Circulating histones are major mediators of multiple organ dysfunction syndrome in acute critical illnesses

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Grants: This work was supported by the Medical Research Council (G0501641), British Heart Foundation (PG/14/19/30751 and PG/16/65/32313) and National Institute of Health Research (II-FS-0110-14061). Scholarships from China Scholarship Council (CSC:20160609156) and Southeast University (YBJJ1740) to ZXC.

Short running head: Circulating histone-mediated multiple organ dysfunction syndrome

Word count: 2993 including Abstract

Keywords: multiple organ dysfunction syndrome (MODS), circulating histones, sepsis, trauma, pancreatitis.

ABSTRACT (294)

Objective: Multiple organ dysfunction syndrome (MODS) is characterised by simultaneous multiple organ failure, which is the leading cause of death in acute critically ill patients. However, what mediates MODS is not fully understood. The discovery of toxic effects by extracellular histones on different individual organs strongly suggests their involvement in MODS. In this study, we investigate whether circulating histones are major mediators of MODS in acute critical illnesses.

Design: Combination of retrospective clinical studies and animal models with intervention.

Setting: Intensive Care Unit (ICU) in a tertiary hospital and research laboratories

Patients: 420 ICU patients, including sepsis (140), severe trauma (63), severe pancreatitis (89) and other admission diagnoses (128).

Laboratory investigation: Cells from major organs treated with calf thymus histones or histone-containing sera. Animal models for sepsis, trauma and acute pancreatitis treated with anti-histone reagents.

Intervention: Anti-histone reagents in in vitro, ex vivo and animal models.

Measurement and Main Results: Retrospective analysis of a prospectively recruited ICU cohort demonstrated a strong correlation between circulating histones and organ injury markers and Sequential Organ Failure Assessment (SOFA) scores. Ex vivo experiments showed that patient sera containing high histone levels were toxic to cultured cells from

different origins, suggesting their universal toxicity to multiple organs. Animal models of sepsis, trauma and pancreatitis further demonstrated a temporal correlation between histone levels and disease severity and multiple organ injury. Importantly, anti-histone reagents, i.e. anti-histone single-chain variable fragment (ahscFv) and non-anticoagulant heparin, could dramatically reduce multiple organ injury, particularly of the heart and lungs, and improve survival in mouse models.

Conclusions: High levels of circulating histones are major mediators of MODS. Our results indicate that monitoring upon ICU admission could inform on disease severity and developing anti-histone therapy holds great potential of reducing MODS and improving survival of critically ill patients.

INTRODUCTION

Multiple organ dysfunction syndrome (MODS) is characterised by dysfunction of two or more organs in acute critically ill patients who require intervention to maintain homeostasis in Intensive Care Units (ICU)(1, 2) . Affected patients have high mortality rates of around 30-100%, depending on the number of dysfunctional organs. Therefore MODS has become the leading cause of morbidity and mortality in current ICU practice (3). Sepsis is the most common primary disease of MODS and causes more deaths than any single type of cancer (4). In the UK, sepsis alone causes 44,000 deaths and costs over 7.6 billion pounds annually. The incidence of sepsis continues to increase by over 30% in the last 2 years (5). Recently, the third international consensus definitions for sepsis have altered previous focus on inflammation to life-threatening multiple organ dysfunction (6). This change reflects the importance of MODS in sepsis.

MODS is mediated by harmful simultaneous effects to multiple organs rather than a chain reaction from one organ to another (7). However, the identification of common mediators remains undefined. Coagulation activation, microcirculatory failure and oxygen deprivation as well as bacterial toxins have been proposed as potential mediators but have not been fully proven (8). In the last decade, the secondary hit theory by damage-associated molecular patterns (DAMPs) (9), particularly histones released from damaged cells, has attracted attention. Histones are well-conserved proteins essential in DNA packaging and gene regulation (10). During cell damage, nuclear chromatin is cleaved into nucleosomes, which are released extracellularly (11) and further degraded into individual histones (12). Nucleosomes/histones are rapidly cleared by the liver (13) and are rarely detected in blood, unless extensive tissue damage or cell death occurs in a short time period, e.g in acute critical illnesses.

Histones are highly cationic proteins that are able to interact with negatively charged phosphate groups of double-stranded DNA and phospholipid bilayers to condense chromatin and disrupt cell membranes, respectively (14, 15). Histone disruption of cell membranes cause abnormal ion flow, leading to loss of membrane potential with calcium overload and consequent cell damage (15). Histones are also the natural ligands of TLR-2, -4 and -9 receptors (16, 17) and serve as the most important DAMPs in activating immune cells, inflammasomes, and release of pro-inflammatory cytokines (18, 19), and enhancing thrombin generation through platelet activation and aggregation (20, 21). Low levels of histones activate endothelium to release von Willebrand factor (VWF) (22), recruit leucocytes/platelets and reduce thrombomodulin-dependent protein C anticoagulant effects (23, 24), whilst high histone levels directly cause endothelial damage (15, 25) and are strongly associated with the development of disseminated intravascular coagulation (DIC) (26).

Although extracellular histones have been reported to damage single organs (15-17, 25, 27-31) and anti-histone reagents, such as neutralizing antibodies (15, 25), heparins (29, 32, 33) and C1 esterase inhibitors (34) are able to reduce histone toxicity, it is not fully clear whether circulating histones are the major secondary hit by mediating MODS development. In this study, we aligned clinical studies with animal models for severe trauma, acute pancreatitis and sepsis, to clarify the roles of circulating histones in MODS development in these common acute critical illnesses.

METHODS

Study design and patients

We retrospectively assessed a prospectively recruited cohort of adult patients admitted to the general ICU at the Royal Liverpool University Hospital (RLUH) between Jan 2008 and Jan 2014. Patients with preconditions that may cause biases were excluded (Supplemental Figure

1). Clinical data and blood samples were collected on admission and then daily up to day 4 in accordance to protocol (Ref: 07/H1009/64) approved by Northwest Research and RLUH Ethics Committees and protocol (Ref: 13/NW/0089) approved by NRES Committee North West-Haydock. MODS was defined as SOFA scores ≥5. Blood samples were also collected from healthy donors according to protocol (RETH000685) approved by Committee on Research Ethics, University of Liverpool. All normal control and patient plasma samples were snap frozen and stored at -80^{o}C until analysis. Samples used for assays in this study had not previously been thawed and re-frozen.

Mice

C57/BL6 male, 8-10 week old mice from Beijing Vital River Laboratory Animal Technology were housed and used in sterile conditions at the Research Centre of Genetically Modified Mice, Southeast University, China. All procedures were performed according to State laws and monitored by local inspectors in compliance with British Home Office laws. CZX holds the full animal license for use of mice.

Disease-specific models

The mouse trauma model by fall of a heavy object (15), sepsis model by Cecal Ligation and Puncture (CLP) (35), and acute pancreatitis models using cerulein and sodium taurochlorate (TCL) (36, 37) was generated with different severities. The detailed procedures are described in Supplemental Data.

Statistical analysis

Human data are presented as median and interquartile ranges [1st, 3rd quartiles]. Differences in medians of continuous clinical variables between two (Mann Whitney U test) or more groups (Kruskall-Wallis test) were tested. Continuous variables used in ex vivo and in vivo

experiments were normally distributed and are presented as mean ± standard deviation (SD). Differences in means between more than two groups were compared using ANOVA test followed by Student-Newman-Keuls test. Correlation between circulating histone and organ injury markers utilized Spearman's rank test for human data and linear regression for mouse data based on the distribution of the data. To test whether circulating histones were predictors of MODS development (48-72h post ICU admission) and 28-day mortality, logistic regression and Receiver Operating Characteristic (ROC) analysis were performed. Survival time comparison was performed using Log rank test. P value (two-tailed) <0.05 was considered statistically significant.

RESULTS

Elevated circulating histone levels in human critical illnesses are associated with MODS

To determine the clinical relevance of circulating histones, we examined a cohort of prospectively recruited 420 ICU patients and their clinical characteristics are described in Supplemental Table 1. Collectively, the data demonstrated that circulating histone levels (median 24.7μg/ml [quartiles 8.0μg/ml, 46.7μg/ml]) are significantly elevated compared to normal healthy donors (1.3 μg/ml [0, 2.1]) (Figure 1A) (P<0.001). Among the subgroups of critical illnesses, circulating histone levels on admission were significantly higher in patients with either an admission diagnosis of sepsis (n=140) (34.0μg/ml [13.2, 60.5]), severe trauma (n=63) (23.8 μg/ml [11.1, 45.2] and severe pancreatitis (n=89) (28.7μg/ml [11.6, 63.8]), compared to others (n=128) (9.2 μg/ml [1.3, 30.0]) (P<0.05). There were no statistical differences in terms of circulating histone levels between sepsis, trauma and pancreatitis patients (Figure 1B). Using Spearman's rank correlation, we found strong correlations between circulating histone levels and clinical organ injury biomarkers, including blood urea nitrogen (BUN for renal function, r=0.496, P<0.0001), alanine aminotransferase (ALT for

liver injury, r=0.545, P<0.0001), cardiac troponin T (cTnT for cardiac injury, r=0.607, P<0.01) and PaO2/FiO2 (P/F ratio for lung function, r=0.360. P=0.015).

Strong correlation between circulating histones and Sequential Organ Failure Assessment (SOFA) scores was also observed (r=0.574, P<0.0001). The levels of circulating histones in patients with MODS (SOFA score ≥5) were significantly higher than those without (median 30.1μg/ml [quartiles 7.3, 63.2] vs 10.8μg/ml [4.3, 30.1], P<0.0001) (Figure 1C). Similarly, circulating histones were higher in patients who died within 28 days of ICU admission than patients who survived (32.7μg/ml [14.4, 66.9] vs 20.1μg/ml [6.7, 40.5], P< 0.0001) (Figure 1D). These data suggest that levels of circulating histones reflect severity of disease and their presence may contribute to MODS and mortality, particularly at high levels. ROC analysis demonstrated that admission histone levels could predict MODS development 48-72h after ICU admission (AUC 0.617, P=0.001), which is supported by a logistic regression model (odds ratio = 1.012 [1.003, 1.020] (P=0.005)). Similarly, ICU admission histone level could predict 28 day mortality (AUC 0.625 (P<0.001), odds ratio = 1.006 (1.002, 1.010) (P<0.001)).**Sera from patients with high histone levels are cytotoxic and not cell-type specific**

Histone-spiked normal sera from healthy donors were found toxic to cultured human endothelial (EAhy926) cells in a dose-dependent manner. At or above 30μg/ml histones, cell viability was significantly reduced, which could be reversed by addition of either 50μg/ml heparin or ahscFv (Figure 2A). When endothelial cells were incubated with ICU patient sera containing circulating histones >30μg/ml, their viability was significantly reduced (Figure 4B). Conversely, cell viability was not significantly reduced when they were incubated with sera with undetectable histones or <30μg/ml. Importantly, addition of ahscFv or non-

anticoagulant heparin could significantly reduce the cytotoxicity by over 90% (Figure 2B), demonstrating that the major toxic factors in these patients' sera were elevated histones.

To demonstrate that circulating histones are also toxic to primary cells from different organs, primary kidney epithelial cells, immortalized liver cells, primary lung epithelial cells, and cardiomyocytes were incubated with 50μg/ml calf thymus histones or pooled patients' sera containing about 50μg/ml circulating histones. Figure 2C shows that cell viability was reduced to comparable levels in all cell types. This data indicates that histones are non-selectively toxic to all tested cells and high histone levels may damage most organs simultaneously.

Circulating histone levels increase with increasing disease severity in mouse models

To comprehensively investigate the role of elevated circulating histones *in vivo*, we used different mouse models for the major acute critical illnesses and found circulating histone levels increased significantly with increasing severity of trauma, sepsis and pancreatitis (Figure 3A-C). In the trauma model, circulating histones increased significantly 1h after trauma to peak at 8h (Supplemental Figure 2A). At 8h, circulating histones were 22.7±8.3μg/ml (Mean±SD) in mild, 80.3±14.2μg/ml in moderate and 242.3±132.9μg/ml in the severe model (Figure 3A, $P<0.01$). In the sepsis models, elevated histone levels were significantly elevated 2h after CLP induction and peaked at 16h (Supplemental Figure 2B). At this time, circulating histone levels were 74.3±15.9μg/ml in less severe and 129.3±43.7μg/ml in severe sepsis (Figure 3B). In the pancreatitis models, cerulein (4x) and cerulein (12x)-induced pancreatitis, circulating histones showed a significant increase at around 6h after the first dose of cerulein and peaked at approximately 14-20h. In TCL-induced severe pancreatitis, circulating histones began to increase 2h following induction and peaked at approximately

16h (Supplemental Figure 2C). At 16h, circulating histones were 3.7±3.5μg/ml in cerulein (4x), 90.2±35.9μg/ml in cerulein (12x) and 155.0±79.1μg/ml in the TCL model (Figure 3C).

Circulating histone levels strongly correlate with organ injury markers in mouse models

Circulating biomarkers for liver, renal and cardiac injury as well as lung injury scores based on H&E stained sections were significantly elevated in these mouse models compared to mock controls (Supplemental Table 2). Typical H&E stained lung sections are presented in Supplemental Figure 3, showing increased thickness of alveolar walls, extensive neutrophil infiltration and hyaline membrane formation. In contrast, mock procedures did not cause obvious changes in lung morphology. No obvious morphological changes were identified in heart, liver and kidney sections (Data not shown). No substantial numbers of apoptotic cells were observed by immuno-histochemical staining (using anti-active caspase-3 from Abcam) of the lungs, liver, heart and kidneys but substantial numbers were found in the spleen and thymus (Supplemental Figure 4). Linear regression analysis demonstrated that elevations in circulating biomarkers for liver (ALT, $r=0.588$, $P<0.001$) (Figure 4A), for renal (BUN, $r=0.539$, $P<0.001$) (Figure 4B), for cardiac (cTnI, $r=0.605$, $P<0.001$) (Figure 4C) and lung injury scores ($r=0.726$, $P<0.001$) (Figure 4D), strongly correlated with circulating histone levels in these mouse models.

Anti-histone reagents significantly reduce multiple organ injury and mortality in mouse models

To examine for a causal relationship between elevated circulating histones and MODS, ahscFv and non-anticoagulant heparin were used (Figure 5A-C, Supplemental Table 2). We found that anti-histone reagents significantly reduced organ injury in severe trauma (5A), severe sepsis (5B) and TCL pancreatitis (5C) models. The cTnI was most affected by anti-histone treatment and its levels were reduced by about 90% compared to untreated models,

whereas BUN and ALT were only reduced by 30-60%. Morphologically, lung injury as indicated by increased alveolar wall thickness, extensive neutrophil infiltration in interstitial tissues and alveoli, and hyaline membrane formation, was also significantly improved by anti-histone treatments (Supplemental Figure 5). Accordingly, lung injury scores were significantly reduced (Figure 5D). In terms of mortality, anti-histone reagents significantly increased the survival time and reduced mortality in 72h after CLP in the severe sepsis model. Administration of anti-histone reagents before CLP (Figure 5E) appeared more effective than at 6h after CLP (Figure 5F) but there was no statistical difference. This data demonstrates that elevations in circulating histones are major mediators of MODS and also contribute to mortality.

DISCUSSION

Multiple but not single organ dysfunction is the most common pathological feature of critical illnesses and appears to be the major contributor of lethality. However, mediators of simultaneous dysfunction of multiple organs are unclear and not fully clarified. In this study, we have investigated the roles of circulating histones in 3 of the most common human critical illnesses in alignment with corresponding animal models where anti-histone treatment could be used. Extracellular histones are not detectable in healthy donors but high histone levels were found in all the 3 common acute critical illnesses, i.e. sepsis, trauma and pancreatitis in both human and animal models. Nearly all critically ill patients showed elevated circulating histones compared to healthy donors. In nearly two-thirds of our ICU population, histone levels were above 30μg/ml, a previously determined threshold for cytotoxicity (26). Furthermore, circulating histone levels were significantly associated with disease severity and MODS as well as to adverse clinical outcomes. The fact that anti-histone reagents could detoxify circulating histones to significantly alleviate MODS and improve survival rates

strongly indicates that high circulating histone levels are not only biomarkers of disease severity but act as a secondary hit to directly contribute to MODS and poor outcomes.

The source of circulating histones is still not clearly defined in the different critical illnesses. It is most likely that in trauma, circulating histones are released from extensively damaged tissues. The early elevation of circulating histones (within 1h) and its significant correlation with the extent of injury strongly supports this argument. In severe pancreatitis, the source of histones is not so obvious. Necrosis of the pancreas is very likely a major source of circulating histones. However, histone levels increase within 24h of onset but no obvious pancreatic necrosis can usually be detected in patients at this time point (38). There may be therefore other sources of histones. We had previously observed that extensive neutrophil loss occurs prior to pancreatic necrosis and correlates with circulating histone levels, within 24h of admission of patients with acute pancreatitis (39). This data could suggest that neutrophil death or NETosis could be another major source of circulating histones, particularly in the early stage of severe pancreatitis. In sepsis, the source of circulating histones also remains unclear. Organ injury may be the source of circulating histones but limited cell death was observed in major organs (Supplemental Figure 4), which therefore suggests that this is not the major source. NETosis or immune cell death, particularly in spleen and thymus was extensive (Supplemental Figure 4) and this might be the major contributor (40-42) but further investigation is required. Since histones themselves can cause cell injury by forming pores on cell membrane, similar to pyroptosis, it is likely that a vicious cycle may exist to damage more cells and release more histones.

The mechanisms of histone-mediated MODS may be divided into direct and indirect effects. In behaving like endogenous pore-forming toxins, circulating histones are non-selectively toxic to cells from different organs (Figure 2) and thereby injure multiple organs

simultaneously (27, 28, 43). Indirect effects may be due to activation of immune responses or coagulation activation (44, 45). An extensive pro-inflammatory state with microcirculatory impairment may especially compromise multiple organs (19). Conversely, histone release also triggers protective mechanisms to recalibrate homeostasis, such as increase in acute phase protein, C-reactive protein, to neutralise histone toxicity, and interaction with complement component 4 to inhibit complement activation (46, 47).

Identification of severely ill patients is important in clinical practice, particularly those that require intensive care support. Irrespective of the source of circulating histones, high levels could represent severe inflammation, infection or tissue damage (15, 25, 36, 48), highlighting their clinical usefulness as a biomarker of disease severity. Previously, we demonstrated that high histone levels in a non-ICU setting could identify patients with pancreatitis at risk of developing persistent organ failure (39). This current ICU-based study demonstrates that this approach could be extended to the majority of critical illnesses. While this study is limited to a single ICU, our demographics and performance are consistent with comparable units according to the Intensive Care National Audit and Research Centre (ICNARC) data. Measuring circulating histones could identify those deteriorating patients that need critical attention.

Anti-histone therapy has been proposed for many years (25) but no specific therapy has become clinically available. One option would be the development of anti-histone antibodies, but there are limitations in terms of production, purification and the risk of host auto-antibodies. Non-anticoagulant heparin has been demonstrated to be a viable and effective alternative anti-histone reagent in histone infusion models (33, 49). Our data in patient ex-vivo experimentation complemented by relevant in vivo modelling advances the translational

potential of neutralising circulating histones using non-anticoagulant heparin to reduce MODS and mortality in critically ill patients.

Acknowledgements: We thank Dr. Steven Lane for guiding our statistical analysis. The authors are indebted to participating patients and their attending staff at Royal Liverpool University Hospital as well as former researchers who worked in our laboratory for their contributions to ethical permission applications and database managements. Special thanks to Professor Philip Rudland at University of Liverpool for critical reading of the manuscript. Thanks to Professor Yi Yang and Professor Haibo Qiu for their supports in supervising the animal experiments.

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Figure legends

Figure 1. Circulating histones are elevated in sepsis, pancreatitis and trauma patients. (A) Circulating histones were quantified in the plasma of normal healthy donors (n=10) and ICU patients (n=420). Mann-Whitney U test shows significant increases in circulating histones in ICU patients compared to healthy controls, $P<0.0001$. (B) Patients were stratified based on an admission diagnosis and circulating histone levels were examined. Kruskall-Wallis test shows significantly elevated circulating histones in sepsis (n=140), pancreatitis (n=89) and trauma (n=63) patients compared to those with other admission diagnoses (n=128), $P<0.01$. Circulating histones levels were analyzed in ICU patients who developed MODS and those who did not (C) along with those who survived and died (D). Mann–Whitney *U* test shows significant difference.

Figure 2. **Sera from patients with high levels of histones are cytotoxic**. (A) Sera from healthy donors spiked with calf thymus histones (A) or sera isolated from patients with high levels of circulating histones (B) were used to treat cultured EAhy926 endothelial cells in the absence or presence of anti-histone reagents, heparin or ahscFv (50 μg/ml) for 1h and then viable cells were detected. (C) Cells derived from different organs, including cardiomyocytes (Heart), lung epithelial cells (Lung), liver cells (Liver) and kidney cells (Kidney), were treated with calf thymus histones (50 μg/ml) or pooled patients' sera with approximately 50 μg/ml endogenous histones. Sera with undetectable histones were used as controls and their resultant cell viability was set as 100%. Means±SD of cell survival rates from at least 3 independent experiments are presented. ANOVA test, $*P<0.05$ when compared to sera without histones. $\#P<0.05$ when compared to histone in sera alone at the indicated concentrations.

Figure 3. Circulating histones are elevated in mouse models along with increasing severity. Comparison of the peak circulating histone levels in normal mice (N=3), mild (N=12), moderate (N=12) and severe (N=15) trauma (A), mock CLP (N=3), less severe (N=12) and severe (N=15) sepsis (B) and saline control (N=3), mock TCL (n=3), cerulein (4x, N=10), cerulein (12x, N=11) and TCL (N=11) pancreatitis (C) mouse models are presented as Means±SD. ANOVA test *P<0.05 when compared to 0h. #P<0.05 when compared to the less severe model.

Figure 4. Circulating histones strongly correlate with organ injury markers in mouse models. The correlation of circulating histones in all 3 mouse disease models (excluding all controls) with parameters of organ injury, ALT for liver (r=0.588, N=92, P<0.001) (A), BUN for renal (r=0.539, N=92, P<0.001) (B), cTnI for cardiac (r=0.605, N=92, P<0.001) (C) and lung injury scores (r=0.726, N=46 (randomly selected from each group), P<0.001) (D) were analyzed using linear regression.

Figure 5. Anti-histone reagents reduce organ injury and mortality in mouse models. Anti-histone reagents, i.e. non-anticoagulant heparin (Sigma, 25 mg/kg) and ahscFv (20 mg/kg) were used to treat mice with severe trauma (A), severe sepsis (B) and taurochlorate (TCL)-induced pancreatitis (C). Changes of organ injury markers are presented as percentage by setting that without anti-histone treatment as 100%. Means±SD are presented. ANOVA test, * P<0.05 when compared to untreated. (D) Changes in lung injury scores before and after treatments are shown. ANOVA test, *P<0.05 when compared with mock group, #P<0.05 when compared with model alone group. (E-F) Survival curves of severe sepsis model untreated or treated with anti-histone reagents starting before CLP (E) or 6h after CLP (F) over a 72h period. Log rank test, P<0.01 when compared to CLP alone.

Figure 1

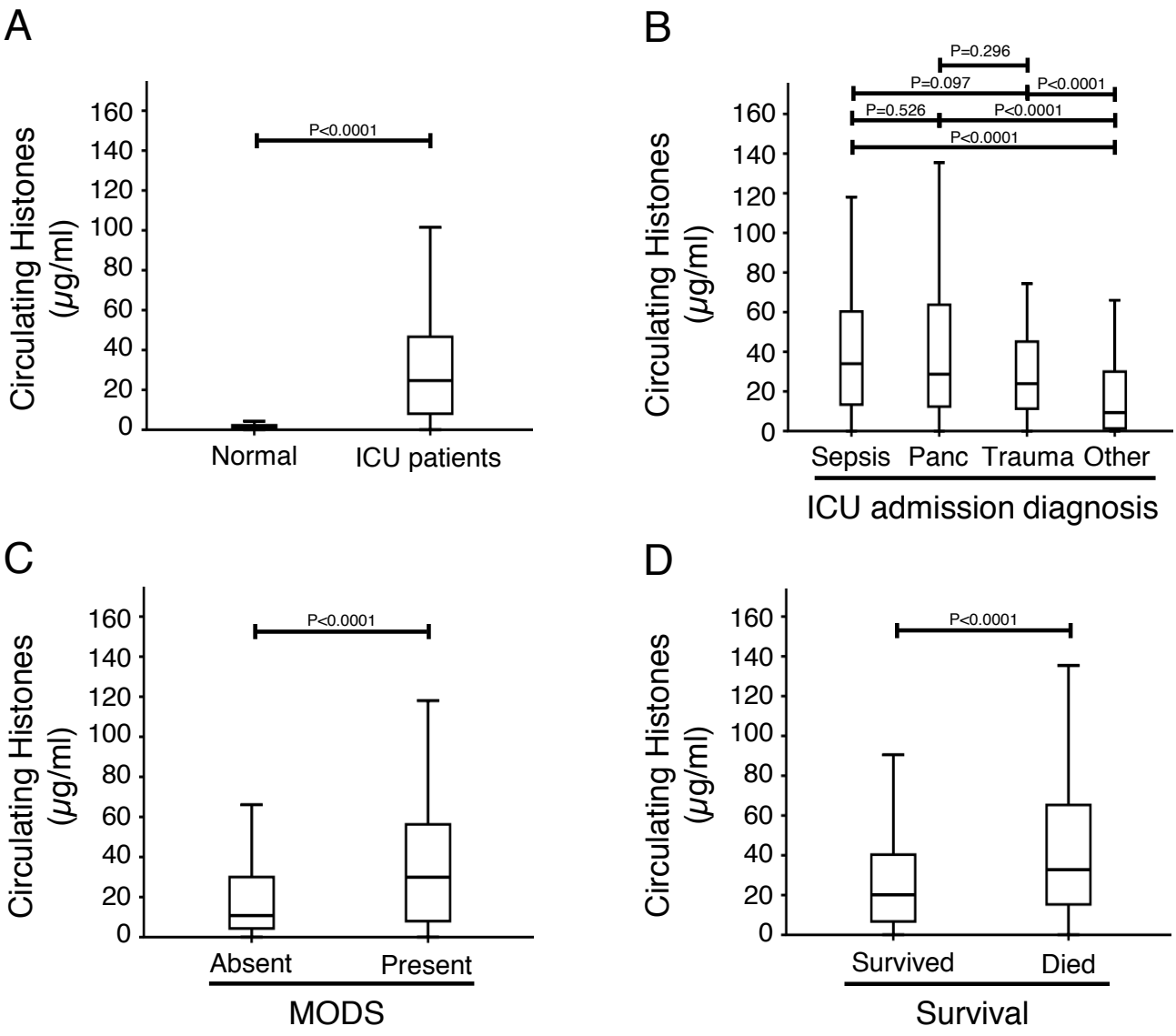

A
Circulating Histones (μg/ml)
160
140
120
100
80
60
40
20
0
P<0.0001
Normal
ICU patients
B
Circulating Histones (μg/ml)
P=0.296
P=0.097
P<0.0001
P=0.526
P<0.0001
P<0.0001
Sepsis
Panc
Trauma
Other
ICU admission diagnosis
C
Circulating Histones (μg/ml)
P<0.0001
Absent
Present
MODS
D
Circulating Histones (μg/ml)
P<0.0001
Survived
Died
Survival

Figure 2

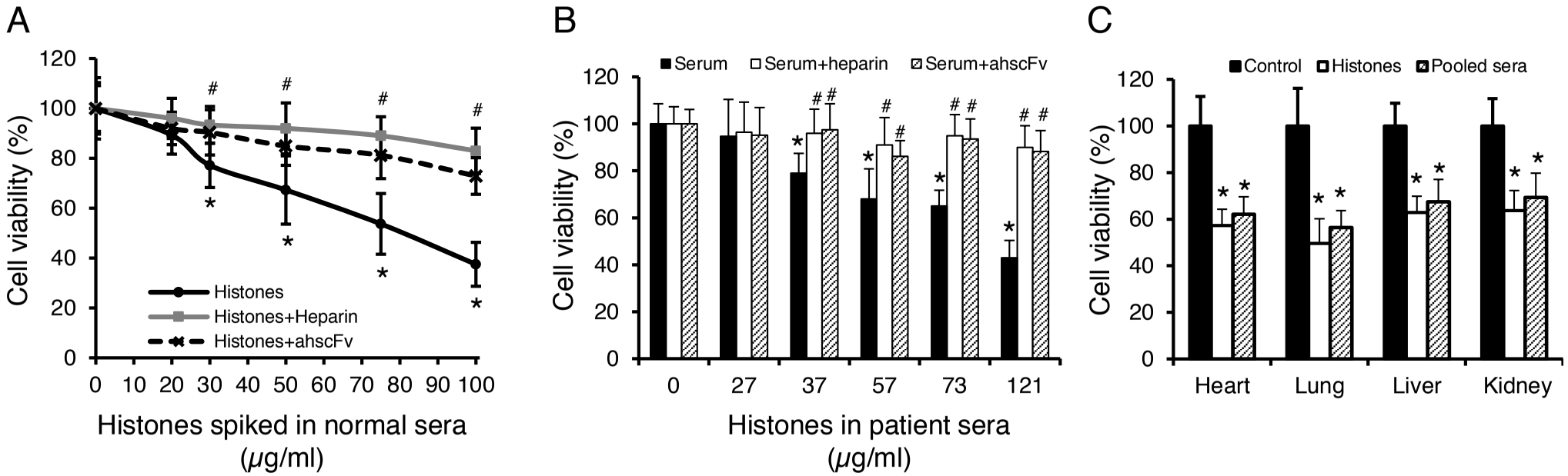

A
Cell viability (%)
120
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100
Histones
Histones+Heparin
Histones+ahscFv
Histones spiked in normal sera
(μg/ml)
B
Serum
Serum+heparin
Serum+ahscFv
Cell viability (%)
0 27 37 57 73 121
Histones in patient sera
(μg/ml)
C
Control
Histones
Pooled sera
Cell viability (%)
Heart
Lung
Liver
Kidney

Figure 3

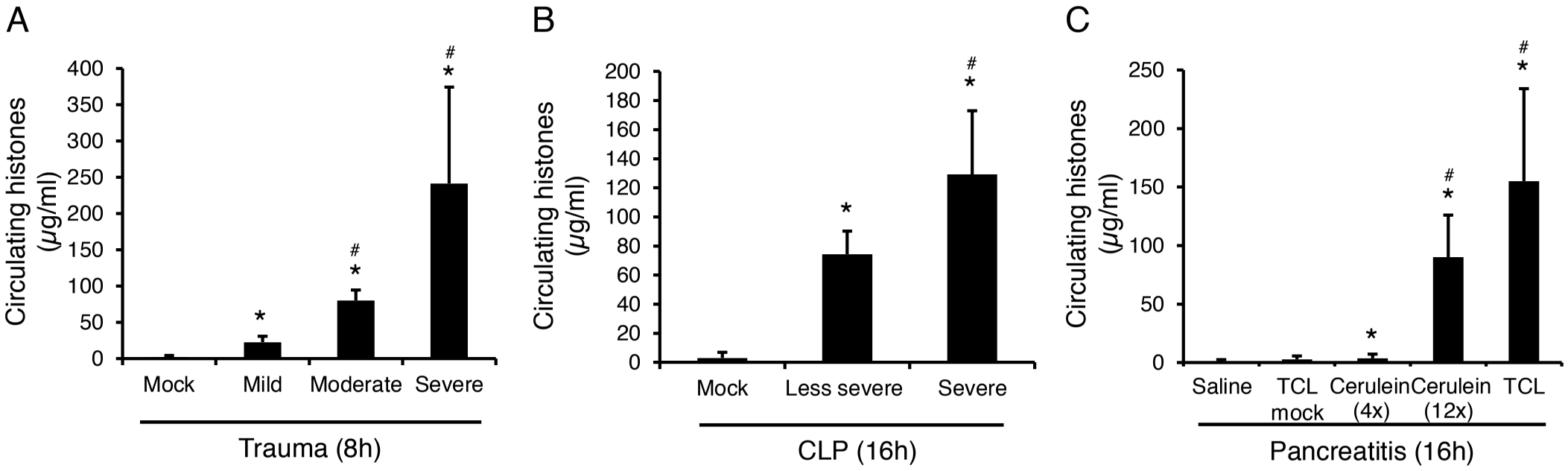

A
Circulating histones (μg/ml)
400
350
300
250
200
150
100
50
0
Mock
Mild
Moderate
Severe
Trauma (8h)
B
Circulating histones (μg/ml)
200
180
160
140
120
100
80
60
40
20
0
Mock
Less severe
Severe
CLP (16h)
C
Circulating histones (μg/ml)
250
200
150
100
50
0
Saline
TCL mock
Cerulein (4x)
Cerulein (12x)
TCL
Pancreatitis (16h)

Figure 4

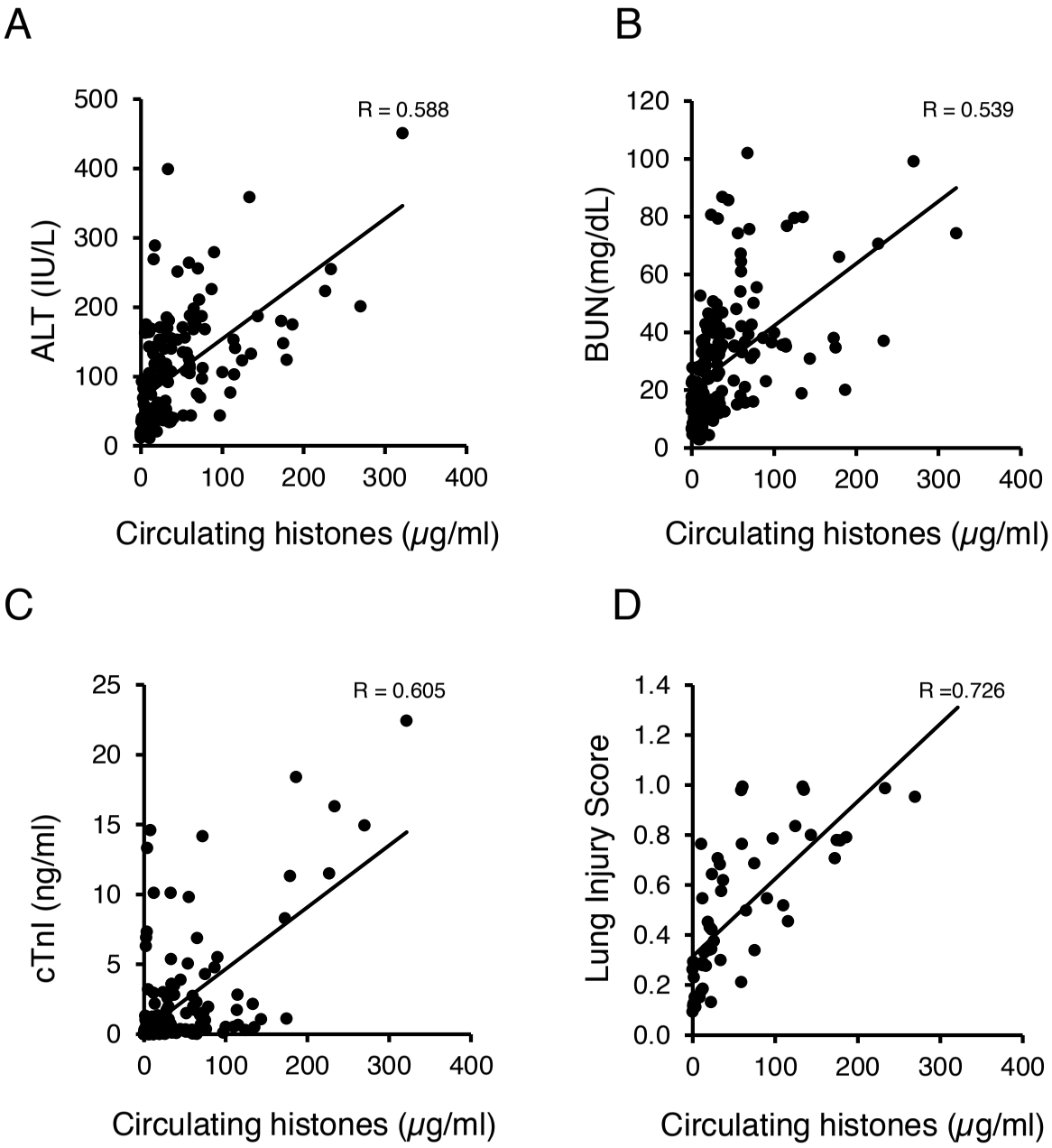
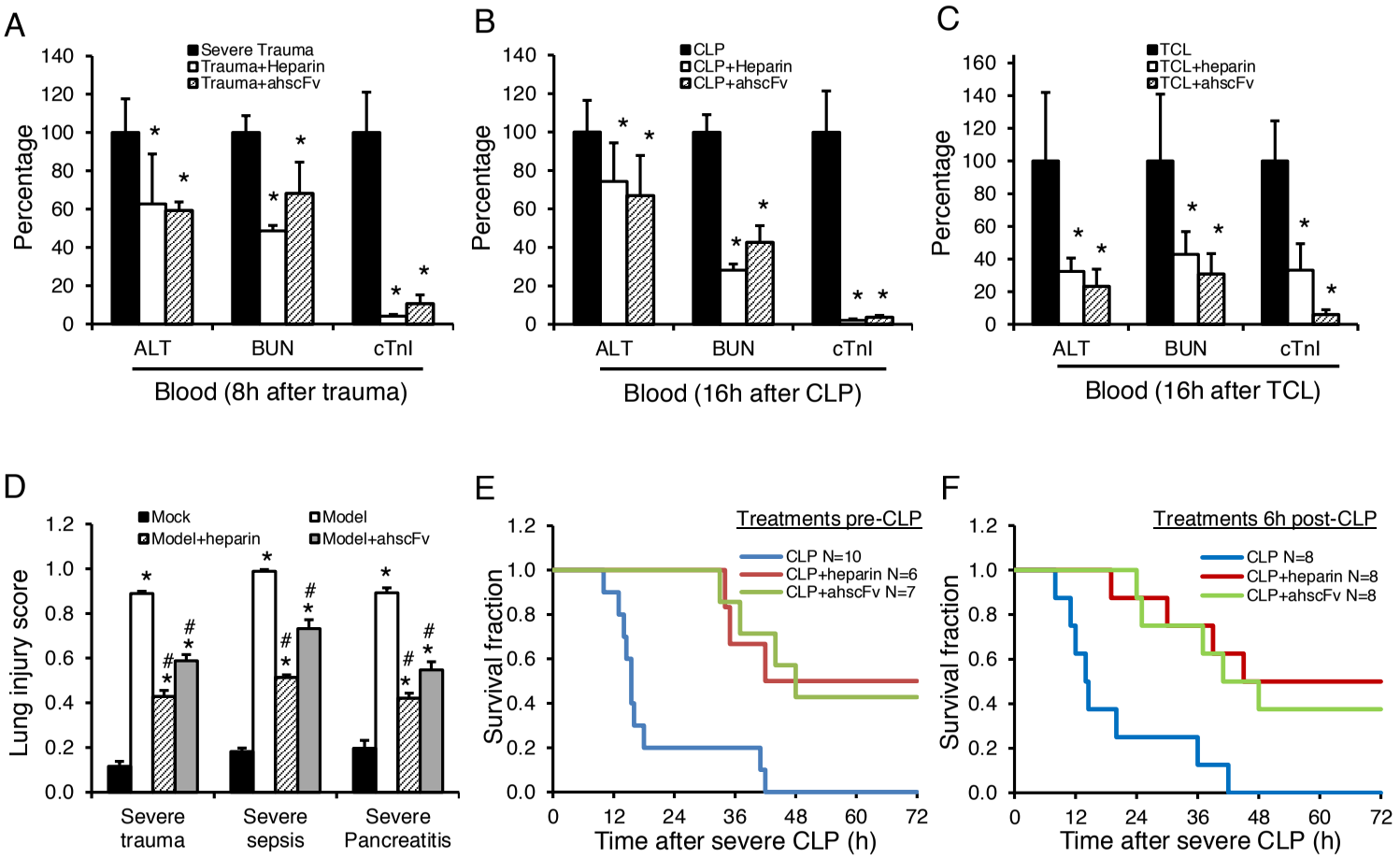

A
B
C
D
ALT (IU/L)
BUN(mg/dL)
cTnI (ng/ml)
Lung Injury Score
Circulating histones (μg/ml)
R = 0.588
R = 0.539
R = 0.605
R =0.726
0
100
200
300
400
500
20
40
60
80
120
5
10
15
20
25
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Figure 5


A
Severe Trauma
Trauma+Heparin
Trauma+ahscFv
Percentage
ALT
BUN
cTnI
Blood (8h after trauma)
B
CLP
CLP+Heparin
CLP+ahscFv
Blood (16h after CLP)
C
TCL
TCL+heparin
TCL+ahscFv
Blood (16h after TCL)
D
Mock
Model
Model+heparin
Model+ahscFv
Lung injury score
Severe trauma
Severe sepsis
Severe Pancreatitis
E
Treatments pre-CLP
CLP N=10
CLP+heparin N=6
CLP+ahscFv N=7
Survival fraction
Time after severe CLP (h)
F
Treatments 6h post-CLP
CLP N=8
CLP+heparin N=8
CLP+ahscFv N=8

Supplemental Table 1

Supplemental Table 2

Supplemental Figure

Supplemental Data File (.doc, .tif, pdf, etc.)