Cytokeratin 18 cell death assays as biomarkers for quantification of apoptosis and necrosis in COVID-19: a prospective, observational study

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ABSTRACT

Background The mechanism by which SARS-CoV-2 triggers cell damage and necrosis are yet to be fully elucidated. We sought to quantify epithelial cell death in patients with COVID-19, with an estimation of relative contributions of apoptosis and necrosis.

Methods Blood samples were collected prospectively from adult patients presenting to the emergency department. Circulating levels of caspase-cleaved (apoptosis) and total cytokeratin 18 (CK-18) (total cell death) were determined using M30 and M65 enzyme assays, respectively. Intact CK-18 (necrosis) was estimated by subtracting M30 levels from M65.

Results A total of 52 COVID-19 patients and 27 matched sick controls (with respiratory symptoms not due to COVID-19) were enrolled. Compared with sick controls, COVID-19 patients had higher levels of M65 (p = 0.046, total cell death) and M30 (p = 0.0079, apoptosis). Hospitalised COVID-19 patients had higher levels of M65 (p= 0.014) and intact CK-18 (p= 0.004, necrosis) than discharged patients. Intensive care unit (ICU)-admitted COVID-19 patients had higher levels of M65 (p= 0.004), M30 (p= 0.004) and intact CK-18 (p= 0.033) than hospitalised non-ICU admitted patients. In multivariable logistic regression, elevated levels of M65, M30 and intact CK-18 were associated with increased odds of ICU admission (OR=22.05, p=0.014, OR=19.71, p=0.012 and OR=14.12, p=0.016, respectively).

Conclusion Necrosis appears to be the main driver of hospitalisation, whereas apoptosis and necrosis appear to drive ICU admission. Elevated levels CK-18 levels are independent predictors of severe disease, and could be useful for risk stratification of COVID-19 patients and in assessment of therapeutic efficacy in early-phase COVID-19 clinical trials.

INTRODUCTION

Recent histopathological studies have demonstrated extensive pulmonary and extrapulmonary tissue damage that drive morbidity and mortality in patients with COVID-19.[1 2] The mechanisms by which SARS-CoV-2 induces cell damage and death are yet to be fully elucidated. In a recent study on transgenic mice models, Li *et al*[3] found an increased number of apoptotic bodies in SARS-CoV-2 infected cells vs control cells, as well as elevated levels of apoptotic markers such as activated caspases-3, 8 and 9, and cleaved poly (ADP-ribose) polymerase 1.[3] Their results, together with findings of necrotic cell debris in histopathological studies, suggest an involvement of multiple pathways of cell death in COVID-19. In the current study, we sought to quantify epithelial cell death in patients with COVID-19 with an estimation of the relative contributions of apoptosis and necrosis, using circulating levels of full length and caspase-cleaved cytokeratin 18 (CK-18). CK-18 is an intermediate filament present in epithelial cells, such as pulmonary alveolar cells, which is leaked into the plasma following cell death. The plasma concentration of CK-18 and caspase-cleaved CK-18 can be measured via M30 and M65 cell death assays. M30 measures caspase-cleaved CK-18 produced during apoptosis, while M65 measures the levels of both intact CK-18 (produced by cells undergoing necrosis) and caspase-cleaved CK-18.[4] We investigated the association of M30 and M65 levels measured at emergency department (ED) presentation and their association with COVID-19 disease progression.

METHODS

Study design

This was a prospective observational cohort study. Adults (≥18 years) presenting to the ED of the University of Cincinnati Medical Center during the initial wave of COVID-19 in this community (April–May 2020) with respiratory symptoms at triage suggestive of COVID-19 and receiving both a standard-of-care nasopharyngeal reverse transcriptase-PCR (RT-PCR) test for COVID-19 and blood draw were enrolled. Patients who presented to the ED and had a positive RT-PCR test for SARS-CoV-2 were enrolled as COVID-19 cases. In contrast, patients with a negative test were further evaluated using a multiple-step algorithm, as previously described,[5] employing both serology (anti-SARS-CoV-2 IgM, IgA, IgG) testing and assessment of pretest probability using the 'corona score' to rule out false-negative RT-PCR tests.[6] Subjects negative by RT-PCR, serology and corona scores were finally enrolled as non-COVID-19 sick controls.

Sample collection, processing and measurements

Blood samples were collected via routine draws for clinical indications in the ED. After collection, blood samples were immediately centrifuged at 2000 g for 15 min and subsequently frozen at −80°C until measurement. The circulating levels of caspase-cleaved (CK-18, ie, apoptosis), as well as total CK-18 (cleaved and intact, ie, total cell

death), were determined using M30 Apoptosense and M65 EpiDeath enzyme assays (PEVIVA, VLVbio/Diapharma, West Chester, Ohio, USA) respectively. Cell death due to necrosis was estimated by subtracting M30 levels from M65. Plasma concentrations of interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor-α (TNF-α) were quantified using Meso Scale Discovery U-Plex assay (Rockville, Maryland, USA). Plasma concentrations of ferritin and C reactive protein (CRP) were measured with a BN II System (Siemens Medical Solutions USA, Malvern, Pennsylvania, USA). Lactate dehydrogenase (LDH) was tested on Dimension RxL Max Integrated Chemistry System (Siemens Medical Solutions USA), while procalcitonin (PCT) was measured with a chemiluminescent immunoassay on Diasorin Liaison XL (DiaSorin S.p.A. Saluggia, Italy).

Outcomes and study definitions

Patients were monitored through hospitalisation until discharge/death if admitted from the ED or for 30 days if discharged from the ED. The primary outcome of interest was the need for intensive care unit (ICU) admission at any point during the follow-up window, while need for hospitalisation from the index ED visit was the secondary outcome.

Statistical analysis

Analysis of the data was carried out using Prism V.8.4.3 (GraphPad Software, San Diego, California, USA) and SPSS (IBM Statistics Software V.25). Categorical data were reported as frequencies (%), while continuous data were reported as median and IQR. Comparison of baseline total CK-18 (M65 levels, total cell death), cleaved CK-18 (M30 levels, apoptosis), intact CK-18 (M65-M30, necrosis) and necrosis-apoptosis ratio (M65-M30 divided by M30) between COVID-19 versus sick controls, hospitalised versus discharged COVID-19 patients, and in ICU versus non-ICU admitted COVID-19 patients was carried out using Mann-Whitney U test. The diagnostic performance of baseline levels of the above variables for predicting the need for ICU admission was assessed using receiver operating characteristics (ROC) curves, with calculation of the area under the curve (AUC) and its 95% CI. Logistic regression was performed to estimate the effect of elevated baseline total CK-18, cleaved CK-18, intact CK-18 and necrosis-apoptosis ratio (>378.5 U/L, >149.5 U/L, >239.6 U/L and >1.450, respectively, based on ROC analysis) on the primary outcome, adjusting for age, sex and comorbidities, and to calculate adjusted ORs with the corresponding 95% Wald CI. Additionally, the correlation between baseline total CK-18, cleaved CK-18, intact CK-18 and necrosis-apoptosis ratio and inflammatory biomarkers (IL-1β, IL-6, IL-10, TNFα, LDH, ferritin, CRP and PCT) was performed using Spearman's correlation.

RESULTS

Patient characteristics

Seventy-nine patients were enrolled in the study, 52 with SARS-CoV-2 infection and 27 sick controls. The median age in the COVID-19 group was 50.5 (IQR: 39.3–66.0) years vs 56 (IQR: 30–64) in the control group (p=0.706). Males were 57.7% of the COVID-19 group and 74.1% of the control group (p=0.134). The comorbidities of the COVID-19 patients are shown in table 1. A total of 16 (32.6%) COVID-19 patients developed the primary outcome (need for ICU admission), while 33 (63.5%) of the COVID-19 patients developed the secondary outcome (need for hospitalisation).

Table 1 Characteristics of the COVID-19 patients and sick controls at admission

COVID-19 group	Sick controls
Age (median, IQR): 50.5 (39.3–66.0)	**Age (median, IQR):** 56 (30-64)
Sex (% male): 58	**Sex (% male):** 74
Comorbidities	**Illnesses**
Hypertension- 26 (50%)	Pneumonia/sepsis/pleural effusion/ pulmonary embolism- 10 (37%)
Coronary artery disease- 8 (16%)	Acute heart failure- 3 (11%)
Heart failure- 9 (17%)	Cancer- 2 (7%)
Chronic kidney disease- 6 (12%)	Diabetic ketoacidosis- 2 (7%)
Chronic liver disease- 7 (13%)	Myasthenia gravis- 1 (4%)
Cerebrovascular disease- 7 (13%)	Encephalitis-1 (4%)
Cancer- 4 (8%)	Postliver transplant- 1 (4%)
Atrial fibrillation- 3 (6%)	Seizure-1 (4%)
Hyperlipidaemia- 15 (29%)	Peptic ulcer bleed-1 (4%)
Diabetes mellitus- 21 (40%)	Haemorrhagic stroke-1 (4%)
Chronic obstructive lung disease- 8 (16%)	Cardiac arrest-1 (4%)
Smoking (current or former)- 23 (44%)	Hyponatraemia-1 (4%)

Cyokeratin-18 levels in COVID-19 patients versus sick controls

Compared with sick controls, COVID-19 patients had significantly higher levels of M65 (384.2 (IQR: 261.2–764.8) vs 275.3 (IQR: 192.5–552.1) U/L; p = 0.046) and M30 (177 (IQR: 111.7–339.8) vs 97.3 (IQR: 76.7–200) U/L; p = 0.0079). The levels of non-cleaved CK-18 (M65-M30) (220.6 (IQR: 103.1–438.8) vs 159.9 (IQR: 62.6–354.3) U/L; p= 0.185) were comparable between the two groups (figure 1A–D).

Cyokeratin-18 levels in hospitalised versus discharged COVID-19 patients

Hospitalised COVID-19 patients had higher baseline levels of M65 (441.1 (IQR: 305.0–811.4) vs 298.0 (IQR: (190.8–464.8) U/L; p= 0.014), non-cleaved CK-18 (M65-M30) (259.5 (IQR: 172.4–527.6) vs 117.2 (IQR: 27.2–355.9) U/L; p= 0.004) and necrosis-apoptosis ratio (1.5 (IQR: 1.2–2.0) vs 0.9 (IQR: 0.2–1.5) U/L; p= 0.008) than patients discharged at index ED visit. The levels of M30 were comparable between the two groups (264.4 (IQR: 118.4–337.4) vs 170.8 (IQR: 108.9–357.4) U/L; p= 0.895) (figure 2A–D).

CK-18 levels in hospitalised ICU-admitted versus non-ICU-admitted COVID-19 patients

Hospitalised ICU-admitted COVID-19 patients had higher baseline levels of M65 (742.6 (IQR: 408.5–966.7) vs 336.2 (IQR: (235.2–659.6) U/L; p= 0.0044), M30 (316.1 (IQR: 188.2–395.6) vs 133.1 (IQR: 96.6–289.6) U/L; p= 0.0039) and non-cleaved CK-18 (434.4 (IQR: 247.8–548.6) vs 206.3 (IQR: 148.7–336.2) U/L; p= 0.0326) compared with hospitalised non-ICU admitted patients. No differences were observed in the necrosis-apoptosis ratio between the two groups (1.588 (IQR: 1.032–2.734) vs 1.454 (IQR: 1.202–1.956) U/L; p= 0.9052) (figure 3A–D).

Diagnostic performance of CK-18 levels for ICU admission

ROC curves were generated for baseline total CK-18 (M65), cleaved CK-18 (M30), intact CK-18 (M65-M30) and necrosis-apoptosis ratio (M65-M30/M30) to predict the need for ICU admission. The AUC was 0.81 (95% CI 0.69 to 0.92; p<0.001)

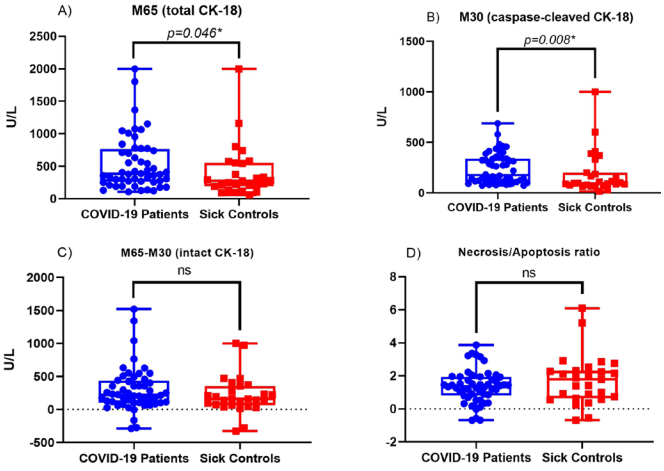

Figure 1 (A–D) Comparison of cytokeratin levels in COVID-19 patients versus sick controls. *Statistically significant. CK-18, cytokeratin 18; ns, not significant.

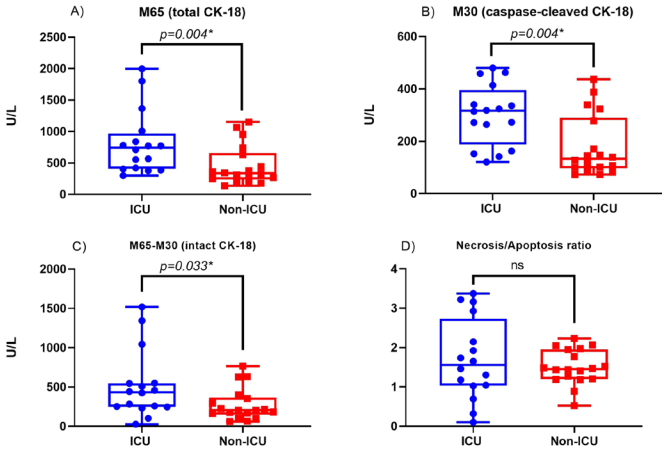

Figure 3 (A–D) Comparison of cytokeratin levels in ICU-admitted versus non-ICU admitted patients. *Statistically significant. CK-18, cytokeratin 18; ICU, intensive care unit; ns, not significant.

for M65. Analysis of ROC data determined that an M65 cut-off of ≥378.5 U/L was associated with 94% sensitivity and 68% specificity for predicting ICU admission, with a positive predictive value (PPV) and negative predictive value (NPV) of 58% and 96%. The AUC for M30 was 0.72 (95% CI 0.58 to 0.85; p=0.013), with ≥149.5 U/L cut-off displaying 88% sensitivity, 58% specificity, 50% PPV and 91% NPV. The AUC for non-cleaved CK-18 (M65-M30) was 0.76 (95% CI 0.60 to 0.91; p=0.003), with a cut-off of ≥239.6 U/L displaying 81% sensitivity and 69% specificity, with a PPV and NPV of 55% and 89% respectively. Lastly, the AUC for necrosis-apoptosis ratio was 0.61 (95% CI 0.43 to 0.78; p=0.227), with a cut-off of ≥1.450 displaying 56% sensitivity, 61% specificity, 40% PPV and 75% NPV (figure 4A–D).

CK-18 levels as a predictor of the need for ICU admission

Multivariable logistic regression (adjusted for age, sex and comorbidities) for baseline M65, M30 and intact CK-18 (M65-M30) using the above ROC curve cut-offs (ie, ≥378.5 U/L, ≥149.5 U/L and ≥239.6 U/L, respectively) as independent predictors of need for ICU admission at time of index ED visit revealed that elevated baseline M65, M30 and intact CK-18 (M65-M30) were associated with increased odds of ICU admission (OR=22.05, 95% CI 1.84 to 263.04, p=0.014, OR 19.71, 95% CI 1.91 to 203.14, p=0.012 and OR 14.12, 95% CI 1.63 to 122.09, p=0.016, respectively) (table 2).

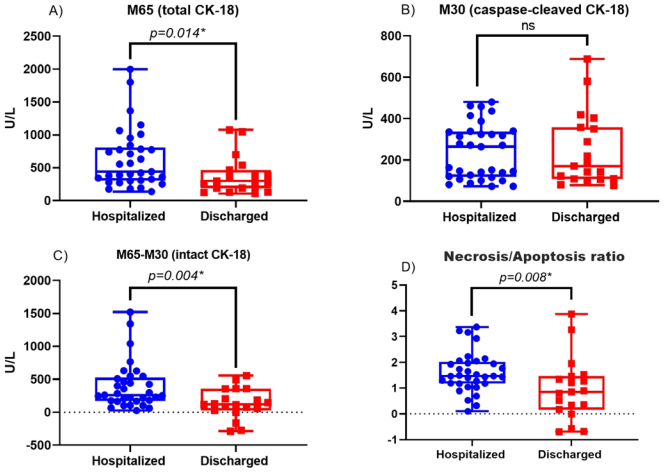

Figure 2 (A–D) Comparison of cytokeratin levels in hospitalised versus discharged COVID-19 patients. *Statistically significant. CK-18, cytokeratin 18; ns, not significant.

Correlation between CK-18 levels and various inflammatory markers

Baseline M65 and non-cleaved CK-18 (M65-M30) positively correlated with all inflammatory biomarkers tested (IL-1β, IL-6, IL-10, TNFα, LDH, ferritin, PCT and CRP) (table 2), whereas necrosis-apoptosis ratio correlated with all but IL-1β and PCT. M30 correlated with IL-1β, IL-6, LDH, ferritin and PCT (table 3).

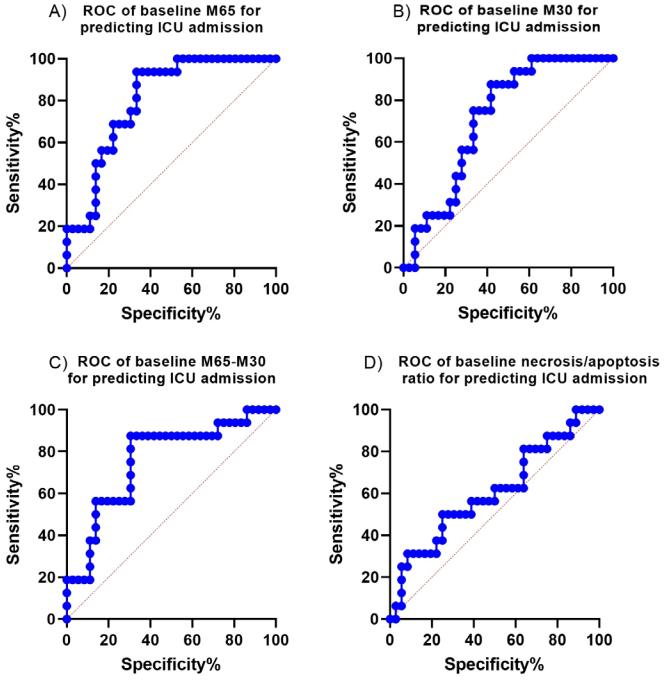

Figure 4 (A–D) ROC for baseline cytokeratin-18 levels for prediction of ICU admission. ICU, intensive care unit; ROC, receiver operating characteristics.

Table 2 Age-adjusted and sex-adjusted multivariable logistic regression for baseline total K-18 (M65), cleaved K-18 (M30), intact K-18 (M65-M30) and necrosis-apoptosis ratio as independent predictors of ICU admission

Variable	Estimate	SE	OR (95% CI)	P value
Elevated M65	3.093	1.265	22.05 (1.84 to 263.04)	0.014*
Elevated M30	2.981	1.190	19.704 (1.91 to 203.14)	0.012*
Elevated M65-M30	2.648	1.101	14.12 (1.63 to 122.09)	0.016*
Elevated Necrosis-Apoptosis Ratio	0.328	0.738	1.388 (0.33 to 5.899)	0.657

*Significant at significance level of 0.05.
ICU, intensive care unit.

DISCUSSION

The findings of the current study provide important preliminary insights into the dynamics of cell death in COVID-19. CK-18 is type I intermediate filaments predominantly expressed in the epithelia of organs such as the lung, kidney, liver and gastrointestinal tract,[7] which are among the most commonly affected organs in COVID-19. CK-18 provides a flexible intracellular scaffold, resists external cellular stresses and plays a role in cellular processes such as apoptosis and mitosis.[7 8] During apoptosis, CK-18 is usually cleaved by caspases, resulting in CK-18 fragments. Cell death by necrosis, however, liberates only full-length (non-cleaved) CK-18[9] (figure 5).

Several observational studies have shown significant elevations in CK-18 levels of critically ill patients with acute respiratory distress syndrome[10] and sepsis,[11–13] which were associated with adverse clinical outcomes. The findings of elevated total CK-18 level in our study suggests that this could also be the case in COVID-19. The supranormal rate of cell death in COVID-19 is attributable to both direct and indirect effects of SARS-CoV-2. The virus has been shown to induce cell death through caspase-mediated apoptotic pathways, as evidenced by high circulating levels of caspases-3, 8 and 9.[3] It is thought that SARS-CoV-2 uses this phenomenon to evade the immune system by suppressing T-cell functions,[14] and this is consistent with observations of lymphopenia in COVID-19 patients.[15] The high rate of cellular injury and death may also be the result of the immune response to viral infection, in which infected cells are directly killed by CD8+ T lymphocytes or indirectly injured by virus-specific antibodies.[14] As COVID-19 is associated with hypercytokinaemia,[16] proinflammatory cytokines such as IL-1, IL-6 and TNFα, which are capable of inducing caspase-mediated cell death,[17] may also be responsible for an increased rate of cell death. This is consistent with the positive correlations between CK-18 assays and inflammatory markers in this study. Lastly, cell death could be a consequence of ischaemic cell injury due to reduced end-organ perfusion in COVID-19 patients, particularly those who develop microvascular and macrovascular thrombotic events.[18 19] In such cases, cytotoxic or ischaemic necrosis would be the predominant pathway of cell death. Necrosis normally results in the release of intracellular contents, which then evokes inflammatory responses,[20] as evidenced by the positive correlation between non-cleaved CK-18 (M65-M30) and all inflammatory biomarkers tested in this study.

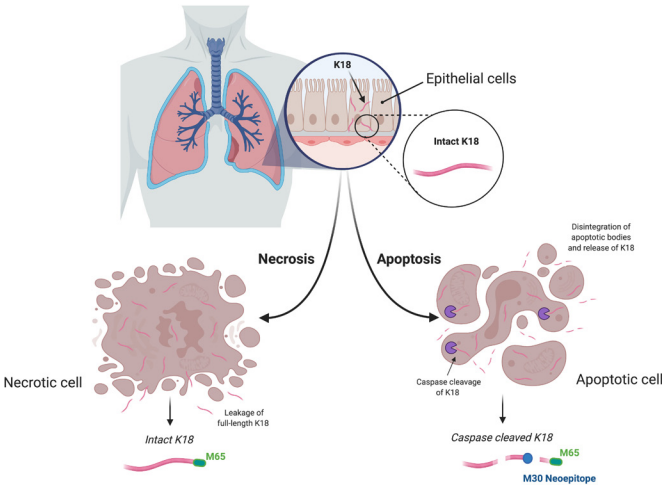

Figure 5 Mechanism of action of M30 and M65 cell death assays.

It is noteworthy that M65 (total cell death) and M30 (apoptosis) were significantly elevated in COVID-19 patients compared with sick controls, while M65-M30 (necrosis) was not, thus suggesting that apoptosis, regardless of COVID-19 severity, is a dominant cell death pathway in comparison to other acute illnesses, consistent with recent preclinical[3] and clinical SARS-CoV-2 studies.[21] M30 (apoptosis) was, however, not significantly elevated in hospitalised COVID-19 patients compared with non-hospitalised COVID-19 patients, thus underpinning that necrosis (M65-M30) could be a major driver of hospitalisation among COVID-19 patients. On top of total (M65), both apoptosis (M30) and necrosis (M65-M30) were significantly elevated in ICU-admitted COVID-19 patients versus hospitalised non-ICU admitted patients. Elevated necrosis levels in hospitalised ICU-admitted COVID-19 patients are consistent with the higher rate of venous and arterial thrombotic events, and subsequent ischaemic injuries, observed in these

Table 3 Correlation between baseline total K-18 (M65), cleaved K-18 (M30), intact K-18 (M65-M30) and necrosis-apoptosis ratio and various inflammatory markers

	IL-1β	IL-6	IL-10	TNFα	CRP	LDH	Ferritin	Procalcitonin
M65	r=0.383 p=0.031*	r=0.555; p<0.001*	r=0.533 p<0.000*	r=0.445; p=0.001*	r=0.414; p=0.002*	r=0.637; p<0.001*	r=0.602; p<0.001*	r=0.356; p=0.010*
M30	r=0.352; p=0.048*	r=0.257; p=0.071*	r=0.262; p=0.064	r=0.201; p=0.156	r=0.009; p=0.951	r=0.468; p<0.001*	r=0.374; p=0.006*	r=0.183; p=0.195
M65-M30	r=0.378; p=0.033*	r=0.650; p<0.001*	r=0.561 p<0.000*	r=0.451; p=0.001*	r=0.566; p<0.001*	r=0.595; p<0.001*	r=0.576; p<0.001*	r=0.312; p=0.024*
Necrosis-Apoptosis ratio	r=0.246; p=0.174	r=0.492; p<0.001*	r=0.371 p=0.007*	r=0.282; p=0.045*	r=0.637; p<0.001*	r=0.285; p=0.040*	r=0.285; p=0.040*	r=0.222; p=0.113

*Significant at significance level of 0.05.
CRP, C reactive protein; IL-1, interleukin 1; LDH, lactate dehydrogenase; TNFα, tumour necrosis factor alpha.

patients.[22–24] Interestingly, the ratio of necrosis to apoptosis was not different between hospitalised COVID-19 patients requiring ICU admission versus those who did not, thus suggesting that it is the overall rate and quantity of cell death driving critical illness, as opposed to any substantial changes in underlying pathophysiology or mechanisms of cell death. Overall, both apoptosis and necrosis appear to drive severity among COVID-19 patients as confirmed by multivariate logistic regression, and M30/M65 levels at ED presentation are independent predictors of disease progression, with high sensitivities (up to 94%). This suggests that CK-18 assays could be useful in risk stratification of COVID-19 patients and, given their relatively short half-life,[25] may also serve as quantitative cell death biomarkers for assessing therapeutic efficacy in early-phase COVID-19 clinical trials, especially given the inconsistent (waxing and waning) pattern of inflammatory biomarkers,[26] along with well-known difficulties in establishing objective clinical outcomes for interventional trials. Cell death pathways also present a potential therapeutic avenue for COVID-19. This is supported by several preclinical mice models of sepsis, in which modulation of Fas/Fas-ligand apoptotic pathways resulted in improved survival.[27–29]

The small sample size limited our study. Additionally, the potential influence of illnesses within the sick controls on baseline CK-18 levels cannot be ruled out. However, this was delimited by age, sex and comorbidity adjusted regression analysis within the COVID-19 group. Lastly, we did not perform serial assays of M30 and M65 over the course of hospitalisation. Future studies should focus on the temporal changes of CK-18 along the clinical course of COVID-19 patients to further clarify the dynamics of cell death among COVID-19 patients.

CONCLUSION

COVID-19 is associated with a higher rate of cell death. Necrosis appears to be the main driver of hospitalisation, whereas apoptosis and necrosis appear to drive ICU admission. Elevated levels of total CK-18 (M65), cleaved CK-18 (M30), intact CK-18 (M65-M30) are independent predictors of the need for intensive care and could be useful in risk stratification of patients with SARS-CoV-2 infection and as biomarkers for assessing therapeutic efficacy in COVID-19 clinical trials.

Take home messages

- Necrosis appears to be the main driver of hospitalisation, whereas apoptosis and necrosis appear to drive intensive care unit admission.
- Elevated levels cytokeratin-18 levels are independent predictors of severe COVID-19.
- Cytokeratin-18 levels could be useful for risk stratification of COVID-19 patients and in assessment of therapeutic efficacy in early-phase COVID-19 clinical trials.

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Contributors Study conception and design: BMH, IC; data collection: BMH; analysis and interpretation of results: BMH, IC; draft manuscript preparation: all authors. All authors reviewed the results and approved the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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REFERENCES

1 Deshmukh V, Motwani R, Kumar A, *et al*. Histopathological observations in COVID-19: a systematic review. *J Clin Pathol* 2021;74:76–83.
2 Bradley BT, Maioli H, Johnston R, *et al*. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington state: a case series. *Lancet* 2020;396:320–32.
3 Li S, Zhang Y, Guan Z, *et al*. SARS-CoV-2 triggers inflammatory responses and cell death through caspase-8 activation. *Signal Transduct Target Ther* 2020;5:235.
4 de Haas EC, di Pietro A, Simpson KL, *et al*. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. *Neoplasia* 2008;10:1041–8.
5 Benoit J, Benoit SW, Lippi G, *et al*. False negative RT-PCR or false positive serological testing in SARS-CoV-2 diagnostics? Navigating between Scylla and Charybdis to prevent misclassification bias in COVID-19 clinical investigations. *Diagnosis* 2020;7:405–7.
6 Kurstjens S, van der Horst A, Herpers R, *et al*. Rapid identification of SARS-CoV-2-infected patients at the emergency department using routine testing. *Clin Chem Lab Med* 2020;58:1587–93.
7 Weng Y-R, Cui Y, Fang J-Y. Biological functions of cytokeratin 18 in cancer. *Mol Cancer Res* 2012;10:485–93.
8 Zhang C, Guo Y, Cui J, *et al*. Cytokeratin 18 is not required for morphogenesis of developing prostates but contributes to adult prostate regeneration. *Biomed Res Int* 2013;2013:1–8.
9 Cheng Y, Qin K, Huang N, *et al*. Cytokeratin 18 regulates the transcription and alternative splicing of apoptotic-related genes and pathways in HeLa cells. *Oncol Rep* 2019;42:301–12.
10 Lee KS, Choi YH, Kim YS, *et al*. Evaluation of bronchoalveolar lavage fluid from ARDS patients with regard to apoptosis. *Respir Med* 2008;102:464–9.
11 Koch A, Yagmur E, Linka J, *et al*. High circulating caspase-cleaved keratin 18 fragments (M30) indicate short-term mortality in critically ill patients. *Dis Markers* 2018;2018:1–8.
12 Roth GA, Krenn C, Brunner M, *et al*. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope M30 in critically ill patients. *Shock* 2004;22:218–20.
13 Lorente L, Martín MM, González-Rivero AF, *et al*. Serum levels of caspase-cleaved cytokeratin-18 and mortality are associated in severe septic patients: pilot study. *PLoS One* 2014;9:e109618.
14 Mortaz E, Tabarsi P, Varahram M, *et al*. The immune response and immunopathology of COVID-19. *Front Immunol* 2020;11:2037.
15 Henry B, Cheruiyot I, Vikse J, *et al*. Lymphopenia and neutrophilia at admission predicts severity and mortality in patients with COVID-19: a meta-analysis. *Acta Biomed* 2020;91:e2020008.
16 Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *Lancet Respir Med* 2020;8:e46–7.
17 Choi C, Kutsch O, Park J, *et al*. Tumor necrosis factor-related apoptosis-inducing ligand induces caspase-dependent interleukin-8 expression and apoptosis in human astroglioma cells. *Mol Cell Biol* 2002;22:724–36.
18 Allegra A, Innao V, Allegra AG, *et al*. Coagulopathy and thromboembolic events in patients with SARS-CoV-2 infection: pathogenesis and management strategies. *Ann Hematol* 2020;99:1953–65.
19 Ciceri F, Beretta L, Scandroglio AM, *et al*. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. *Crit Care Resusc* 2020;22:95–7.
20 Chan FK-M, Moriwaki K, De Rosa MJ. Detection of necrosis by release of lactate dehydrogenase activity. *Methods Mol Biol* 2013;979:65–70.

21 Cardot-Leccia N, Hubiche T, Dellamonica J, *et al*. Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection. *Intensive Care Med* 2020;46:1777–8.
22 Klok FA, Kruip MJHA, van der Meer NJM, *et al*. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. *Thromb Res* 2020;191:148-150–50.
23 Klok FA, Kruip MJHA, van der Meer NJM, *et al*. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 2020;191:145–7.
24 Cheruiyot I, Kipkorir V, Ngure B, *et al*. Arterial thrombosis in coronavirus disease 2019 patients: a rapid systematic review. *Ann Vasc Surg* 2021;70:273–81.
25 Olofsson MH, Ueno T, Pan Y, *et al*. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. *Clin Cancer Res* 2007;13:3198–206.
26 Zeng F, Huang Y, Guo Y, *et al*. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis* 2020;96:467–74.
27 Hotchkiss RS, Chang KC, Swanson PE, *et al*. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nat Immunol* 2000;1:496–501.
28 Chung C-S, Song GY, Lomas J, *et al*. Inhibition of Fas/Fas ligand signaling improves septic survival: differential effects on macrophage apoptotic and functional capacity. *J Leukoc Biol* 2003;74:344–51.
29 Wesche-Soldato DE, Chung C-S, Lomas-Neira J, *et al*. In vivo delivery of caspase-8 or Fas siRNA improves the survival of septic mice. *Blood* 2005;106:2295–301.