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

Short title: Circulating histones in severe COVID-19

Rebecca J. Shaw[1,2]*, Simon T. Abrams[1]*, James Austin[1]*, Joseph M. Taylor[3], Steven Lane[4], Tina Dutt[2], Colin Downey[3], Min Du[1], Lance Turtle[1,5], J. Kenneth Baillie[6,7], Peter JM Openshaw[8], Guozheng Wang[1], Malcolm G. Semple[1,9#] and Cheng-Hock Toh[1,2#] on behalf of the ISARIC4C investigators.

[1]Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, Liverpool, UK.

[2]Roald Dahl Haemostasis & Thrombosis Centre, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK.

[3]Liverpool Clinical Laboratories, Liverpool University Hospitals NHS Foundation Trust, Liverpool, UK.

[4]Department of Biostatistics, University of Liverpool, Liverpool, UK.

[5]Infectious Diseases Unit, Royal Liverpool University Hospital, Liverpool, UK.

[6]Roslin Institute, University of Edinburgh, Edinburgh, UK.

[7]Intensive Care Unit, Royal Infirmary Edinburgh, Edinburgh, UK.

[8]National Heart and Lung Institute, Imperial College London, UK.

[9]Respiratory Medicine, Alder Hey Children's Hospital NHS Foundation Trust, Liverpool, UK.

*These authors contributed equally to this work

[#]Co-senior authors

Corresponding Author: Cheng-Hock Toh, MD, Department of Clinical Infection, Microbiology and Immunology, University of Liverpool, Ronald Ross Building, 8 West Derby St, Liverpool L69 7BE, United Kingdom (toh@liverpool.ac.uk).

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COVID-19 has highlighted the lethal consequences of immunothrombosis; i.e. the cross-talk between coagulation, inflammation and the innate immune system. These patients have significant immune cell death[1], which can release pro-coagulant[2] and cytotoxic[3] histones. Histones are small positively charged proteins, typically found within the cell nucleus, 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 were significantly elevated with increasing severity of COVID-19 infection (Mild, median=2.6μg/ml [IQR=0.7-7.6], Moderate, 10.5μg/ml [3.5-27.2], Critical, 20.0μg/ml [6.2-33.0], Non-survivors, 29.6μg/ml [11.2-60.0]; P<.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 hospitalised 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 2 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 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, 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, dyspnoea OR tachypnoea AND admitted to a healthcare

facility[11]. Patients were categorised 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 (dyspnoea, 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) and 4) Non-survivors (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 analysed. Severity of infection was determined by the patient's most severe clinical state throughout the 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 given 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 for comparison of continuous variables, presented as median [interquartile range; IQR]; the Fishers Exact/Chi squared 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 analysed either as continuous variables or categorised based on a previously determined threshold for

cytotoxicity (30μg/mL)[3, 7]. Mann-Whitney U test was used to compare categorical histone levels to continuous clinical variables. Correlation analysis was performed using Spearman's rank. 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 analyse the probability of mortality over time. Statistical tests were performed on SPSS (IBM, version 25). A 2-tailed P value of <.05 was considered significant.

One hundred and thirteen COVID-19 patients were studied (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), characterised by elevated D-dimer (P=.017) and prolonged prothrombin time (P=.005), and a pro-inflammatory phenotype characterised by elevated CRP (P<.001) and IL-6 (P=.002) on hospital admission, as well as with hypoxia and cardiac injury (Table 1). Median hospital length of 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=2.9μg/ml [IQR=1.5-3.3]; Mild, 2.6μg/ml [0.7-7.6]; Moderate, 10.5μg/ml [3.5-27.2]; Critical, 20.0μg/ml [6.2-33.0]; Non-survivors, 29.6μg/ml [11.2-60.0]; P<.001). Circulating histone levels strongly correlated with D-dimer levels (R=.606), indicating potential involvement of extracellular histones in COVID-19 coagulopathy. Positive association with organ injury markers, including bilirubin (R=.531), creatinine (R=.501) and cardiac troponin (R=.486), indicates the possible role of histone-induced cytotoxicity in multiple organ injury. Strong associations with fibrinogen (R=.632), CRP (R=.735) and IL-6 (R=.677) confirmed histone-initiated acute phase response[9]. Negative

correlation with lymphocyte count (R=-.446) suggests that lymphocyte and other immune cell death might be a major source of circulating histones in COVID-19 infection.

Using a 30μg/ml cytotoxic histone threshold[3, 7], patients over the threshold (n=29) had significantly higher D-dimer (2267.0ng/ml [1227.0-5235.0] vs 1128.0ng/ml [589.0-1844.3], P=.001), fibrinogen (6.6g/L [4.6-7.6] vs 4.8g/L [3.9-5.7], P=.012), IL-6 (226.2pg/ml [90.6-518.9] vs 71.8pg/ml [35.2-111.4], P<.001) and CRP levels (186mg/L [108.5-247.5] vs 48.0mg/L [10.0-107.5], P<.001) than those patients below the threshold (Table 2). These patients also had significantly reduced SpO_2 than those with circulating histones <30 μg/ml (oxygen saturations 92.0% [85.8-94.0] vs 95.0% [93.5-97.0], P=.001), required critical care admission (P<.001), with longer duration of mechanical ventilation (R=.635) and hospital stay (R=.654).

Circulating histone levels were significantly higher in non-survivors than those who survived (29.6μg/ml [11.2-60.0] vs 8.6 μg/ml [3.1-24.8], P=.002), and accordingly, patients with histones >30μg/ml were more likely to die (13/29 [44.8%] vs 12/84 [14.3%], P=.001). Patients who died were significantly older than those who survived (Table 2, 76 years [66-86] vs 59 years [46-72] P<.001). Compared to survivors, non-survivors had evidence of consumptive coagulopathy with lower platelet counts (P=.003), prolonged prothrombin time (P=.028), elevated D-dimer (P=.017) and reduced antithrombin levels (P=.048). Furthermore, in non-survivors, lymphocyte counts (P=.001), and oxygen saturations (P=.005) were significantly reduced, and IL-6 (P=.021), CRP (P=.013), troponin (P<.001), bilirubin (P=.041) and creatinine (P=.024) were elevated when compared to survivors (Table 2).

Univariate analysis using continuous circulating histones demonstrated that rising histone levels were associated with mortality (odds ratio =1.031 (95% CI=1.013-1.049, P=.001). Using categorical data where patients were stratified based on ≥30μg/ml threshold[3, 7], similar

results were obtained (Figure 1C, OR=4.875 (95% CI=1.879-12.649, P=.001), demonstrating that patients with high circulating histone levels on admission had higher risks 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=.001) or categorical variables (odds ratio=5.404; 95% CI=1.852-15.770, P=.002). ROC curve analysis shows an area under the curve [AUC] of .708 (95% CI=.589-.827, P=.002). Kaplan-Meier survival curve demonstrated a significant increase in the probability of mortality during 28-days in patients with histones ≥30μg/ml (Figure 1D, P=.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, suggesting 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 the 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 seen with 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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	Total	Mild	Moderate	Critical	Non-survivors	*P* value[a]
Total number (n)	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] *,†	**<.001**
Male, No. [%]	65 [57.5]	15 [50.0]	20 [52.6]	14 [70.0]	16 [64.0]	.428
White ethnicity, No. [%]	96 [85.0]	26 [86.7]	35 [92.1]	11 [55.0]	24 [96.0]	**.001**
Smoking history, No. [%]	38 [33.6]	10 [33.3]	16 [42.1]	4 [20.0]	8 [32.0]	**.033**
Hypertension, No. [%]	36 [31.9]	8 [26.7]	12 [31.6]	5 [25.0]	11 [44.0]	.474
Asthma/COPD, No. [%]	29 [25.7]	14 [46.7]	10 [26.3]	1 [5.0]	4 [16.0]	**.005**
Diabetes mellitus, No. [%]	29 [25.7]	5 [16.7]	10 [26.3]	5 [25.0]	9 [36.0]	.443
Ischaemic heart disease, No.[%]	16 [14.2]	3 [10.0]	8 [21.1]	0 [0.0]	5 [20.0]	.116
Chronic kidney disease, No. [%]	15 [13.3]	3 [10.0]	10 [26.3]	0 [0.0]	2 [8.0]	**.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] *,¥	**<.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]	.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]	.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] *,¥,†	**.009**
Haemoglobin (g/L), Median [IQR]	129.0 [117.8, 145.3]	126.0 [119.0, 145.0]	123.0 [113.8, 139.8]	134.5 [131.0, 146.0] ¥	136.0 [107.0, 147.0]	.122
Platelets (x10^9/L), Median [IQR]	236.5 [170.3, 296.0]	253.0 [177.0, 311.0]	243.5 [113.8, 139.8]	250.5 [207.3, 299.3]	174.0 [124.0, 250.0] *,¥,†	**.026**
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] *	**.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]	.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] †	**.010**
D-dimer (ng/ml), Median [IQR]	1227.0 [687.0, 2141.5]	755.5 [431.5, 1744.0]	1315.0 [832.5, 2176.3] *	950.0 [602.0, 1728.0]	1630.0 [1117.0, 4334.0] *,†	**.017**
Antithrombin (%), Median [IQR]	80.0 [61.0, 100.0]	81.0 [57.5, 98.5]	80.0 [61.5, 97.5]	98.0 [80.3, 114.8] *¥	70.0 [59.0, 87.0] †	**.024**
Pro-inflammatory markers						
IL-6 (pg/ml), Median [IQR]	79.0 [40.5, 131.9]	53.2 [15.0, 83.1]	70.5 [41.9, 115.0]	166.7 [75.6, 214.7] *	107.7 [81.3, 269.8] *,¥	**.002**
C-reactive protein (mg/L), Median [IQR]	61.0 [21.0, 153.5]	16.0 [3.5, 53.8]	52.0 [23.3, 146.3] *	145.0 [97.0, 202.5] *,¥	105.0 [71.0, 192.0] *,¥	**<.001**
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] *,†	**<.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] *	.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]	.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] *	.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] *	**<.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] *	..	**<.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]	**<.001**

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

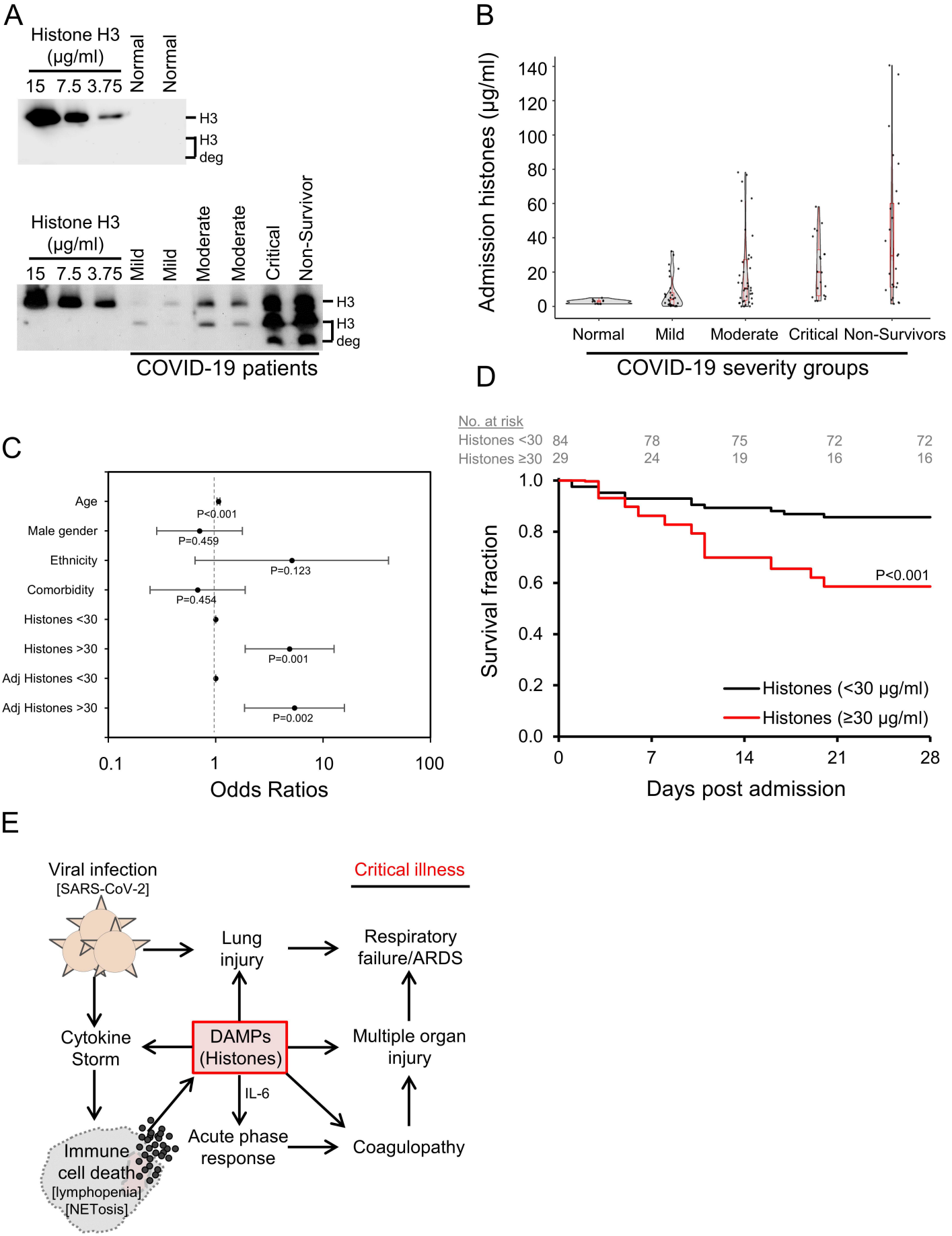
	Survivors	Non-survivors	*P* value[a]	Histones <30µg/ml	Histones ≥30µg/ml	*P* value[b]
Total number (n)	88	25	..	84	29	..
Demographics & Comorbidities						
Age (years), Median [IQR]	59.0 [45.8, 72.3]	76.0 [66.0, 86.0]	**<.001**	63.0 [47.8, 76.0]	66.0 [57.0, 80.0]	.224
Male, No. [%]	49 [55.7]	16 [64.0]	.458	48 [57.1]	17 [58.6]	.890
White ethnicity, No. [%]	72 [81.8]	24 [96.0]	.113	73 [86.9]	23 [79.3]	.324
Smoking history, No. [%]	30 [34.1]	8 [32.0]	.845	28 [33.3]	10 [34.5]	.910
Hypertension, No. [%]	25 [28.4]	11 [44.0]	.140	28 [33.3]	8 [27.6]	.567
Asthma/COPD, No. [%]	25 [28.4]	4 [16.0]	.301	25 [29.8]	4 [13.8]	.138
Diabetes mellitus, No. [%]	20 [22.7]	9 [36.0]	.180	21 [25.0]	8 [27.6]	.783
Ischaemic heart disease, No. [%]	11 [12.5]	5 [20.0]	.343	13 [15.5]	3 [10.3]	.758
Chronic kidney disease, No. [%]	13 [14.8]	2 [8.0]	.515	11 [13.1]	4 [13.8]	>.999
Histones (µg/ml), Median [IQR]	8.6 [3.1, 24.8]	29.6 [11.2, 60.0]	**.002**	6.1 [2.0, 13.5]	51.6 [38.2, 72.8]	**<.001**
Peripheral blood cell counts						
White blood cells ($x10^9$/L), Median [IQR]	8.7 [6.1, 11.8]	8.1 [5.2, 11.3]	.387	8.0 [5.7, 11.0]	9.8 [6.7, 13.3]	.084
Neutrophils ($x10^9$/L), Median [IQR]	6.2 [4.0, 8.9]	7.2 [4.0, 11.2]	.563	5.7 [3.6, 8.2]	9.1 [6.1, 12.2]	**.001**
Lymphocytes ($x10^9$/L), Median [IQR]	1.1 [0.8, 1.7]	0.7 [0.4, 1.1]	**.001**	1.2 [0.8, 1.7]	0.8 [0.5, 1.1]	**.007**
Haemoglobin (g/L), Median [IQR]	128.0 [118.0, 144.0]	136.0 [107.0, 147.0]	.740	128.0 [118.0, 145.0]	131.0 [116.0, 147.0]	.740
Platelets ($x10^9$/L), Median [IQR]	248.0 [181.0, 299.0]	174.0 [124.0, 250.0]	**.003**	237.5 [174.3, 295.8]	215.0 [155.8, 296.8]	.410
Coagulation parameters						
PT (seconds), Median [IQR]	13.0 [11.8, 14.1]	14.1 [12.4, 20.7]	**.028**	12.8 [11.8, 14.0]	13.8 [13.3, 15.6]	**.005**
aPTT (seconds), Median [IQR]	30.9 [28.4, 32.9]	30.0 [28.2, 37.6]	.858	30.7 [28.7, 34.0]	29.5 [28.0, 32.6]	.268
Fibrinogen (g/L), Median [IQR]	5.3 [4.1, 6.5]	4.5 [3.1, 4.9]	.091	4.7 [3.9, 5.7]	6.6 [4.6, 7.6]	**.012**
D-dimer (ng/ml), Median [IQR]	1166.0 [619.0, 2038.0]	1630.0 [1117.0, 4334.0]	**.017**	1128.0 [589.0, 1844.3]	2267.0 [1227.0, 5235.0]	**.001**
Antithrombin (%), Median [IQR]	83.0 [62.5, 102.5]	69.5 [55.8, 81]	**.048**	82.0 [59.0, 100.4]	77.0 [69.0, 99.0]	.971
Pro-inflammatory markers						
IL-6 (pg/ml), Median [IQR]	73.9 [36.6, 125.4]	107.7 [81.3, 269.8]	**.021**	71.8 [35.2, 111.4]	226.2 [90.6, 518.9]	**<.001**
C-reactive protein (mg/L), Median [IQR]	50.0 [15.3, 149.0]	105.0 [71.0, 192.0]	**.013**	48.0 [10.0, 107.5]	186.0 [108.5, 247.5]	**<.001**
Organ injury markers						
Troponin T (ng/L), Median [IQR]	5.0 [10.0, 23.0]	35.0 [17.0, 58.0]	**<.001**	10.0 [5.0, 24.0]	25.0 [9.8, 57.3]	**.011**
Bilirubin (µmol/L), Median [IQR]	8.0 [5.0, 13.0]	12.0 [8.0, 16.5]	**.041**	8.0 [5.0, 13.0]	11.0 85.0, 16.3]	**.016**
ALT (U/L), Median [IQR]	25.0 [12.8, 45.0]	28.5 [15.8, 44.3]	.727	20.5 [12.8, 38.3]	36.5 [25.5, 55.3]	.062
Creatinine (µmol/L), Median [IQR]	76.0 [61.0, 96.8]	102.0 [71.0, 180.0]	**.024**	76.0 [62.5, 99.3]	96.0 [65.0, 154.0]	.127
SpO2 (%), Median [IQR]	95.0 [93.0, 97.0]	92.0 [78.5, 96.0]	**.005**	95.0 [93.5, 97.0]	92.0 [85.8, 94.0]	**.001**
Outcomes						
Length of stay (days), Median [IQR]	10.0 [3.0, 20.0]	..	..	8.0 [2.5, 15.5]	28.0 [13.0, 41.5]	**<.001**
Ventilator support (days), Median [IQR]	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]	**<.001**
Mortality at 28 days, No. [%]	0 [0]	25 [100]	**<.001**	12 [14.3]	13 [44.8]	**.001**

Table 2. Demographics, peripheral blood measurements and outcomes of COVID-19 patients. [a] P value for survivors vs non-survivors. [b] P value for toxic histone levels vs. non-toxic. Performed using Mann-Whitney U test for continuous variables and Fishers Exact/Chi squared tests for categorical variables.

Figure legends

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) and critical disease (n=20) and non-survivors (n=25) with COVID-19 infection. Circulating histone levels were higher with increasing disease severity (P<.001). Histone levels were higher in non-survivors compared to the moderate (P=.023), mild groups (P<.001) and to normal healthy controls (P<.001). Histone levels were higher in the critical group compared to mild groups (P<.001) and normal healthy controls (P<.001). Histone levels were higher in the moderate group compared to the mild group (P=.007) and normal healthy controls (P=.002). **(C)** Multivariate analysis of crude and adjusted odds ratios (with patients adjusted for age, gender, BAME ethnicity and comorbidities including smoking, hypertension, asthma/COPD, diabetes, ischaemic heart disease and chronic kidney disease). Circulating histone levels >30μg/ml were independently associated with 28-day mortality. **(D)** Kaplan-Meier survival curve for the probability of mortality during 28 days. Patients were stratified based on circulating histones levels on admission (<30μg/ml vs ≥30μg/ml). **(E)** Diagram to propose that circulating histones play a central pathological role in the development of severe COVID-19.

Figure 1


A
Histone H3 (µg/ml)
15 7.5 3.75
Normal
Normal
H3
H3 deg
Histone H3 (µg/ml)
15 7.5 3.75
Mild
Mild
Moderate
Moderate
Critical
Non-Survivor
H3
H3 deg
COVID-19 patients
B
Admission histones (µg/ml)
140
120
100
80
60
40
20
0
Normal
Mild
Moderate
Critical
Non-Survivors
COVID-19 severity groups
C
Age
P<0.001
Male gender
P=0.459
Ethnicity
P=0.123
Comorbidity
P=0.454
Histones <30
Histones >30
P=0.001
Adj Histones <30
Adj Histones >30
P=0.002
0.1
1
10
100
Odds Ratios
D
No. at risk
Histones <30 84 78 75 72 72
Histones ≥30 29 24 19 16 16
Survival fraction
1.0
0.8
0.6
0.4
0.2
0.0
P<0.001
Histones (<30 µg/ml)
Histones (≥30 µg/ml)
0
7
14
21
28
Days post admission
E
Viral infection
[SARS-CoV-2]
Critical illness
Lung injury
Respiratory failure/ARDS
Cytokine Storm
DAMPs (Histones)
Multiple organ injury
IL-6
Immune cell death
[lymphopenia]
[NETosis]
Acute phase response
Coagulopathy

Appendix 1

The ISARIC4C investigators

Contributions

1. **Consortium Lead Investigator:** J Kenneth Baillie

2. **Chief Investigator**: Malcolm G Semple

3. **Co-Lead Investigator**: Peter JM Openshaw

4. **ISARIC Clinical Coordinator**: Gail Carson

5. **ISARIC Co-Investigators**: Beatrice Alex, Benjamin Bach, Wendy S Barclay, Debby Bogaert, Meera Chand, Graham S Cooke, Annemarie B Docherty, Jake Dunning, Ana da Silva Filipe, Tom Fletcher, Christoper A Green, Ewen M Harrison, Julian A Hiscox, Antonia Ying Wai Ho, Peter W Horby, Samreen Ijaz, Saye Khoo, Paul Klenerman, Andrew Law, Wei Shen Lim, Alexander J Mentzer, Laura Merson, Alison M Meynert, Mahdad Noursadeghi, Shona C Moore, Massimo Palmarini, William A Paxton, Georgios Pollakis, Nicholas Price, Andrew Rambaut, David L Robertson, Clark D Russell, Vanessa Sancho-Shimizu, Janet T Scott, Thushan de Silva, Louise Sigfrid, Tom Solomon, Shiranee Sriskandan, David Stuart, Charlotte Summers, Richard S Tedder, Emma C Thomson, AA Roger Thompson, Ryan S Thwaites, Lance CW Turtle, Maria Zambon

6. **Project Management Team**: Hayley Hardwick, Chloe Donohue, Ruth Lyons, Fiona Griffiths, Wilna Oosthuyzen

7. **Data Analysis Team**: Lisa Norman, Riinu Pius, Thomas M Drake, Cameron J Fairfield, Stephen R Knight, Kenneth A Mclean, Derek Murphy, Catherine A Shaw

8. **Data Architecture Team**: Jo Dalton, Michelle Girvan, Egle Saviciute, Stephanie Roberts, Janet Harrison, Laura Marsh, Marie Connor, Sophie Halpin, Clare Jackson, Gary Leeming, Andrew Law, Murray Wham, Sara Clohisey, Ross Hendry, James Scott-Brown

9. **Data Analysis and Management Team**: William Greenhalf, Victoria Shaw, Sara McDonald, Seán Keating

10. **Outbreak Laboratory Staff and Volunteers**: Katie A. Ahmed, Jane A Armstrong, Milton Ashworth, Innocent G Asiimwe, Siddharth Bakshi, Samantha L Barlow, Laura Booth, Benjamin Brennan, Katie Bullock, Benjamin WA Catterall, Jordan J Clark, Emily A Clarke, Sarah Cole, Louise Cooper, Helen Cox, Christopher Davis, Oslem Dincarslan, Chris Dunn, Philip Dyer, Angela Elliott, Anthony Evans, Lorna Finch, Lewis WS Fisher, Terry Foster, Isabel Garcia-Dorival, William Greenhalf, Philip Gunning, Catherine Hartley, Rebecca L Jensen, Christopher B Jones, Trevor R Jones, Shadia Khandaker, Katharine King, Robyn T. Kiy, Chrysa Koukorava, Annette Lake, Suzannah Lant, Diane Latawiec, Lara Lavelle-Langham, Daniella Lefteri, Lauren Lett, Lucia A Livoti, Maria Mancini, Sarah McDonald, Laurence McEvoy, John McLauchlan, Soeren Metelmann, Nahida S Miah, Joanna Middleton, Joyce Mitchell, Shona C Moore, Ellen G Murphy, Rebekah Penrice-Randal, Jack Pilgrim, Tessa Prince, Will Reynolds, P. Matthew Ridley, Debby Sales, Victoria E Shaw, Rebecca K Shears, Benjamin Small, Krishanthi S Subramaniam, Agnieska Szemiel, Aislynn Taggart, Jolanta Tanianis-Hughes, Jordan Thomas, Erwan Trochu, Libby van Tonder, Eve Wilcock, J. Eunice Zhang, Lisa Flaherty, Nicole Maziere, Emily Cass, Alejandra Doce Carracedo, Nicola Carlucci, Anthony Holmes, Hannah Massey

11. **Local Principal Investigators**: Kayode Adeniji, Daniel Agranoff, Ken Agwuh, Dhiraj Ail, Ana Alegria, Brian Angus, Abdul Ashish, Dougal Atkinson, Shahedal Bari, Gavin Barlow, Stella Barnass, Nicholas Barrett, Christopher Bassford, David Baxter, Michael Beadsworth, Jolanta Bernatoniene, John Berridge, Nicola Best, Pieter Bothma, David Brealey, Robin Brittain-Long, Naomi Bulteel, Tom Burden, Andrew Burtenshaw, Vikki Caruth, David Chadwick, Duncan Chambler, Nigel Chee, Jenny Child, Srikanth Chukkambotla, Tom Clark, Paul Collini, Catherine Cosgrove, Jason Cupitt, Maria-Teresa Cutino-Moguel, Paul Dark, Chris Dawson, Samir Dervisevic, Phil Donnison, Sam Douthwaite, Ingrid DuRand, Ahilanadan Dushianthan, Tristan Dyer, Cariad Evans, Chi Eziefula, Chrisopher Fegan, Adam Finn, Duncan Fullerton, Sanjeev Garg, Sanjeev Garg, Atul Garg, Effrossyni Gkrania-Klotsas, Jo Godden, Arthur Goldsmith, Clive Graham, Elaine Hardy, Stuart Hartshorn, Daniel Harvey, Peter Havalda, Daniel B Hawcutt, Maria Hobrok, Luke Hodgson, Anil Hormis, Michael Jacobs, Susan Jain, Paul Jennings, Agilan Kaliappan, Vidya Kasipandian, Stephen Kegg, Michael Kelsey, Jason Kendall, Caroline Kerrison, Ian Kerslake, Oliver Koch, Gouri Koduri, George Koshy, Shondipon Laha, Steven Laird, Susan Larkin, Tamas Leiner, Patrick Lillie, James Limb, Vanessa Linnett, Jeff Little, Michael MacMahon, Emily MacNaughton, Ravish Mankregod, Huw Masson, Elijah Matovu, Katherine McCullough, Ruth McEwen, Manjula Meda, Gary Mills, Jane Minton, Mariyam Mirfenderesky, Kavya Mohandas, Quen Mok, James Moon, Elinoor Moore, Patrick Morgan, Craig Morris, Katherine Mortimore, Samuel Moses, Mbiye Mpenge, Rohinton Mulla, Michael Murphy, Megan Nagel, Thapas Nagarajan, Mark Nelson , Igor Otahal, Mark Pais, Selva Panchatsharam, Hassan Paraiso, Brij Patel, Natalie Pattison, Justin Pepperell, Mark Peters, Mandeep Phull, Stefania Pintus, Jagtur Singh Pooni, Frank Post, David Price, Rachel Prout, Nikolas Rae, Henrik

Reschreiter, Tim Reynolds, Neil Richardson, Mark Roberts, Devender Roberts, Alistair Rose, Guy Rousseau, Brendan Ryan, Taranprit Saluja, Aarti Shah, Prad Shanmuga, Anil Sharma, Anna Shawcross, Jeremy Sizer, Manu Shankar-Hari, Richard Smith, Catherine Snelson, Nick Spittle, Nikki Staines, Tom Stambach, Richard Stewart, Pradeep Subudhi, Tamas Szakmany, Kate Tatham, Jo Thomas, Chris Thompson, Robert Thompson, Ascanio Tridente, Darell Tupper-Carey, Mary Twagira, Andrew Ustianowski, Nick Vallotton, Lisa Vincent-Smith, Shico Visuvanathan, Alan Vuylsteke, Sam Waddy, Rachel Wake, Andrew Walden, Ingeborg Welters, Tony Whitehouse, Paul Whittaker, Ashley Whittington, Meme Wijesinghe, Martin Williams, Lawrence Wilson, Sarah Wilson, Stephen Winchester, Martin Wiselka, Adam Wolverson, Daniel G Wooton, Andrew Workman, Bryan Yates, Peter Young