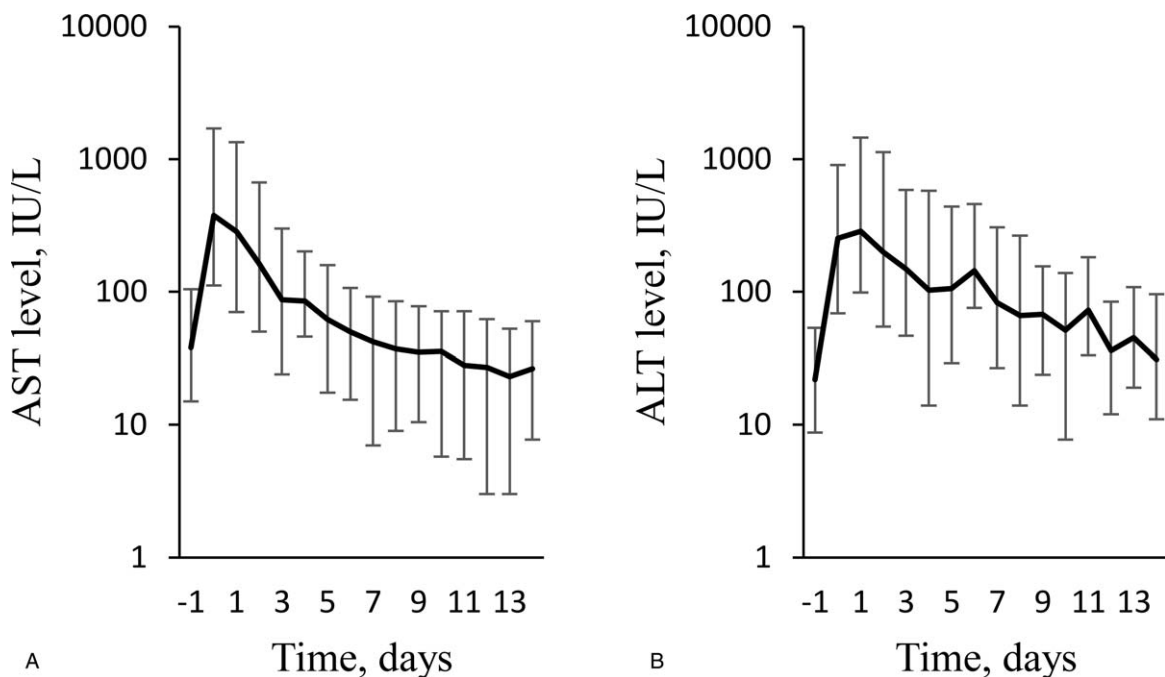

Figure 2. Changes in aminotransferase levels in the ALI group (N=20). Aminotransferase levels were recorded before ICU admission (day–1), at ICU admission (day 0), and repeatedly for 14 days. ALI, acute liver injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ICU, intensive care unit.

Table 3

Etiologies of acute liver injury in the study (N = 20).

Causes	Number of patients
Post-liver surgery	
Hepatocellular carcinoma	2
Metastatic liver tumor	4
Donors in LDLT	2
Recipients in LDLT	2
Hypoxic hepatitis	
Gastrointestinal bleeding	4
Sepsis	3
Pulmonary thromboembolism	1
Acute bile duct obstruction	2

LDLT = living donor liver transplantation.

hepatitis.[9,10] Activated NET formation in damaged liver might be a common pathological mechanism in ALI due to diverse etiologies.

Interestingly, HMGB1 levels in the ALI patients were significantly lower than those in the non-ALI patients. In addition to histones and HMGB1, we evaluated the levels of sTM, an endothelium-specific membrane protein, based on a previous study showing that sTM binds HMGB1 via an N-terminal lectin-like domain to exert anti-inflammatory effects.[13] Then, we showed that sTM levels were significantly increased in the ALI patients and that there was a positive correlation between sTM and histone levels, which might reflect histone-induced endothelial damage.[14] After adjustment of confounding factors for HMGB1 including sTM, we found that HMGB1 levels were not independently associated with ALI. Previous experimental studies reported that administration of recombinant human sTM significantly decreased serum HMGB1 levels in rats with ALI induced by endotoxemia or ischemia-reperfusion insult.[34,35] Increased levels of sTM might contribute to the decrease in HMGB1 levels, consequently attenuating the production of inflammatory cytokines in ALI patients. Indeed, the findings of the current study indicated that the IL-6 levels in the ALI patients
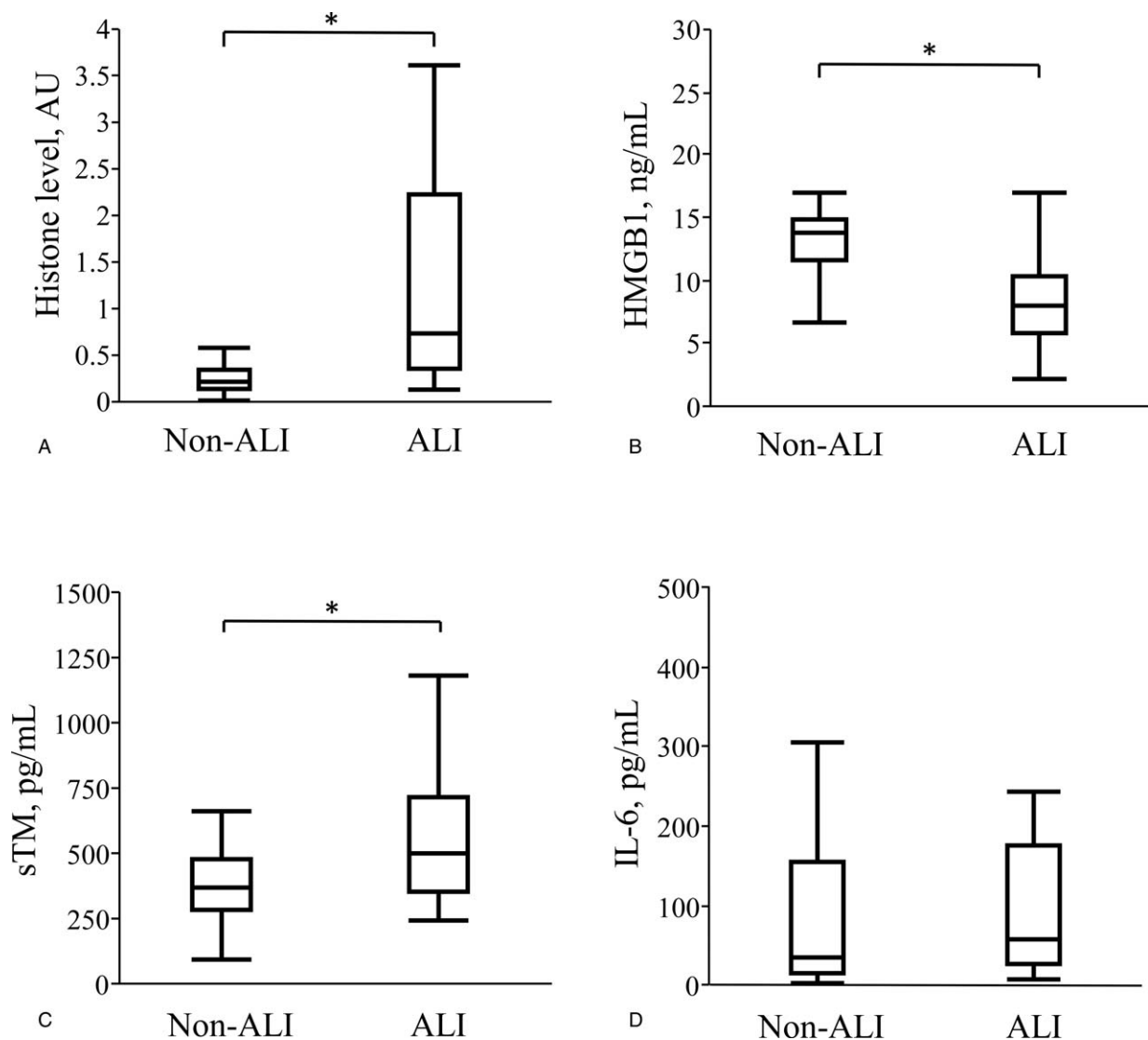

Figure 3. Extracellular histone (A), HMGB1 (B), sTM (C), and IL-6 (D) levels in patients with ALI (N = 20) and without ALI (N = 40). ALI, acute liver injury; HMGB1, high morbidity group box-1; IL-6, interleukin-6; sTM, soluble thrombomodulin. *P < .05 by Wilcoxon rank-sum test. No significant difference in the levels of IL-6 was observed between the groups by Wilcoxon rank-sum test (P = .35).

Table 4

Spearman's correlation between damage-associated molecular patterns and aminotransferase levels (N=60).

	Histone		HMGB1	
	ρ	P	ρ	P
AST	0.645	<.001	−0.538	<.001
ALT	0.604	<.001	−0.447	<.001

ALT = alanine aminotransferase, AST = aspartate aminotransferase, HMGB1 = high morbidity group box-1.
Correlations between parameters were assessed with Spearman rank correlation.

Table 5

Univariate and multivariate logistic regression model for factors associated with the diagnosis of acute liver injury.

	Univariate			Multivariate		
Variables	OR	95% CI	P	OR	95% CI	P
Age	1.0	0.97–1.03	.91			
Sex (male)	0.74	0.25–2.17	.58			
SOFA score	1.4	0.68–3.07	.38			
Histone	6.0	2.62–18.3	<.001	6.75	1.68–27.1	0.007
HMGB1	0.035	0.004–0.21	<.001	0.06	0.004–1.02	0.052
sTM	4.17	1.20–14.5	.014	2.14	0.46–10.1	0.34
Chronic liver diseases	8.14	1.47–45.2	.017	7.65	0.82–71.2	0.074
Hematopoietic diseases	0.65	0.03–5.46	.71			
COPD or IP	1.37	0.17–8.98	.74			
SLE or RA	0.65	0.03–5.46	.71			
Cancer	2.02	0.57–7.10	.27			
Presence of shock	3.0	0.97–9.30	.057			
Sepsis	0.46	0.12–1.56	.22			
ARDS	0.64	0.19–1.97	.45			
AKI	2.33	0.77–7.20	.13			
Stroke	0.25	0.01–1.55	.15			
DIC	3.14	0.94–10.9	.063			
PRBC	3.0	0.97–9.55	.055			
FFP	10.1	2.53–51.9	<.001	3.76	0.36–39.0	0.27
PC	2.43	0.66–9.08	.18			

AKI = acute kidney injury, ARDS = acute respiratory distress syndrome, CI = confidence interval, COPD = chronic obstructive pulmonary disease, DIC = disseminated intravascular coagulation, FFP = fresh frozen plasma, HMGB1 = high morbidity group box 1, IP = interstitial pneumonia, OR = odds ratio, PC = platelet concentration, PRBC = packed red blood cells, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SOFA = sequential organ failure assessment, sTM = soluble thrombomodulin.

were not different from those in the non-ALI patients. Elevated sTM could be a protective mechanism against the progression of ALI, via the induction of histone release into the extracellular space. However, the multivariate logistic analysis also showed elevated sTM levels did not show any association with the presence of ALI. A previous study reported that HMGB1-binding antibodies could not only interfere detection of HMGB1 by ELISA but also modulate cytokine activity of HMGB1.[36] It may be possible that the auto-antibodies might reduce the values of HMGB1 measured by ELISA in the ALI patients.

Extracellular histones have been recognized as important prognostic factors in various disorders including sepsis and

Table 6

Prognosis of the study patients.

Outcome	ALI (N=20)	Non-ALI (N=40)	P
Mortality, n (%)			
28 day	4 (20)	3 (8)	.21
Hospital	5 (25)	3 (8)	.11
Length of ICU stay, days			
All	4.5 (3–11.5)	7 (3–12)	.70
Survivors only	6 (3–16)	7 (3–11)	.74

ALI = acute liver injury, ICU = intensive care unit.

trauma.[32,37] Previous studies in ALI models suggested the detrimental effect of histones on the outcomes of ALI.[6,8,10,33] However, our study found no significant differences in 28-day and in-hospital mortality rates between the ALI and the non-ALI patients, irrespective of the higher levels of extracellular histones detected in the ALI patients. This finding might be due to the relatively small number of cases for the detection of potential changes in mortality rates in the current study. In addition, liver injury observed in the current study was generally attenuated at 7 to 10 days after ICU admission. Reversible and transient liver injury might not have a significant impact on mortality. The potential protective action of sTM elevation described above might also play a role in the recovery from liver injury. Our finding may be supported by the previous clinical study showing that elevated aminotransferase levels at ICU admission were not independent determinants of 30 day mortality after adjustment of acute physiology and chronic health evaluation II score.[4] Further investigation is needed to clarify whether elevated histone levels in ALI might have an impact on clinically important outcomes.

Several limitations should be acknowledged that might affect the results of the current study. First, this study was conducted at a single ICU, and the number of patients was relatively small. Given the small sample size, this study is exploratory in nature. In

addition, high cost of histone measurement did not allow to analyze many patients in this study. High throughput measurement with lower cost for histone measurement needs to be developed. Second, since the elevation of extracellular histones and HMGB1 can be multifactorial, it is difficult to identify a single source of DAMPs in critically ill patients. Third, the levels of extracellular histones and HMGB1 were determined only at ICU admission. Many of DAMPs have time dependency and can fluctuate due to varying degree of organ dysfunction in ICU patients. Repeated-measures analysis will provide superior insight into changes in the levels of DAMPs over time following liver insults and during recovery. Fourth, this observational study could not determine the causal relationship between DAMPs and ALI development. Fifth, because of inadequate specificity of serum aminotransferase, the possibility of overestimation of the ALI cases cannot be excluded. An accurate interpretation of elevated aminotransferase levels remains challenging in ICU patients. Finally, the processes and severity of liver damage were heterogeneous in the ALI group. There might be a critical difference between ALI caused by extra-hepatic insults including sepsis or gastrointestinal bleeding and that from liver surgery or trauma in terms of pathogenic mechanism and clinical course. Following this preliminary proof-of-concept study, further investigation with larger cohorts in the respective etiology of ALI should be done to confirm our findings.

In conclusion, this study showed that the levels of extracellular histones were significantly and independently associated with the presence of ALI. Increased histones may contribute to the development of ALI in ICU patients, although many other mechanisms may also play crucial roles in ALI development. Further investigation is necessary to elucidate the pathological roles of DAMPs and their impact on prognosis in clinical ALI.

Acknowledgments

Author contributions

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