**Circulating histones are major mediators of cardiac injury in patients with sepsis**

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**ABSTRACT**

**Objective:** To investigate theimpact of circulating histones on cardiac injury and dysfunction in a murine model and patients with sepsis.

**Design:** Prospective, observational clinical study with *in vivo* and *ex vivo* translational laboratory investigations.

**Setting:** General intensive care unit and University research laboratory.

**Patients, subjects and interventions:** Serial blood samples from 65 septic patients were analyzed and left ventricular (LV) function was assessed by echocardiography. Patients’ sera were incubated with cultured cardiomyocytes in the presence or absence of anti-histone antibody and cellular viability was assessed. Murine sepsis was initiated by intra-peritoneal *E.coli* injection (108 CFU/mouse) in 12-week old male C57BL/6N mice and the effect of anti-histone antibody (10 mg/kg) was studied. Murine blood samples were collected serially and LV function was assessed by intra-ventricular catheters and electrocardiography (ECG).

**Measurements and Main Results:** Circulating histones and cardiac troponins in human and murine plasma were quantified. In 65 septic patients, circulating histones were significantly elevated compared to healthy controls (n=27) and linearly correlated with cardiac troponin T levels (*rs*=0.650,*p*<0.001), noradrenaline doses required to achieve haemodynamic stability (*r*s=0.608,*p*<0.001), SOFA scores (*p*=0.028) and mortality (*p*=0.008). In a subset of 36 septic patients without prior cardiac disease, high histone levels were significantly associated with new-onset LV dysfunction (*p*=0.001) and arrhythmias (*p*=0.01). LV dysfunction only predicted adverse outcomes when combined with elevated histones or cardiac troponin levels. Furthermore, patients’ sera directly induced histone-specific cardiomyocyte death *ex vivo*, which was abrogated by anti-histone antibodies. *In vivo* studies on septic mice confirmed the cause-effect relationship between circulating histones and the development of cardiac injury, arrhythmias and LV dysfunction.

**Conclusion:** Circulating histones are novel and important mediators of septic cardiomyopathy, which can potentially be utilized for prognostic and therapeutic purposes.

**INTRODUCTION**

Sepsis is the host response to infection and a major cause of mortality worldwide. Most deaths in sepsis are attributed to the development of multiple organ failure (MOF) (1) and cardiac involvement is very common. This includes cardiomyocyte injury, arrhythmia and echocardiographic abnormalities (2-4). In terms of cardiomyocyte injury, cardiac troponin T (cTnT) or I (cTnI) are routinely used as specific biomarkers in clinical practice. As a complex of three regulatory proteins, i.e. C, I and T that are present only in cardiomyocytes (5), elevated levels of cardiac troponins (cTn) can be detected in ~60% of septic patients without coronary artery disease (6). Indeed, very high levels of more than 10000 pg/ml can be reached in sepsis as opposed to a threshold of 40 pg/ml cTnI (14 pg/ml for cTnT) for diagnosing acute coronary syndrome (5, 7, 8). More importantly, it is widely recognised that elevated cTn levels are strongly associated with cardiac dysfunction and poor prognosis although their use as an independent predictor of mortality remains arguable (6, 9-14).

New-onset cardiac arrhythmias occur frequently in sepsis (15). In particular, new-onset atrial fibrillation (AF) may increase mortality by up to 3 fold (16). There is, however, less consensus on the clinical significance of cardiac dysfunction in sepsis, at least if defined as impaired systolic performance. Parker *et al* observed that low left ventricular (LV) ejection fraction and LV dilatation were associated with survival in a group of 20 septic patients (18). However, further studies did not support this “protective compensatory” mechanism (19-22) but showed that cardiac output would increase in early sepsis in compensatory response to reduced systemic vascular resistance (SVR). When SVR changes were accounted for, septic cardiomyopathy (observed in 20-40% of septic patients) provided prognostic value (23) with its presence increasing the mortality rate to 70-90% (24, 25). The well-recognized association between cardiac dysfunction and elevated cTn in sepsis (10, 11, 13, 14) further suggests that sepsis-induced myocardial injury and dysfunction, adversely affect prognosis.

The underlying mechanisms of the septic cardiomyopathy are multi-factorial, but remain incompletely understood (26). Impaired coronary perfusion with endothelial and coagulation activation, as well as the effect of endotoxins and cytokines have been proposed as possible mechanisms, but are not universally accepted (27-30). More recently, toxic effects on endothelial and haematopoietic cells with increased vascular permeability, pro-coagulant and pro-inflammatory consequences (31-34) have been attributed to extracellular histones in the circulation. Furthermore, a recent study provided evidence that histones can induce direct myocardial dysfunction in septic mice (35). Histones occur normally inside the nucleus as a family of five proteins. During sepsis, histones can be released into the circulation due to extensive inflammation and cellular death as a form of damage-associated molecular patterns (DAMPs) (31, 35, 36). In mouse models of sepsis, mortality and cardiac dysfunction can be ameliorated by anti-histone antibodies (31, 35).

In this study, we examine the hypothesis that circulating histone levels in septic patients correlate with parameters of myocardial injury and LV dysfunction to predict adverse cardiac events and outcome. The investigative approach blends clinical studies on septic patients with pathophysiological *in vivo* studies in septic mice and *ex vivo* translational studies using septic patients’ sera incubated with cultured cardiomyocytes.

**MATERIALS AND METHODS**

**Patients**

Healthy donors and septic patients admitted to the general Intensive Care Unit (ICU) at the Royal Liverpool University Hospital between November 2010 and October 2011 were recruited prospectively according to the protocol approved by the Local Research Ethics (North West Centre of Research Ethics Committee, UK) and the Hospital Governance Committees. Written informed consent was obtained from patients or their next of kin.

The diagnosis of sepsis was made according to the definition of the American College of Chest Physicians/Society for Critical Care Medicine (37, 38). Clinical history was examined in septic patients for pre-existing cardiac disease and/or arrhythmia. Those with acute coronary events on admission or in the preceding 6 months were excluded. Cardiac events were defined as arrhythmias (atrial fibrillation/flutter, recurrent ectopic beats, supraventricular/ventricular tachycardia) (39, 40) and/or impaired LV function (reduced LV ejection fraction “EF”, LV hypokinesia, LV dilatation) as judged by echocardiography and detailed clinical evaluation by a senior cardiologist and intensivist, using criteria published before (20). These were considered new-onset if arising in those without pre-existing cardiac disease. Clinical information, including the start date of the septic episode, SOFA score, APACHE II score, drug history and the development of any cardiovascular complications were collected.

**Blood sample collection**

Upon ICU admission, surplus blood samples were collected prospectively every 24h from all patients up to 7 days of ICU stay. Further details are described in Supplemental Digital Content.

**Measurement of circulating histones and cTnT in patients**

Circulating histones were measured as described previously (32). cTnT was quantified by MODULAR ANALYTICS EVO analyser (Roche, Indianapolis, USA) based on electrochemiluminescence immunoassay, with a cut-off value of 14 pg/ml to suggest cardiac injury and a lower detection limit of 3 pg/ml. These were performed blind to the clinical information.

**Mouse septic model and animal experiments**

All animal experiments were performed in accordance with state laws and monitored by local inspectors in compliance with British Home Office laws. Twelve-week old male C57BL/6N mice were obtained from SLAC Experimental Animal Centre (Shanghai, China) and were housed and used for experiments under sterile conditions at the Research Centre of Genetically Modified Mice (Southeast University, Nanjing, China). To generate sepsis, a bacterial peritonitis mouse model was used by injecting mice intraperitoneally (i.p.) with fresh, cultured *E.coli* (41) (K-12, 108 CFU/mouse, n=6 for septic mice, n=8 for septic mice with anti-histone antibody, n=6 for sham mice injected with normal saline). LV function assessments of mice using pressure-volume intra-ventricular catheters and quantification of histones and cTns in murine plasma are described in Supplemental Digital Content.

**HL-1 cardiomyocyte culture and viability assay**

This is described in Supplemental Digital Content.

**Statistical analysis**

For clinical data, Mann-Whitney U test and Fisher Exact test were used to assess statistical differences in median values of different groups and for categorical groups, respectively. Circulating histones and cTnT levels are presented as “median [1st,3rd quartiles]”. Circulating histones-cTn linear correlation was analysed using Spearman rank correlation. Intergroup differences were analysed using ANOVA test. Receiver operating characteristics (ROC) curves were constructed to examine the performance of circulating histone concentrations on mortality, new-onset LV dysfunction and arrhythmias. Statistical significance was defined as *p* value of <0.05.

**RESULTS**

**Circulating histones correlate with cardiac troponin levels in septic patients and mediate cardiomyocyte injury *ex vivo***

Sixty-five septic patients were recruited over 12-months and their characteristics are shown in Table 1. Their levels of circulating histones (Fig.1A) were significantly elevated (63.5 μg/ml [22.2,78.3]) compared to normal controls (n=27) (1.5 μg/ml [0.7,2.1]) (*p*<0.001). Significantly higher concentrations of cTnT were also found in septic patients (37.3 pg/ml [15.5,84.4]) compared to normal controls (5.3 pg/ml [0,9.9]) (*p*<0.001) (Fig. 1B). Distinctively, there was significant linear correlation between circulating histones and cTnT (Fig. 1C, *rs*=0.650, *p*<0.001). Furthermore, we observed that septic patients with high circulating histones (≥75 µg/ml), a level that we previously reported to induce direct cytotoxicity on endothelial cells (32) and which was recently shown to induce structural changes and damage to cardiomyocytes *ex vivo* (35), had much higher cTnT levels (147.8 pg/ml [62.3,204.2]) compared to those with circulating histones <75 µg/ml (28.8 [14.9,64.1]) (Fig. 1D,*p*<0.001).

There was also a significant linear correlation between histone levels and noradrenaline doses required to achieve a stable mean arterial pressure of >65 mmHg (Fig. 1E, *rs*=0.608, *p*<0.001). Likewise, septic patients with histone levels ≥75 µg/ml required ~2.5 fold higher doses of noradrenaline (19.7 µg/kg/h [7.0,24.0]) to achieve haemodynamic stability than those with histones <75 µg/ml (7.0 µg/kg/h [0,12.8]) (*p*=0.006). We have previously shown that high circulating histones in patients’ sera cause direct damage to endothelial cells (32), which could explain the contribution of high circulating histone levels to peripheral vascular dysfunction.

Here, we extend upon those findings to show that high circulating histones in septic patients’ sera can be injurious to cardiomyocytes (Fig. 1F). In particular, we find significant reductions in cardiomyocyte viability when incubated with septic sera containing high circulating histones (≥75 µg/ml) compared to septic sera with low histone levels (<75 µg/ml) (Fig. 1F). Furthermore, the inclusion of anti-histone single chain variable fragment (ahscFv) antibody (32) significantly attenuated these toxic effects (Fig. 1F) which confirms the direct toxicity of high circulating histones on cardiomyocytes.

**Circulating histones and cardiac troponins correlate with sepsis severity and outcome**

Further analysis revealed that septic patients with histone levels ≥75 µg/ml had significantly higher Sequential Organ Failure Assessment (SOFA) scores (11 [7,13.5]) compared to those with histones levels <75 µg/ml (7.5 [5.5,10]) (*p*=0.028) (Fig. 2A). This suggests that high circulating histones correlate with disease severity. Indeed, histones were significantly higher in non-survivors (74 µg/ml [44.2,102]) than in survivors (28.8 μg/ml [18.8,55.9]) (*p*=0.008) (Fig. 2B) as were cTnT levels (75.9 pg/ml [43.6, 165.6] versus 36.1 pg/ml [7.8,53.7] (*p*=0.014) (Fig. 2C). Circulating histones in septic patients were also strong predictors of mortality and the area under curve (AUC) was 0.744 (*p*=0.003) (Fig. 2D) with a histone cut-off value at 75 µg/ml having sensitivity and specificity of 60% and 86.1%, respectively. Furthermore, both circulating histones and cTnT levels correlated with sepsis severity (37, 38) and were significantly higher in septic shock patients than in those with sepsis or severe sepsis (Figs. 2E-2F). These results are in-line with a previous report demonstrating higher levels of nuclear breakdown products (nucleosomes) in septic shock patients (42).

Interestingly, there was no significant association between LV dysfunction and mortality (*p*=0.083) but this became significant when concomitant elevation in circulating histones (*p*=0.036) or cTn (*p*=0.02) were included (Fisher Exact test). Collectively, these data suggest that circulating histone levels provide clinical value in the assessment of cardiac injury and outcome of septic patients.

**Patients with new-onset left ventricular dysfunction have higher levels of circulating histones**

It has been reported that cardiac events arising during the septic episode are highly relevant to outcome (16, 22). Of the 65 septic patients, 16 died with 11 of them (68.8%) developing new-onset cardiac events (LV dysfunction and/or arrhythmia). This is in comparison to only 18.3% (9/49) of survivors (*p*<0.001) developing new-onset cardiac events.

To explore the potential contribution of histones to new-onset cardiac events, analysis of the sub-group of 36 septic patients with no history of cardiac disease and/or arrhythmia was performed to exclude the effects of underlying cardiac disease (Supplemental Digital Content 1, Supplemental Fig. 1). Among these 36 patients, 11 (30.5%) had features of LV dysfunction (Table 2). Histone levels in these 11 patients (113.9 µg/ml [80.9,135.7] were significantly higher than those without LV dysfunction (n=25) (32.6 µg/ml [18.8,61.9]) (*p*=0.001) (Fig. 3A) as were cTnT levels (93.7 pg/ml [54.8,161.2] versus 22.0 pg/ml [12.1,44.4] (*p*=0.001) (Fig. 3B). Indeed, 75% (9/12) of patients with histone levels ≥75 µg/ml developed LV dysfunction compared to 8.3% (2/24) (*p*<0.001) of patients with histones <75 µg/ml (Fig. 3C). Furthermore, the AUC for circulating histones in new-onset LV dysfunction was 0.865 (*p*=0.001) (Fig. 3D). A cut-off value of 75 µg/ml histones had sensitivity and specificity of 81.8% and 92%, respectively.

**High levels of circulating histones are associated with a higher incidence of new-onset arrhythmias**

New-onset arrhythmias developed in 19 (52.8%) of the 36 septic patients with no prior history of cardiac disease (Table 2). Circulating histone levels in these patients were significantly higher (77.8 µg/ml [40.5,114.8]) than in those without arrhythmia (n=17) (32.6 μg/ml [20.1,55.8]) (*p*=0.01) (Fig. 4A). Similarly, cTnT levels were higher in patients with new-onset arrhythmia (45.4 pg/ml [15.6,76.3] compared to those without 15.4 pg/ml [6.4,24.8]) (*p*=0.03) (Fig. 4B). The incidence of new-onset arrhythmia also correlated significantly with histone levels ≥75 µg/ml (Table 2). Strikingly, 83.3% (10/12) patients with histones levels ≥75 µg/ml developed arrhythmia compared to 37.5% (9/24) of patients with histones <75 µg/ml (*p*=0.009) (Fig. 4C). In 5 of the 19 patients (26.3%), the new-onset arrhythmia presented as paroxysmal AF and histone levels in these patients were particularly high (113.4 µg/ml [96.8,117.3]) when compared to patients without AF (35.8 µg/ml [20.3,74.9]) (*p*=0.014) (Fig. 4D). All non-survivors within the new-onset AF group (4/5 patients) had circulating histone levels exceeding 75 µg/ml. Table 2 also illustrates the potential association between high circulating histones and the appearance of ventricular tachycardia (VT) (*p*=0.02) and frequent new-onset atrial/ ventricular ectopic beats (*p*=0.042). Figure 4E shows circulating histones to have an AUC of 0.813 (*p*=0.001) for predicting new-onset arrhythmias and 75 µg/ml histones cut-off providing sensitivity and specificity of 72.7% and 94.1%, respectively.

Taken together, these clinical observations suggest that high levels of circulating histones strongly correlate with and may predict the development of new-onset cardiac events during sepsis.

**Parallel and concomitant elevation of circulating histones and cardiac troponins in septic mice**

The potential clinical link between histones and cardiac abnormalities led us to examine if this could be reproduced and specifically targeted in *in vivo* sepsis models. For this purpose, we have used an established bacterial peritonitis-sepsis mouse model (41) with i.p. injection of *E.coli*. Figures 5A-5B show that high circulating histone levels could be determined from 10h after initiation of sepsis and peaked between 16 and 24h at values of ~500 µg/ml. Importantly, in these septic mice, a parallel and concomitant increment in both circulating histones and cardiac troponins (I and T) was observed (Fig. 5C), which showed strong linear correlation (*rs*=0.877,*p*<0.001) (Fig. 5D). These data in septic mice further support our clinical findings of a linear correlation between circulating histones and cTn in septic patients. Arrhythmias in septic mice often occurred after 12h, and most frequently presented as ectopic beats and Io or IIo atrio-ventricular (AV) block (Supplemental Digital Content 2, Supplemental Fig. 2).

**Anti-histone intervention attenuates cardiac injury and dysfunction in septic mice**

To establish the cause-effect relationship between circulating histones and cardiac injury and dysfunction, septic mice were treated with anti-histone antibodies (10 mg/kg intravenously 8 and 16h after sepsis induction) to examine for potential protective effects. The direct infusion of anti-histone antibody significantly reduced cTnI levels by ~70% (from ~500 pg/ml in septic mice to 120.8±107.3 pg/ml) (Fig. 6A,*p*=0.002). This finding strongly supports a direct effect of histones on the development of cardiac injury. LV contractility, measured using catheters at 20h after sepsis induction, showed significant reduction of LV dp/dtmax (maximum rate of LV pressure rise) (43) (Fig. 6B) and LV dp/dtmin (maximum rate of LV pressure drop) (Fig. 6C) with significant increases in Tau (a preload-independent parameter of LV isovolumic relaxation (44, 45)) (Fig. 6D). These changes were significantly alleviated by ahscFv (Fig. 6B-6D) to indicate that high histone levels are indeed involved in LV dysfunction that is independent of pre or after-load changes during sepsis.

**DISCUSSION**

This clinically-centred study describes for the first time the important role played by circulating histones in cardiac injury, arrhythmias and LV dysfunction in septic patients. Our findings highlight the clinical predictive and prognostic significance of circulating histones. A key discovery is that concentrations of circulating histones strongly correlate with elevated levels of cTnT in septic patients. Elevation of circulating cTn in septic patients is clinically important as it indicates cardiomyocyte injury and arguably has prognostic implications (10, 11, 13, 14). However the causes of cTn release are poorly understood and indeed, remain controversial (4). One theory is based on global myocardial ischemia but studies using thermodilution catheters placed in the coronary sinus of septic shock patients do not support this hypothesis (27). Although the varying dynamics and magnitude of cardiac dysfunction in clinical sepsis is unlikely to be explained by one mechanism, we have identified a time-dependent association between histones and cTn levels in septic patients which may likely contribute to septic cardiac events.

This observation was further consolidated by our demonstration of a time-dependent increment in circulating histones and cardiac troponins (I and T) in murine sepsis which translated into strong linear correlation between circulating histones and cTn levels. The ability of histones to *directly* injure cardiomyocytes is confirmed by the ability of anti-histone antibodies to significantly reduce cTn release in septic mice and also prevent cardiomyocyte death *ex vivo* when incubated with septic patients’ sera containing high level of circulating histones. Previous studies have demonstrated that histone-mediated toxicity is mainly attributed to cell membrane binding, calcium influx and overload (32, 34), which is a well-known trigger for cardiomyocyte injury and dysfunction (46, 47). However, the detailed mechanism requires further investigation.

Furthermore, the significant association between high circulating histones with SOFA scores, sepsis severity and mortality indicates that organ dysfunction, associated with high histone levels, contributes to adverse outcomes. As such, our data further confirm previous reports highlighting the association between circulating histone levels (and other nuclear breakdown products) and sepsis severity (36, 42). As to the source of circulating histones, Kalbitz *et al* (35) has suggested that this is most likely released from neutrophil extracellular traps (NETs). However, the relative and/or temporal contribution from other cells and organs is not known, which requires further investigation.

This study also confirms and extends on previous studies showing that new-onset cardiac events significantly correlate with poor prognosis and high mortality (16, 22, 23, 40, 48, 49). Specifically, this work provides novel data suggesting that the release of histones may represent a new pathological mechanism for cardiac injury, dysfunction and arrhythmias associated with sepsis. Cardiac events were more frequently observed in septic patients with circulating histones ≥75 µg/ml (19 of 65 patients, ~30%), a cut-off value that also provided robust sensitivity and specificity to predict the development of new-onset cardiac events and mortality. Therefore, accurate clinical quantification of circulating histones would be required for translational impact. Our study is limited by a relatively small sample size of 65 septic patients, of which 36 patients had no previous cardiac disease. Although we back up our clinical observations with *in vivo* studies in septic mice, our results may require further validation with bigger cohort of septic patients and with extended echocardiographic studies and measurements of new markers of cardiac damage to fully assess cardiac injury and dysfunction.

Myocardial dysfunction in sepsis can be caused by direct cardiomyocyte injury or indirect effects from systemic haemodynamic changes, which are common during sepsis. The degree to which each contributes to myocardial dysfunction can vary according to the time or stage of sepsis and is a topic of ongoing debate. However, it is plausible that in the presence of significant cardiac injury, when cTn and circulating histone levels are high, cardiac dysfunction would be directly associated with a poor outcome (11, 13, 14). In our group of patients, the non-significant association between LV dysfunction and mortality became significant only when concomitant elevation in cTn or circulating histones were considered. This would suggest that the accuracy of an echocardiographic diagnosis of myocardial dysfunction can be further improved by determination of cTn concentrations, as has been suggested by others (10, 11, 14). Measurement of circulating histone levels may further add to a reliable evaluation of direct cardiac injury and outcome prediction in patients with sepsis. Future translational and clinical studies will have to be tailored to further investigate this.

Further evidence of the direct role of histones in causing myocardial dysfunction is provided by our LV catheter findings in septic mice, particularly from ±dP/dt and Tau, which is the volume-independent parameter of LV contractile performance. Their significant alterations strongly indicate the existence of primary LV dysfunction in sepsis, which was abrogated using anti-histone antibodies. Indeed, these volume-independent changes are consistent with a recent report illustrating the role of circulating histones in inducing LV dysfunction using a cecal ligation and puncture (CLP) septic mouse model (35). However, the circulating histone levels described in that CLP model were relatively low (<50 µg/ml) and many *ex vivo* experiments were performed at much higher concentrations (100-2000 µg/ml) (35). Therefore, the overall translational relevance of these studies was not clear. The trauma caused by surgical procedures in the CLP model may induce histone release, and we have therefore used a septic mouse model based on intraperionteal injection of *E.coli* (41). Nevertheless, the results from both septic models strongly implicate histones in the pathogenesis of septic cardiomyopathy.

Although we have demonstrated LV dysfunction caused by histones, their effects on right ventricular (RV) function remain to be investigated.

A further finding of our study is the strong association between the development of new-onset arrhythmias and mortality in sepsis (*p*=0.003) which is in agreement with the existing literature (15, 16, 49). We further extend on these findings by showing that high circulating histones in septic patients strongly correlate (and predict) the development of new-onset arrhythmias. Of interest, those patients with the highest circulating histone levels who developed new-onset AF were least likely to survive, which is in keeping with a recent report highlighting the importance of new-onset AF as an indicator of poor prognosis in septic patients (49). The arrhythmias recorded in our mouse model were mainly ectopic beats and AV blocks. These findings are in-line with a recent report documenting the development of ventricular ectopic beats and sinus bradycardia in mouse hearts perfused with histones *ex vivo* (35). The absence of AF in our mouse model may be due to species differences as AF is not typically seen in mice and requires large animal models to be studied (50). Although supraventricular arrhythmias during sepsis have traditionally not been considered a sign of cardiac dysfunction, there is growing evidence and discussion on its association with cardiac impairment, adverse consequences and impact on outcome in septic patients (16, 49). Our study supports this notion and proposes circulating histones as possible predictors and prognosticators.

**CONCLUSION**

In summary, this study shows that in septic patients, cardiac injury and dysfunction correlate with high circulating histone levels, a finding which may have implications in other critical illnesses. Cardiac dysfunction in the presence of elevated cTn or histone levels predicts a worse outcome and measurement of circulating histones may improve the diagnostic and prognostic accuracy of cardiac dysfunction in septic patients. In addition, this study illustrates that reversing cardiac depression using anti-histone reagents as exemplified by anti-histone antibody administration (32, 35) may offer novel therapeutic options to ameliorate the consequences of septic cardiomyopathy and improve the overall prognosis of septic patients.

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**FIGURE LEGENDS**

**Figure 1.** Elevated circulating histones in septic patients correlate with circulating cardiac troponin T and doses of vasospressors administered to achieve haemodynamic stability**. A,** Total circulating histones and **B,** Cardiac troponin T (cTnT) in healthy donors (normal, n=27) and septic patients (Sepsis, n=65) measured during the first 3 days of ICU stay (\**p*<0.001,° represents outliers). **C,** Linear correlation between cardiac troponin T (cTnT) and circulating histones (*rs*=0.650, *p*<0.001) in septic patients (n=65) during the first 3 days of ICU stay. **D,** Cardiac troponin T (cTnT) levels in septic patients with histone levels ≥75 µg/ml (n=19) or <75µg/ml (n=46) (\**p*<0.001,° represents outliers). **E,** Linear correlation between doses of noradrenaline required to achieve haemodynamic stability (stable mean arterial pressure >65 mmHg) and circulating histones in septic patients (*rs*=0.608, *p*<0.001) (n=65). **F,** HL-1 cardiomyocytes were incubated with sera from healthy volunteers and from septic patients with histones < and ≥75 µg/ml (< and ≥75, respectively) with or without anti-histone antibody (75+A) (n=4 each group) and cell viability was assessed after 1 hour by WST-8 cell proliferation kit. Viability of cells treated with normal serum was set as 100%. (\**p*<0.01 compared to normal serum, # *p*<0.01 compared to septic sera containing histones >75 µg/ml).

**Figure 2.** Circulating histones correlate with severity and outcome in septic patients. **A,** SOFA scores in septic patients with histones < (n=46) and ≥75 µg/ml (n=19)(*p*=0.028). **B,** Circulating histones and **C,** Cardiac troponin T (cTnT) in septic patients who survived (survival) and those who died (death) (\**p*=0.008 and \**p*=0.014 respectively,° represents outliers). **D,** Receiver operating characteristic curve for prediction of 28-day mortality in septic patients (area under curve AUC=0.744, 95% confidence interval 0.609-0.880, *p*=0.003). Cut-off value of 75 µg/ml circulating histones is arrowed (sensitivity 60%, specificity 86.1%). **E,** Circulating histone levels in patient with sepsis (30.8 µg/ml [21.1, 60]) (n=20), severe sepsis (50.4 µg/ml [22.1, 58.4]) (n=17) and septic shock (69.3 µg/ml [33.4, 102]) (n=28) (\**p*=0.026 vs sepsis, #*p*=0.03 vs severe sepsis,° represents outliers). **F,** Cardiac troponin T (cTnT) levels in patients with sepsis (23.5 pg/ml [12.4, 45.6]) (n=20), severe sepsis (33 pg/ml [14, 56.5]) (n=17) and septic shock (65 pg/ml [41.2, 173.1]) (n=28) (\**p*=0.003 vs sepsis, *#p*=0.011 vs severe sepsis,° represents outliers).

**Figure 3.** Highcirculating histones and cardiac troponin levels are associated with higher incidence of new-onset left ventricular dysfunction in septic patients. In 36 septic patients with no history of cardiac disease and/or arrhythmia, **A,** Circulating histones and **B,** Cardiac troponin T (cTnT) levels were compared between those without (Absence, n=25) and those developing (Presence, n=11) left ventricular (LV) dysfunction (\**p*<0.01). **C,** Incidence of left ventricular (LV) dysfunction was 75% (9/12) and 8.3% (2/24) in patients with histone levels ≥75 or <75 µg/ml, respectively (*p*<0.001). **D,** Receiver operating characteristic curve for prediction of new-onset left ventricular (LV) dysfunction in septic patients by circulating histone levels (area under curve AUC=0.865, 95% confidence interval 0.703- 1.000, *p*=0.001). Cut-off value of 75 µg/ml circulating histones is arrowed (sensitivity 81.8%, specificity 92%).

**Figure 4.** High circulating histones levels are associated with higher incidence of new-onset arrhythmias in septic patients. **A,** Circulating histones**,** and **B,** Cardiac troponin T (cTnT) levels in septic patients without (Absence, n=17) or with (Presence, n=19) new-onset arrhythmia (\**p*=0.01 and *p*=0.03 respectively,° represents outliers). **C,** Incidence of new-onset arrhythmia was 83.3% (10/12) and 37.5% (9/24) in septic patients with histone levels ≥75 or <75µg/ml, respectively (*p*=0.009). **D,** Circulating histone levels in septic patients who developed new-onset atrial fibrillation (AF) (Presence, n=5) as compared to those who developed other types of new-onset arrhythmias but not AF (Absence, n=14) (\**p*=0.014,° represents outliers). **E,** Receiver operating characteristic curve for prediction of new-onset arrhythmias in septic patients by circulating histone levels (area under curve AUC=0.813, 95% confidence interval 0.673- 0.953, *p*=0.001). Cut-off value of 75 µg/ml circulating histones is arrowed (sensitivity 72.7%, specificity 94.1%).

**Figure 5.** Parallel and concomitant elevation of histones and cardiac troponins in the circulation of septic mice. Three groups of mice (10 per group) were used to investigate levels of circulating histones and cardiac troponins. **A,** Representative Western blotting showing typical levels of histone 3 (H3) at different time points after initiation of sepsis in mice. Recombinant H3 standards were run on the same gel to quantity H3 levels in mice samples. **B,** Band quantification histogram of total histone levels in septic mice at different time points after initiation of sepsis (n=10). **C,** Western blots of dynamic changes in cardiac troponin I and T (cTnI, cTnT), and histones 3 (H3) in blood taken from sham group (injection of same volume of saline i.p.) and sepsis (108 CFU *E.coli* K12 intra-peritoneal) with immunoglobulin-G (IgG) as an endogenous control. **D,** Linear correlation (*rs*=0.877, *p*<0.001) between circulating levels of histones and cardiac troponin I (cTnI) in septic mice at different time points (n=53 events).

**Figure 6.** Anti-histone intervention alleviates left ventricular dysfunction and injury in murine sepsis. **A,** Mean ± SD of cardiac troponin I (cTnI) levels in sham mice (n=6), septic mice (sepsis) (n=6) and septic mice rescued by ahscFv (10 mg/kg) injected intra-venously at 8 and 16 hours after sepsis induction (Sepsis+A) (n=8). (\**p*<0.05 compared with sham, #*p*<0.05 compared with sepsis).  **B-D,** Left ventricle haemodynamic changes as measured by catheter in different groups of mice: sham: intra-peritoneal saline at 0 hour, intra-venous saline at 8 and 16 hours (n=6); Sepsis: intra-peritoneal *E.coli* as above at 0 hour, intra-venous saline at 8 and 16 hours (n=6); Sepsis+A: intra-peritoneal *E.coli* and intra-venous ahscFv (10 mg/kg) as above (n=8). Left ventricular maximum rate of pressure rise (dp/dtmax)(B), maximum rate of left ventricular pressure drop (dp/dtmin) **(**C**)** and pre-load independent left ventricular relaxation time (Tau) **(**D**)** are presented as Mean ± SD. (\**p*<0.05 compared with sham, #*p*<0.05 compared with sepsis). No blood was taken and no fluid resuscitation was carried out in these groups of mice.

**SUPPLEMENTAL DIGITAL CONTENT LEGENDS**

**Supplemental Figure 1.** Schematic presentationof the subgroups of septic patients with or without new-onset cardiac events.

**Supplemental Figure 2.** Typical ECG traces in septic mice recorded between 16 to 22 hours after sepsis induction showing frequent ectopic beats (upper trace) and atrio-ventricular (AV) block (lower trace).