Circulating histones play a central role in COVID-19-associated coagulopathy and mortality

COVID-19 has highlighted the lethal consequences of immunothrombosis; i.e., the cross-talk between coagulation, inflammation and the innate immune system. Patients with immunothrombosis have significant immune cell death,[1] which can release pro-coagulant[2] and cytotoxic[3] histones. Histones are small, positively-charged proteins that are typically found within the cell nucleus and which bind to negatively-charged DNA. We hypothesize that circulating histones play a central role in critically-ill COVID-19 patients. This translational study demonstrates that admission histone levels are significantly elevated with increasing severity of COVID-19 infection (Mild, median=2.6 μg/mL [IQR=0.7-7.6], Moderate, 10.5 μg/mL [3.5-27.2], Critical, 20.0 μg/mL [6.2-33.0], Non-survivors, 29.6 μg/mL [11.2-60.0]; P<0.001). Circulating histones associated with severe coagulopathy, inflammation and organ injury markers, including cardiac troponin. Extracellular histone levels on admission are associated with poor outcomes and independently predict 28-day mortality of hospitalized COVID-19 patients. This is the first report to indicate that circulating histones, released following immune cell death, may play a central pathological role in severe SARS-CoV-2 infection.

COVID-19 was the cause of more than two million deaths worldwide by February 2021,[4] resulting from respiratory and multi-organ failure,[5] with evidence of pulmonary thrombosis at post-mortem.[6] These patients have extensive immune cell death,[1] a strong acute-phase inflammatory response and coagulopathy, as well as cardiac injury.[1,5] Cell death can release histones, and extracellular histones are cytotoxic, pro-inflammatory[7] and pro-coagulant,[2] leading to pulmonary thrombosis.[8] Extracellular histones also trigger interleukin-6 (IL-6) release to induce an acute phase response, including elevation of C-reactive protein (CRP), which, in turn, reduces histone toxicity.[9] High levels of circulating histones initiate an alternative coagulation pathway during sepsis,[2] mediate multiple organ injury[3] and correlate with adverse clinical outcomes, including death.[10] We therefore hypothesized that high levels of histones are present in severe SARS-CoV-2 infection, and act as major mediators of coagulopathy and mortality in COVID-19 disease.

In this study, adult COVID-19 patients (n=113) were recruited at the Royal Liverpool University Hospital from 30th March 2020 to 16th May 2020. Patients were selected using the ISARIC WHO Clinical Characterisation Protocol for Severe Emerging Infections in the UK. Inclusion criteria were: (1) swab positive or high likelihood of infection or (2) ≥1 of the following symptoms: fever ≥38°C, new cough, dyspnea or tachypnea and admitted to a healthcare facility.[11] Patients were categorized into four groups: 1) Mild (minor respiratory symptoms to exclude shortness of breath OR incidental finding, where the patient required admission to hospital for reasons other than COVID-19 (such as for frailty) and was otherwise asymptomatic of COVID-19); 2) Moderate (dyspnea, i.e., patient symptomatic with shortness of breath OR hypoxia, defined by oxygen saturations on pulse oximeter of ≤93% or requiring supplementary oxygen to maintain oxygen saturations ≥96%); 3) Critical disease (respiratory failure requiring the administration of continuous positive airway pressure (CPAP) to maintain oxygen saturations ≥96% OR invasive ventilation in a critical care setting); 4) Non-survivors (patients who died within 28 days of hospital admission).

Circulating histones were quantified in patient plasma on admission (as described previously)[8,12] and associations with severity of infection, coagulation, inflammatory and organ injury markers were analyzed. Severity of infection was determined by the patient's most severe clinical state throughout hospital admission, according to the previously described definitions. Cytokines were measured using a Luminex-based bead array, as per manufacturer's instructions (Thermo-Fisher Scientific). Outcome measures included ventilator-support days, length of hospital stay, and 28-day mortality. Ethical approval was provided by the South Central - Oxford C Research Ethics Committee in England (Ref 13/SC/0149), the Scotland A Research Ethics Committee (Ref 20/SS/0028), and the WHO Ethics Review Committee (RPC571 and RPC572, 25 April 2013). Local approval was granted by the North West - Haydock Research Ethics Committee (REC reference 20/NW/0332).

The Kruskall-Wallis test was used to compare continuous variables, presented as median (interquartile range; IQR); the Fisher Exact/χ^2 test for comparison of categorical variables, presented as counts (percentage). Circulating histone levels were measured by Western Blot, using purified histone as the standard, and analyzed either as continuous variables or categorized based on a previously-determined threshold for cytotoxicity (30 μg/mL).[3,7] The Mann-Whitney U test was used to compare categorical histone levels to continuous clinical variables. Correlation analysis was performed using Spearman's rank. A Receiver Operating Characteristic (ROC) curve analysis and multivariate regression (adjusted for age, gender, ethnicity and co-morbidities) assessed admission histone levels in predicting 28-day mortality. Kaplan-Meier survival curve analysis was performed to analyze the probability of mortality over time. Statistical tests were performed on SPSS (IBM, version 25). A 2-tailed P value of <0.05 was considered significant.

The study involved 113 COVID-19 patients (Table 1): median age 65.0 years (IQR=51.0-78.0 years), 65 patients were male (57.5%), 96 of white ethnicity (85.0%). Disease severity was associated with coagulation activation (Table 1), characterized by elevated D-dimer (P=0.017) and prolonged prothrombin time (P=0.005), and a pro-inflammatory phenotype characterized by elevated CRP (P<0.001) and IL-6 (P=0.002) on hospital admission, as well as with hypoxia and cardiac injury (Table 1). The median hospital stay was 10 days (IQR, 3-20 days) and 25 patients (22.1%) died within 28 days.

Circulating histone levels on admission were significantly elevated in COVID-19 patients compared to normal controls and were associated with increasing severity of infection (Figure 1A and B; Healthy controls, median=2.9 μg/mL [IQR=1.5-3.3]; Mild, 2.6 μg/mL [0.7-7.6]; Moderate, 10.5 μg/mL [3.5-27.2]; Critical, 20.0 μg/mL [6.2-33.0]; Non-survivors, 29.6 μg/mL [11.2-60.0]; P<0.001). Circulating histone levels strongly correlated with D-dimer levels (R=0.606), indicating the potential involvement of extracellular histones in COVID-19 coagulopathy. Positive association with organ injury markers, including bilirubin (R=0.531), creatinine (R=0.501) and cardiac troponin (R=0.486), indicates the possible role of histone-induced cytotoxicity in multiple organ injury. Strong associations with fibrinogen (R=0.632), CRP (R=0.735) and IL-6 (R=0.677) confirmed histone-initiated acute phase response.[9] Negative correlation with lymphocyte count (R=-0.446) suggests that lymphocyte and other immune cell death might be a major source of circulating histones in COVID-19 infection.

Adopting a 30 μg/mL cytotoxic histone threshold,[3,7] patients over the threshold (n=29) had significantly higher D-dimer (2267.0 ng/mL [1227.0-5235.0] *vs.* 1128.0 ng/ml [589.0-1844.3], *P*=.001), fibrinogen (6.6 g/L [4.6-7.6] *vs.* 4.8 g/L [3.9-5.7], *P*=0.012), IL-6 (226.2 pg/mL [90.6-518.9] *vs.* 71.8 pg/mL [35.2-111.4], *P*<0.001) and CRP levels (186 mg/L [108.5-247.5] *vs.* 48.0 mg/L [10.0-107.5], *P*<0.001) than those patients below the threshold (Table 2). These patients also had significantly reduced SpO2 compared to those with circulating histones <30 μg/mL (oxygen saturations 92.0% [85.8-94.0] *vs.* 95.0% [93.5-97.0], *P*=0.001), required critical care admission (*P*<0.001), with a longer duration of mechanical ventilation (R=0.635) and longer hospital stay (R=0.654).

Circulating histone levels were significantly higher in non-survivors than those who survived (29.6 μg/mL

Table 1. Demographics, peripheral blood measurements and outcomes for disease severity groups in COVID-19 infection.

	Total	Mild	Moderate	Critical	Non-survivors	*P* value[a]
Total number	113	30	38	20	25	–
Demographics & Comorbidities						
Age (years), Median [IQR]	65.0 [51.0, 78.0]	63.5 [42.0, 70.0]	67.0 [57.5, 81.5]	51.0 [42.8, 54.5]*,¥	76.0 [66.0, 86.0]*,†	**<0.001**
Male, No. [%]	65 [57.5]	15 [50.0]	20 [52.6]	14 [70.0]	16 [64.0]	0.428
White ethnicity, No. [%]	96 [85.0]	26 [86.7]	35 [92.1]	11 [55.0]	24 [96.0]	**0.001**
Smoking history, No. [%]	38 [33.6]	10 [33.3]	16 [42.1]	4 [20.0]	8 [32.0]	**0.033**
Hypertension, No. [%]	36 [31.9]	8 [26.7]	12 [31.6]	5 [25.0]	11 [44.0]	0.474
Asthma/COPD, No. [%]	29 [25.7]	14 [46.7]	10 [26.3]	1 [5.0]	4 [16.0]	**0.005**
Diabetes mellitus, No. [%]	29 [25.7]	5 [16.7]	10 [26.3]	5 [25.0]	9 [36.0]	0.443
Ischemic heart disease, No.[%]	16 [14.2]	3 [10.0]	8 [21.1]	0 [0.0]	5 [20.0]	0.116
Chronic kidney disease, No. [%]	15 [13.3]	3 [10.0]	10 [26.3]	0 [0.0]	2 [8.0]	**0.025**
Histones (μg/mL), Median [IQR]	10.8 [3.2, 29.9]	2.6 [0.7, 7.6]	10.5 [3.5, 27.2]*	20.0 [6.2, 33.0]*	29.6 [11.2, 60.0]*,¥	**<0.001**
Peripheral blood cell counts						
White blood cells (x10^9/L), Median [IQR]	8.5 [5.8, 11.8]	8.2 [6.6, 10.7]	9.8 [5.9, 12.3]	8.1 [6.5, 10.8]	8.1 [5.2, 11.3]	0.623
Neutrophils (x10^9/L), Median [IQR]	6.4 [4.0, 9.3]	5.9 [3.8, 8.0]	7.0 [4.1, 9.8]	6.4 [4.0, 9.0]	7.2 [4.0, 11.2]	0.748
Lymphocytes (x10^9/L), Median [IQR]	1.0 [0.7, 1.6]	1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	1.1 [0.9, 2.1]	0.7 [0.4, 1.1]*,¥,†	**0.009**
Haemoglobin (g/L),	129.0	126.0	123.0	134.5	136.0	0.122
Median [IQR]	[117.8, 145.3]	[119.0, 145.0]	[113.8, 139.8]	[131.0, 146.0]¥	[107.0, 147.0]	
Platelets (x10^9/L),	236.5	253.0	243.5	250.5	174.0	**0.026**
Median [IQR]	[170.3, 296.0]	[177.0, 311.0]	[113.8, 139.8]	[207.3, 299.3]	[124.0, 250.0]*,¥,†	
Coagulation parameters						
PT (seconds), Median [IQR]	13.2 [12.1, 14.4]	12.1 [11.2, 13.0]	13.1 [12.1, 14.4]*	13.4 [13.1, 14.2]*	14.1 [12.4, 20.7] *	**0.005**
aPTT (seconds), Median [IQR]	30.6 [28.2, 33.6]	31.0 [28.9, 32.7]	30.5 [28.3, 32.6]	32.0 [29.1, 33.7]	30.0 [28.2, 37.6]	0.775
Fibrinogen (g/L), Median [IQR]	4.8 [3.9, 6.5]	4.2 [2.8, 5.4]†	4.8 [4.4, 6.7]	6.5 [5.4, 6.6]*	4.5 [3.1, 4.9]†	**0.010**
D-dimer (ng/mL),	1227.0	755.5	1315.0	*950.0	1630.0	**0.017**
Median [IQR]	[687.0, 2141.5]	[431.5, 1744.0]	[832.5, 2176.3]	[602.0, 1728.0]	[1117.0, 4334.0]*,†	
Antithrombin (%),	80.0	81.0	80.0	98.0	70.0	**0.024**
Median [IQR]	[61.0, 100.0]	[57.5, 98.5]	[61.5, 97.5]	[80.3, 114.8]*,¥	[59.0, 87.0]†	
Pro-inflammatory markers						
IL-6 (pg/ml),	79.0	53.2	70.5	166.7	107.7	**0.002**
Median [IQR]	[40.5, 131.9]	[15.0, 83.1]	[41.9, 115.0]	[75.6, 214.7]*	[81.3, 269.8]*,¥	
C-reactive protein (mg/L),	61.0	16.0	52.0	145.0	105.0	**<0.001**
Median [IQR]	[21.0, 153.5]	[3.5, 53.8]	[23.3, 146.3]*	[97.0, 202.5]*,¥	[71.0, 192.0]*,¥	
Organ injury markers						
Troponin T (ng/L), Median [IQR]	12.0 [5.0, 35.0]	8.0 [5.0, 16.0]	16.0 [6.8, 47.3]*	6.5 [5.0, 10.5]¥	35.0 [17.0, 58.0]*,†	**<0.001**
Bilirubin (μmol/L), Median [IQR]	9.0 [6.0, 14.0]	8.0 [4.5, 13.0]	8.0 [6.0, 15.0]	9.0 [6.0, 12.5]	12.0 [8.0, 16.5]*	0.142
ALT (U/L), Median [IQR]	25.5 [14.5, 45.0]	21.0 [11.5, 55.0]	19.0 [11.5, 38.0]	33.5 [29.0, 59.5]¥	28.5 [15.8, 44.3]	0.163
Creatinine (μmol/L), Median [IQR]	77.0 [63.0, 105.0]	74.5 [62.0, 82.3]	78.0 [60.8, 104.3]	80.0 [57.8, 96.0]	102.0 [71.0, 180.0]*	0.125
SpO2 (%), Median [IQR]	95.0 [92.0, 97.0]	97.0 [95.0, 98.0]	94.5 [92, 96]*	94.0 [92.0, 96.5]*	92.0 [78.5, 96.0]*	**<0.001**
Outcomes						
Length of stay (days), Median [IQR]	10.0 [3.0, 20.0]	2.0 [1.0, 13.8]	10.0 [6.0, 22.0]*	17.0 [9.5, 43.8]*	–	**<0.001**
Ventilator support (days), Median [IQR]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	2.0 [0.0, 9.3]	0.0 [0.0, 8.0]	**<0.001**

[a]*P* value for comparisons mild *vs.* moderate *vs.* critical disease *vs.* non-survivors, collectively. Performed using Kruskall-Wallis test. *Significant *vs.* mild disease. ¥Significant vs moderate disease. †Significant *vs.* critical disease.

[11.2-60.0] *vs.* 8.6 µg/ml [3.1-24.8], *P*=0.002), and, accordingly, patients with histones >30 µg/mL were more likely to die (13/29 [44.8%] *vs.* 12/84 [14.3%], *P*=0.001). Patients who died were significantly older than those who survived (Table 2, 76 years [66-86] *vs.* 59 years [46-72] *P*<0.001). Compared to survivors, non-survivors had evidence of consumptive coagulopathy with lower platelet counts (*P*=0.003), prolonged prothrombin time (*P*=0.028), elevated D-dimer (*P*=0.017) and reduced antithrombin levels (*P*=0.048). Furthermore, in non-survivors, lymphocyte counts (*P*=0.001), and oxygen saturations (*P*=0.005) were significantly reduced, and IL-6 (*P*=0.021), CRP (*P*=0.013), troponin (*P*<0.001), bilirubin (*P*=0.041) and creatinine (*P*=0.024) were elevated when compared to survivors (Table 2).

Univariate analysis using continuous circulating his-

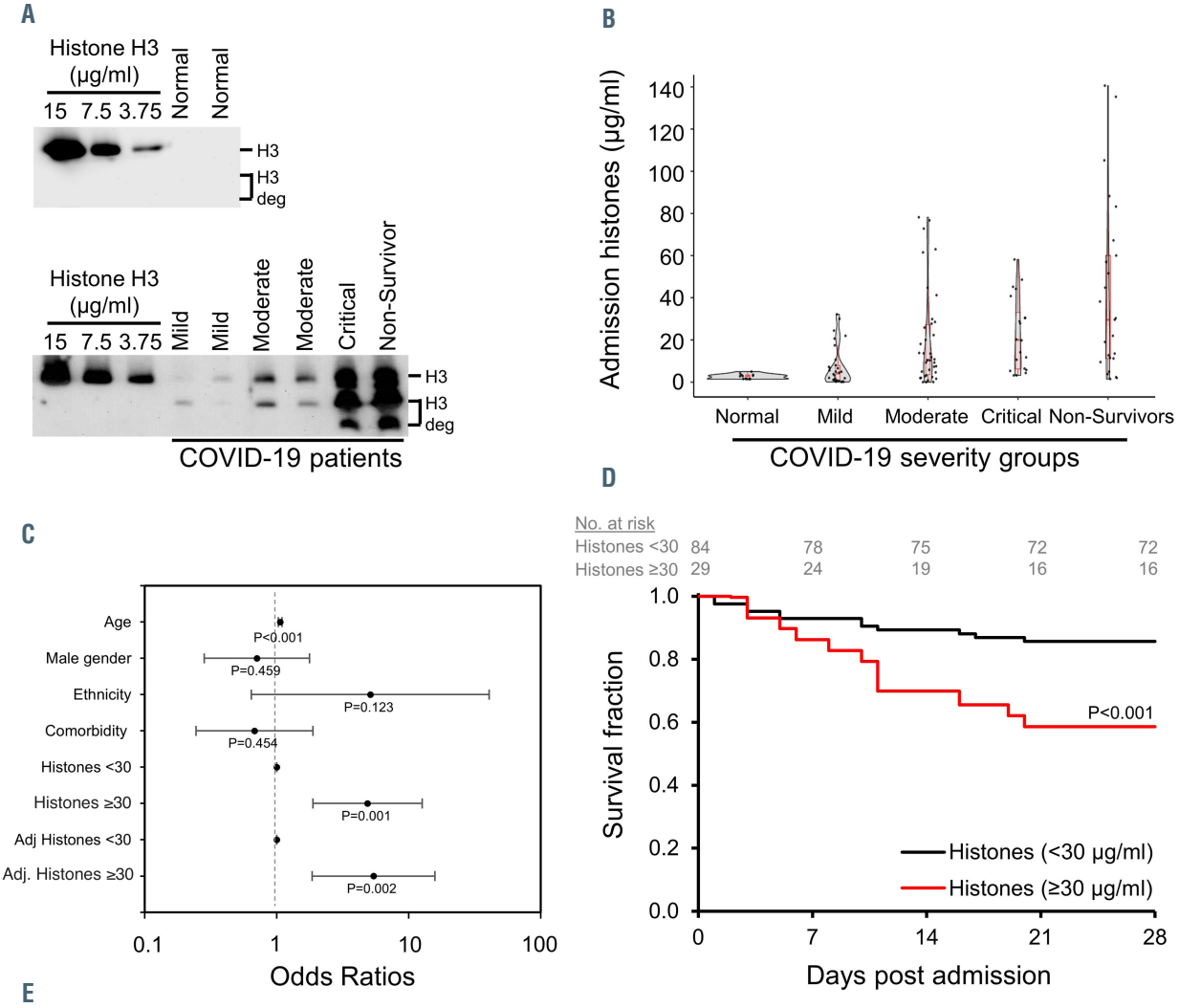

Figure 1. High levels of circulating histones on hospital admission are associated with disease severity and mortality in COVID-19. Typical Western Blots (A) and quantification (B) of histone levels in healthy controls (n=12), mild (n=30), moderate (n=38), critical disease (n=20) and non-survivors (n=25) with COVID-19 infection. Circulating histone levels were higher with increasing disease severity (*P*<0.001). Histone levels were higher in non-survivors compared to the moderate (*P*=0.023), mild groups (*P*<0.001) and to normal healthy controls (*P*<0.001). Histone levels were higher in the critical group compared to mild groups (*P*<0.001) and normal healthy controls (*P*<0.001). Histone levels were higher in the moderate group compared to the mild group (*P*=0.007) and normal healthy controls (*P*=0.002). (C) Multivariate analysis of crude and adjusted odds ratios (with patients adjusted for age, gender, Black and Ethnic Minorities (BAME) and comorbidities including smoking, hypertension, asthma/COPD, diabetes, ischemic heart disease and chronic kidney disease). Circulating histone levels ≥30 µg/mL were independently associated with 28-day mortality. (D) Kaplan-Meier survival curve for the probability of mortality during the 28-day period. Patients were stratified based on circulating histones levels on admission (<30 µg/mL *vs.* ≥ 30 µg/mL). (E) Diagram to propose that circulating histones play a central pathological role in the development of severe COVID-19.

Table 2. Demographics, peripheral blood measurements and outcomes of COVID-19 patients.

	Survivors	Non-survivors	*P* value[a]	Histones <30 μg/mL	Histones ≥30 μg/mL	*P* value[b]
Total number (n)	88	25	–	84	29	–
Demographics & Comorbidities						
Age (years), n.	59.0	76.0	**<0.001**	63.0	66.0	0.224
Median [IQR]	[45.8, 72.3]	[66.0, 86.0]		[47.8, 76.0]	[57.0, 80.0]	
Male, No. [%]	49 [55.7]	16 [64.0]	0.458	48 [57.1]	17 [58.6]	0.890
White ethnicity, No. [%]	72 [81.8]	24 [96.0]	0.113	73 [86.9]	23 [79.3]	0.324
Smoking history, No. [%]	30 [34.1]	8 [32.0]	0.845	28 [33.3]	10 [34.5]	0.910
Hypertension, No. [%]	25 [28.4]	11 [44.0]	0.140	28 [33.3]	8 [27.6]	0.567
Asthma/COPD, No. [%]	25 [28.4]	4 [16.0]	0.301	25 [29.8]	4 [13.8]	0.138
Diabetes mellitus, No. [%]	20 [22.7]	9 [36.0]	0.180	21 [25.0]	8 [27.6]	0.783
Ischemic heart disease, No. [%]	11 [12.5]	5 [20.0]	0.343	13 [15.5]	3 [10.3]	0.758
Chronic kidney disease, No. [%]	13 [14.8]	2 [8.0]	0.515	11 [13.1]	4 [13.8]	>0.999
Histones (μg/mL),	8.6	29.6	**0.002**	6.1	51.6	**<0.001**
Median [IQR]	[3.1, 24.8]	[11.2, 60.0]		[2.0, 13.5]	[38.2, 72.8]	
Peripheral blood cell counts						
White blood cells (x10^9/L),	8.7	8.1	0.387	8.0	9.8	0.084
Median [IQR]	[6.1, 11.8]	[5.2, 11.3]		[5.7, 11.0]	[6.7, 13.3]	
Neutrophils (x10^9/L),	6.2	7.2	0.563	5.7	9.1	**0.001**
Median [IQR]	[4.0, 8.9]	[4.0, 11.2]		[3.6, 8.2]	[6.1, 12.2]	
Lymphocytes (x10^9/L),	1.1	0.7	**0.001**	1.2	0.8	**0.007**
Median [IQR]	[0.8, 1.7]	[0.4, 1.1]		[0.8, 1.7]	[0.5, 1.1]	
Haemoglobin (g/L),	128.0	136.0	0.740	128.0	131.0	0.740
Median [IQR]	[118.0, 144.0]	[107.0, 147.0]		[118.0, 145.0]	[116.0, 147.0]	
Platelets (x10^9/L),	248.0	174.0	**0.003**	237.5	215.0	0.410
Median [IQR]	[181.0, 299.0]	[124.0, 250.0]		[174.3, 295.8]	[155.8, 296.8]	
Coagulation parameters						
PT (seconds), Median [IQR]	13.0 [11.8, 14.1]	14.1 [12.4, 20.7]	**0.028**	12.8 [11.8, 14.0]	13.8 [13.3, 15.6]	**0.005**
aPTT (seconds), Median [IQR]	30.9 [28.4, 32.9]	30.0 [28.2, 37.6]	0.858	30.7 [28.7, 34.0]	29.5 [28.0, 32.6]	0.268
Fibrinogen (g/L), Median [IQR]	5.3 [4.1, 6.5]	4.5 [3.1, 4.9]	0.091	4.7 [3.9, 5.7]	6.6 [4.6, 7.6]	**0.012**
D-dimer (ng/mL),	1166.0	1630.0	**0.017**	1128.0	2267.0	**0.001**
Median [IQR]	[619.0, 2038.0]	[1117.0, 4334.0]		[589.0, 1844.3]	[1227.0, 5235.0]	
Antithrombin (%),	83.0	69.5	**0.048**	82.0	77.0	0.971
Median [IQR]	[62.5, 102.5]	[55.8, 81]		[59.0, 100.4]	[69.0, 99.0]	
Pro-inflammatory markers						
IL-6 (pg/mL),	73.9	107.7	**0.021**	71.8	226.2	**<0.001**
Median [IQR]	[36.6, 125.4]	[81.3, 269.8]		[35.2, 111.4]	[90.6, 518.9]	
C-reactive protein (mg/L),	50.0	105.0	**0.013**	48.0	186.0	**<0.001**
Median [IQR]	[15.3, 149.0]	[71.0, 192.0]		[10.0, 107.5]	[108.5, 247.5]	
Organ injury markers						
Troponin T (ng/L), Median [IQR]	5.0 [10.0, 23.0]	35.0 [17.0, 58.0]	**<0.001**	10.0 [5.0, 24.0]	25.0 [9.8, 57.3]	**0.011**
Bilirubin (μmol/L), Median [IQR]	8.0 [5.0, 13.0]	12.0 [8.0, 16.5]	**0.041**	8.0 [5.0, 13.0]	11.0 [85.0, 16.3]	**0.016**
ALT (U/L), Median [IQR]	25.0 [12.8, 45.0]	28.5 [15.8, 44.3]	0.727	20.5 [12.8, 38.3]	36.5 [25.5, 55.3]	0.062
Creatinine (μmol/L), Median [IQR]	76.0 [61.0, 96.8]	102.0 [71.0, 180.0]	**0.024**	76.0 [62.5, 99.3]	96.0 [65.0, 154.0]	0.127
SpO2 (%), Median [IQR]	95.0 [93.0, 97.0]	92.0 [78.5, 96.0]	**0.005**	95.0 [93.5, 97.0]	92.0 [85.8, 94.0]	**0.001**
Outcomes						
Length of stay (days),	10.0	–	–	8.0	28.0	**<0.001**
Median [IQR]	[3.0, 20.0]			[2.5, 15.5]	[13.0, 41.5]	
Ventilator support (days), Median [IQR]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.347	0.0 [0.0, 0.0]	0.0 [0.0, 8.0]	**<0.001**
Mortality at 28 days, No. [%]	0 [0]	25 [100]	**<0.001**	12 [14.3]	13 [44.8]	**0.001**

[a]*P* value for survivors *vs.* non-survivors. [b]*P* value for toxic histone levels *vs.* non-toxic. Performed using the Mann-Whitney U test for continuous variables and Fisher Exact/χ^2 tests for categorical variables.

tones demonstrated that rising histone levels were associated with mortality (odds ratio =1.031 (95% CI=1.013-1.049, *P*=0.001). Using categorical data where patients were stratified based on a ≥30 μg/mL threshold,[3,7] similar results were obtained (Figure 1C, OR=4.875 (95% CI=1.879-12.649, *P*=0.001), demonstrating that patients with high circulating histone levels on admission had a higher risk of mortality. Subsequent multivariate analysis demonstrated that histones were independently associated with mortality after adjustment for age, gender, ethnicity and co-morbidity when histone levels were treated as either continuous (odds ratio=1.032; 95% CI=1.013-1.051, *P*=0.001) or categorical variables (odds ratio=5.404; 95% CI=1.852-15.770, *P*=0.002). ROC curve analysis shows an area under the curve [AUC] of 0.708 (95% CI=0.589-0.827, *P*=0.002). A Kaplan-Meier survival curve demonstrated a significant increase in the probability of mortality during the 28-day period in patients with histones ≥30 μg/mL (Figure 1D, *P*<0.001).

Coagulopathy has emerged as a key feature of severe COVID-19 and has been linked to increased mortality.[13] It has been documented that extracellular histones, released following cell death, are drivers of coagulation by activating platelets,[7] generating thrombin[2] and damaging endothelial cells[8] to induce coagulopathy in critical illness.[3] This is the first report to demonstrate high levels of circulating histones in SARS-CoV-2 infection, with levels strongly associated with coagulopathy. This suggests their involvement in thrombosis in severe cases.[14]

High levels of circulating histones reflect the extent of cellular death, such as lymphopenia or NETosis,[15] which may be a major source of circulating histones in COVID-19. Histone release following cell death triggers IL-6 release to induce an acute-phase response.[8] We found that circulating histone levels significantly correlated with IL-6 and acute-phase protein levels, including fibrinogen and CRP, indicating histone-induced acute phase response in patients with COVID-19.

Extracellular histones disrupt cell membranes through phospholipid binding to induce cytotoxic effects on cells, including endothelial cells[8] and cardiomyocytes.[12] This study demonstrates circulating histones associated with cardiac injury, which is frequently observed in severe COVID-19 and associated with poor outcomes.[5] Therefore, the cytotoxic and pro-coagulant properties of circulating histones may be an underlying molecular mechanism contributing to disease severity and poor outcomes (Figure 1E).

In conclusion, this is the first report to quantify high levels of circulating histones in viral infection and demonstrate that extracellular histones play a central role in the development of immunothrombosis and critical illness in COVID-19.

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