Preliminary predictive criteria for COVID-19 cytokine storm

Roberto Caricchio ,[1] Marcello Gallucci ,[2] Chandra Dass,[3] Xinyan Zhang,[1] Stefania Gallucci ,[4] David Fleece,[5] Michael Bromberg,[6] Gerard J Criner,[7] Temple University COVID-19 Research Group

Handling editor Josef S Smolen

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2020-218323).

For numbered affiliations see end of article.

Correspondence to
Professor Roberto Caricchio, Medicine/Rheumatology, Temple University School of Medicine, Philadelphia, PA 19140, USA; roc@temple.edu

Received 16 June 2020
Revised 7 September 2020
Accepted 8 September 2020
Published Online First 25 September 2020

ABSTRACT

Objectives To develop predictive criteria for COVID-19-associated cytokine storm (CS), a severe hyperimmune response that results in organ damage in some patients infected with COVID-19. We hypothesised that criteria for inflammation and cell death would predict this type of CS.

Methods We analysed 513 hospitalised patients who were positive for COVID-19 reverse transcriptase PCR and for ground-glass opacity by chest high-resolution CT. To achieve an early diagnosis, we analysed the laboratory results of the first 7 days of hospitalisation. We implemented logistic regression and principal component analysis to determine the predictive criteria. We used a 'genetic algorithm' to derive the cut-offs for each laboratory result. We validated the criteria with a second cohort of 258 patients.

Results We found that the criteria for macrophage activation syndrome, haemophagocytic lymphohistiocytosis and the HScore did not identify the COVID-19 cytokine storm (COVID-CS). We developed new predictive criteria, with sensitivity and specificity of 0.85 and 0.80, respectively, comprising three clusters of laboratory results that involve (1) inflammation, (2) cell death and tissue damage, and (3) prerenal electrolyte imbalance. The criteria identified patients with longer hospitalisation and increased mortality. These results highlight the relevance of hyperinflammation and tissue damage in the COVID-CS.

Conclusions We propose new early predictive criteria to identify the CS occurring in patients with COVID-19. The criteria can be readily used in clinical practice to determine the need for an early therapeutic regimen, block the hyperimmune response and possibly decrease mortality.

INTRODUCTION

COVID-19 is the cause of the pandemic declared by the WHO in January 2020.[1] As of 3 August 2020, there have been 18 million confirmed cases and 688 000 deaths worldwide (coronavirus.jhu.edu). While most of these cases are mild, a sizeable number of patients develop a severe acute hyperimmune response characterised by a cytokine storm (CS).[2] Previous epidemics induced by coronaviruses SARS-CoV-1 and Middle Eastern respiratory syndrome-CoV were also associated with a CS.[3] CS occurs in several conditions, including autoimmune diseases, malignancies and infections.[2] Two forms of CS, haemophagocytic lymphohistiocytosis (HLH) and macrophage activation syndrome (MAS), rely on well-established criteria to identify their occurrence.[4 5] Results from recent reports suggest that COVID-19-associated CS is a unique form of a hyperinflammatory response, which needs further clinical and laboratory characterisation as well as classification criteria.[6] It has been suggested that the 2016 MAS classification criteria are not applicable to patients with COVID-19,[7–9] while it remains to be determined whether the 2004 HLH criteria and the HScore may be more helpful.[10–12] Reports from COVID-19 cohorts and autopsies highlight significant diffuse inflammation and widespread tissue damage, such as renal, cardiac and muscular damage, in addition to pulmonary impairment.[13–17] These findings underscore the need for criteria that should include not only the respiratory status but also markers of inflammation and tissue damage. The latter were recently reported to be associated with higher mortality in COVID-19.[18] We therefore designed a novel statistical strategy based on our clinical experience at Temple University Hospital[19] and developed preliminary criteria that can be used to identify the CS during COVID-19 infection.

METHODS

Patients and data collection

The first cohort used in this study included patients admitted to Temple University Hospital from 10 March 2020 to 17 April 2020. The 513 patients enrolled in the cohort and considered eligible must have met the following criteria on hospital admission: (1) signs and symptoms of COVID-19 infection (fever, generalised malaise, cough and shortness of breath) up to 1 week prior to hospital admission[20] and (2) presence of ground-glass opacity (GGO) by high-resolution CT (HRCT) of the chest as per radiology reading and reverse transcriptase PCR (RT-PCR) for COVID-19 RNA. A positive RT-PCR was not required due to the high percentage (15%) of false negatives in our cohort and in the literature.[21]

Age, sex, race/ethnicity and comorbidities were all collected on admission. Sixty-two laboratory variables, such as complete blood count with differential, complete metabolic panel, inflammatory and respiratory markers, were collected daily. Interleukin (IL)-6 was measured only in a subset of patients, before any biological treatment.

All 513 patients received oxygen supplementation, low-dose (0.5 mg/kg) prednisone and azithromycin

► http://dx.doi.org/10.1136/annrheumdis-2020-219448

To cite: Caricchio R, Gallucci M, Dass C, *et al*. *Ann Rheum Dis* 2021;**80**:88–95.

on admission and for at least the first 7 days of hospitalisation. Eighty-two patients were enrolled in clinical trials with biologics and their laboratory results were initially included in the analyses. The rest of the patients were clinically followed up and 64 were considered in CS by a consensus between the pulmonologists and rheumatologists. The initial consensus was based on the application of both MAS and HLH criteria; however, among the first few patients, very few met these criteria despite worsening clinical status and elevation of inflammatory markers. Hence, a newly devised consensus was based on (1) worsening respiratory status defined as increased oxygen supplementation required to maintain SpO_2>93% and (2) elevation above threefold the upper normal level of at least two of the following markers: C reactive protein (CRP), ferritin, D-dimer, lactate dehydrogenase (LDH) and cardiac troponin. Patients in this group were retrospectively selected as the basis for the following statistical analyses.

To validate the results, an additional cohort of 258 patients was collected from all the patients admitted to Temple University Hospital from 18 April 2020 to 30 April 2020. Inclusion criteria were the same of the first cohort.

Statistical analyses

A series of univariate logistic regressions were used to assess the association between each laboratory variable and the presence of the CS, with the criterion of the clinical consensus of the medical group indicating the presence of the storm (see previous discussion). The predictors were the laboratory variables, aggregated by using each patient average up to the day when the clinicians made the consensus of CS or the first 7 days of hospitalisation for patients not diagnosed in storm. Due to the presence of missing values, only predictors obtained in at least 300 patients were considered.

Predictors showing a significant odds ratio, at alpha-level 0.05, were then analysed by principal component analysis (PCA) with promax rotation to cluster them in coherent groups. We retained the components (clusters) having eigenvalues larger than 1. Each laboratory variable was associated with the cluster in which it featured the highest factor loading.

Cut-off values for each individual laboratory variable were estimated using a *genetic algorithm*[22] as implemented by Scrucca[23] (for more details, see online supplemental methods). In the algorithm, a population of 500 sets of cut-off values was defined, with mutation probability of 0.1 and crossover probability of 0.8. In each generation, 5% of the sets of cut-off values were selected based on their fitness. The fitness function maximised the geometric mean of the sensitivity and specificity of the classification (confusion table) predicting the (COVID-19 cytokine storm (COVID-CS)) groups obtained with a given set of cut-off values. The stopping rule was set to 200 generations with no improvement in fitness. In order to develop cut-offs that can be feasibly used in the clinic, daily laboratory data were used in the genetic algorithm fitness function. A patient was classified as COVID-CS positive when the criteria were met at least for 1 day. When a laboratory value was not present for a patient 1 day, the most recent available value was used.

To evaluate the stability of the cut-off values, a bootstrap procedure was employed to compute the CI classification statistics (accuracy, sensitivity and specificity). For each statistic, a distribution of bootstrap estimates was created across 5000 bootstrap samples, and the 95% CIs were obtained by setting the 2.5th and 97.5th percentiles of the bootstrap distribution as the interval boundaries. Finally, the criteria and cut-off values were also validated on the second cohort of patients.

Table 1 Demographics and comorbidities in the cohort of patients with COVID-19

Patients with COVID-19	All	Clinical consensus		P value
		No storm	Storm	
Numbers	513	449	64	*<0.001*
%	100	88	12	
Sex (%)				
Females	43	45	33	*<0.001*
Males	57	55	66	
Age (years)	58.3	57.7	62.2	*0.041*
Race/ethnicity (%)				
AA	53	54	53	n.s.
EA	11	11	14	n.s.
Hispanic	23	22	19	n.s.
Other	9	9	9	n.s.
Unknown	4	4	5	n.s.
Comorbidities (%)				
Lung disease	26	24	36	0.077
Hypertension	69	68	72	n.s.
Obesity	52	51	55	n.s.
Heart disease	25	25	25	n.s.
Smoking history	42	43	36	n.s.
Diabetes	48	50	36	0.057

Patients with diagnosis of COVID-19 infection and chest high-resolution CT with ground-glass opacity were divided according to a consensus of clinicians for a diagnosis of cytokine storm. P values were calculated using χ^2 test for frequencies and t-test for age.
Italics indicate significant p values.
AA, African–American; EA, European–American; n.s., not significant at $\alpha \geq 0.05$.

RESULTS

Description of the cohort

In this retrospective study, we investigated 513 patients who presented GGOs by chest HRCT. Ninety-five per cent of the patients were also COVID-19 positive by RT-PCR. Of these 513, 64 patients were eventually determined to be in CS and treated with biologics, such as monoclonal antibodies against IL-6R and recombinant IL-1R antagonist (table 1). Table 1 shows the demographics of the cohort, comparing patients who reached or not a clinical consensus for CS. As previously reported in COVID-19 pneumonia, more patients were male, and the average age was 58.3. Mirroring the population that our hospital serves, most patients were African–American and Hispanic. Frequent comorbidities included hypertension, obesity, diabetes and smoking history. We did not find any statistically significant difference in the distribution of race and comorbidities between the patients in storm or not, while older male patients were slightly more likely to develop CS, suggesting that sex and age, but not race and specific comorbidities, increase the risk of developing CS during COVID-19 infection.

COVID-CS does not meet the 2004 HLH criteria and HScore

To understand the type of CS occurring during COVID-19 infection, we determined the number of patients in our cohort who fulfilled the HLH criteria and had an HScore ≥169 (online supplemental tables S1 and S2)[4 24] using the averages of laboratory tests performed during the first 7 days of hospitalisation. We found that only 10 out of 513 patients fit the 2004 HLH criteria (table 2), and most patients (8/10) did not fulfil the clinical consensus of COVID-19 storm. We also found that 43 out of 513 patients had an HScore of >169, but only 12 also met the

Table 2 HLH, HScore and MAS criteria applied to the COVID-19 cohort

	Clinical consensus storm		H Score	Clinical consensus storm		MAS	Clinical consensus storm	
HLH	No	Yes		No	Yes		No	Yes
No	441	62	No	418	52	No	443	63
Yes	8	2	Yes	31	12	Yes	6	1

For HLH criteria, the specificity was 0.98 and the sensitivity was 0.2. For HScore, the specificity was 0.93 and the sensitivity was 0.28. For MAS, the specificity was 0.98 and the sensitivity was 0.14.
HLH, haemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome.

clinical consensus of COVID-19 storm (table 2). In our analyses of the HLH criteria and HScore, most patients admitted with COVID-19 infection did not have splenomegaly (not shown) nor cytopenias affecting at least two cell lineages in the peripheral blood. On the contrary, they had normal absolute numbers of monocytes and increased numbers of neutrophils. They also had normal or increased levels of fibrinogen, typically low in HLH, and mostly normal triglycerides, which are frequently increased in HLH[4] (online supplemental table S4). All patients with COVID-19 had high levels of serum ferritin. In addition, CRP, which is not included in the HLH 2004 criteria (online supplemental table S1), was also elevated. We could not evaluate natural killer (NK)-cell activity and the level of soluble interleukin-2 receptor (sIL-2R), since the results of these tests were not rapidly available at our medical centre or in most hospitals. Nevertheless, reports of other cohorts of patients with COVID-19 showed sIL-2R levels below those considered in the HLH criteria.[25] The analysis of haemophagocytosis in the bone marrow or in secondary lymphoid organs was deemed unnecessary, considering its invasiveness. Even if the tests that we did not perform were hypothetically positive, the majority of patients did not meet the five out of eight criteria of HLH because the majority fulfilled only two—fever and hyperferritinemia. Therefore, our results suggest that most patients in our cohort who developed a CS did not meet the HLH criteria and the HScore[24] performed poorly as previously suggested (table 2).[12]

COVID-CS does not meet the 2016 MAS criteria

We analysed whether our cohort fulfilled the MAS criteria, reported in online supplemental table S3,[5] and found that only 7/513 did (table 2). Six out of seven of these patients were not clinically found in storm, therefore fulfilling neither the laboratory nor the clinical judgement of MAS. These patients did not fit the MAS criteria due to the uncommon presence of thrombocytopenia, increased levels of fibrinogen and the relatively normal levels of triglycerides in the COVID-19-infected patients (online supplemental table S4).

Criteria to predict the COVID-CS

Since most patients with COVID-19 in CS did not meet the classification criteria of HLH, HScore or MAS, we next followed the strategy depicted in figure 1 and analysed the predictive power of 62 laboratory tests available in our hospital (table 3 and online supplemental table S4). We aimed to find novel criteria to identify patients in CS. In order to reach a predictive power that can be clinically useful to diagnose a COVID-CS, we used the mean values of laboratory results of the first 7

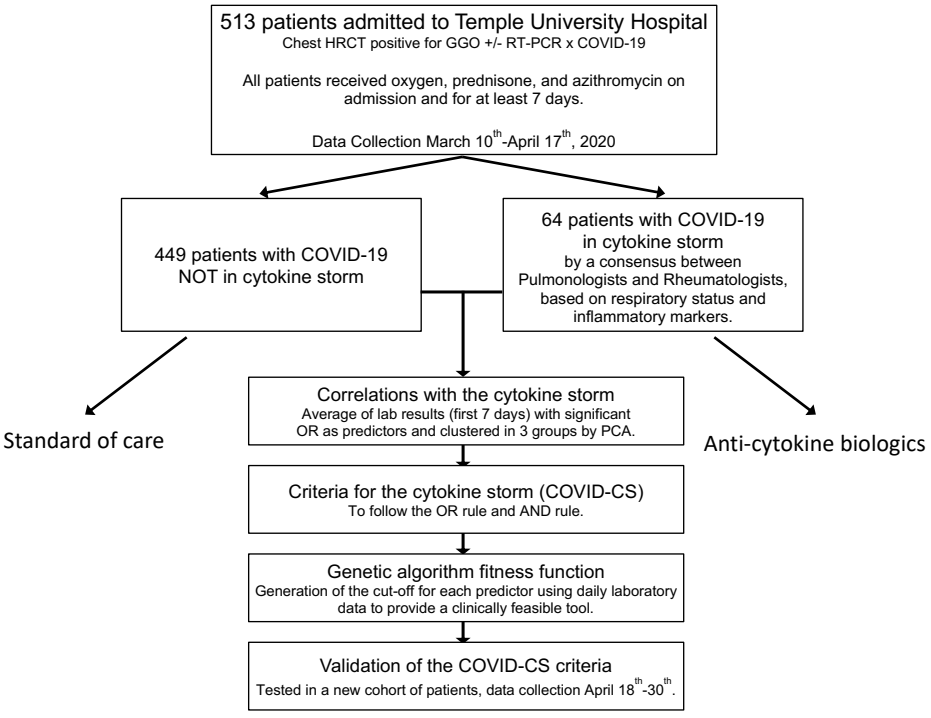

Figure 1 Research strategy. Flowchart of the experimental strategy followed in the generation of the new criteria aimed to recognise the cytokine storm in patients with COVID-19. COVID-CS, COVID-19 cytokine storm; GGO, ground-glass opacity; HRCT, high-resolution CT; PCA, principal component analysis; RT-PCR, reverse transcriptase PCR.

Table 3 Laboratory parameters in the cohort of patients with COVID-19

	Normal range	All	Clinical consensus		OR	P value
			No Storm	Storm		
Albumin	3.2–4.6 g/dL	2.9±0.6	2.9±0.6	2.7±0.5	0.637	*0.001*
ALT	16–61 U/L	45±46	43±37	58±86	1.254	*0.04*
Anion gap	6–16 mmol/L	7.6±3.0	7.8±3.0	7±3.1	0.734	*0.04*
AST	15–37 U/L	54±92	50±80	82±145	1.249	*0.028*
BUN:creatinine ratio	10–20 ratio	18.9±8.3	18.5±8	21±10	1.295	*0.03*
Chloride	101–111 mmol/L	104±5	104±5	106±5	1.316	*0.032*
CRP	0–0.4 mg/dL	7.2±6.4	6.9±6.4	9.1±6.1	1.341	*0.016*
D-dimers	0–500 ng/mL	3,227±11,306	2,396±7,851	8,817±23,356	1.41	*0.002*
LDH	84–246 U/L	323±169	305±153	447±212	1.892	*<0.001*
Lymphocytes Abs	1–4.8 K/mm^3	1.23±2.16	1.28±2.30	0.86±0.41	0.058	*<0.001*
Lymphocytes (%)	20%–40%	18±10	19±11	12±7	0.389	*<0.001*
Neutrophil Abs	1.8–7.8 K/mm^3	6±3.6	5.8±3.6	7.23±3.5	1.4	*0.004*
Potassium	3.5–5.2 mmol/L	4.09±0.5	4.07±0.51	4.23±0.59	1.392	*0.019*
Troponin I	0.045–0.1 ng/mL	0.23±2.29	0.1±0.38	1.07±6.1	2.727	*0.045*

Average and SD of the laboratory parameters collected up to the 24 hours within reaching the clinical consensus of CS or in the first 7 days of hospitalisation in patients with COVID-19 who never reached the clinical consensus of CS. ORs and p values were calculated by logistic regression. Normal range of values is shown for our laboratory as reference.
Abs, absolute numbers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C reactive protein; CS, cytokine storm; LDH, lactate dehydrogenase.

days of hospitalisation or up to the 24 hours within reaching the clinical consensus of CS. Using the logistic regression, we found that 12 laboratory parameters predict development of CS and by PCA, we determined that these 12 variables could be included in three coherent clusters (table 4). Based on factor analysis, we considered the parameters belonging to the same cluster as alternative indicators (OR rule), with the rationale that parameters of the same cluster highly correlate and may be indicators of the same condition or mechanism. We considered parameters belonging to the different clusters instead as necessary indicators (AND rule) because they represent conditions or mechanisms that should be met. Our analyses highlighted three clusters of laboratory results, and the alteration of one parameter for each cluster predicts the development of COVID-CS (table 4).

Table 4 Predictive criteria for COVID-19 cytokine storm

Entry criteria (must be all met)	Cut-off values
+Signs/symptoms of COVID-19	
±RT-PCR positive for COVID-19	
+GGO by HRCT (or chest X-ray)	
Ferritin	>250 ng/mL
C reactive protein	>4.6 mg/dL
AND (one variable from each cluster)	
Cluster I	
Albumin	<2.8 g/dL
Lymphocytes (%)	<10.2
Neutrophil Abs	>11.4 K/mm^3
Cluster II	
ALT	>60 U/L
AST	>87 U/L
D-dimers	>4,930 ng/mL
LDH	>416 U/L
Troponin I	>1.09 ng/mL
Cluster III	
Anion gap	<6.8 mmol/L
Chloride	>106 mmol/L
Potassium	>4.9 mmol/L
BUN:creatinine ratio	>29 ratio

Criteria are met when patients fulfil all the entry criteria and at least one criterion per each cluster. Cut-off values were calculated using a genetic algorithm.
Abs, absolute numbers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GGO, ground-glass opacity; HTCT, high-resolution CT; LDH, lactate dehydrogenase; RT-PCR, reverse transcriptase PCR.

The first cluster included decreased levels of albumin and percentage of lymphocytes, along with increased absolute numbers of neutrophils in patients in storm compared with patients who did not develop a storm (tables 3 and 4). The absolute number of lymphocytes formed a separated component and correlated with the first cluster, and we excluded it from the criteria because of its close correlation and redundancy with the percentage of lymphocytes. The second cluster included the increased levels of alanine aminotransaminase (ALT), aspartate aminotransferase (AST), D-dimers, LDH and troponin I. The third cluster included the decreased anion gap and increased levels of chloride, potassium and blood urea nitrogen (BUN):creatinine ratio (tables 3 and 4). These results highlight an important component of tissue damage occurring during the COVID-CS.

In order to develop cut-offs that can be used in clinical practice, we used daily laboratory parameters and estimated the cut-off for each individual laboratory parameter using a genetic algorithm.[22] The predictive requirement for the first cluster consisted of an albumin<2.87 mg/mL OR lymphocytes<10.2%, OR neutrophil absolute number>11.4×10^3/mL. For the second cluster, ALT>60 IU/L, OR AST>87 IU/L, OR D-dimers>4930 ng/mL, OR LDH>416 U/L OR troponin I>1.09 ng/mL were required. For the third cluster, anion gap<6.8 mmol/L, OR chloride>106 mmol/L, OR potassium>4.9 mmol/L OR BUN:creatinine ratio>29 were required (table 4).

Interestingly, ferritin and CRP had the widest ranges and had discriminatory power only if transformed by logarithmic scale. They were therefore added as such in the analyses. Although the performance of their predictive algorithm did not add any power, for clinical reassurance of an ongoing systemic inflammation, we propose to add them to the predictive criteria of COVID-CS (table 4).

Table 5 Validation of the novel criteria of COVID-CS

COVID-CS new criteria					Consensus storm	
	N	%	LoS (days)*	Mortality (%)*	No	Yes
All group						
No	340	66	5.7±6.7	6.6	330	10
Yes	173	34	15.1±13	28.8	119	54
					SP=0.73	SE=0.84
						ACC=0.75
No trials group						
No	308	71.5	5.3±6.7	6.4	300	8
Yes	123	28.5	15.3±13.7	28.1	78	45
					SP=0.79	SE=0.85
						ACC=0.80

The new COVID-CS criteria were applied to the cohort (left) and to the same patients divided according to the clinical consensus of cytokine storm (right). All group includes all the COVID-19 cohort (513, 64 patients who reached and 449 who did not reach the clinical consensus of COVID-19 cytokine storm). No trials group includes 431 patients of the cohort because it excludes 82 patients with COVID-19 who were recruited in clinical trials testing biologic therapies. The new criteria identified patients with significantly greater LoS and mortality.
*P<0.0001.
ACC, accuracy; COVID-CS, COVID-19 cytokine storm; LoS, length of stay; SE, sensitivity; SP, specificity.

Preliminary validation of the novel criteria of COVID-CS

We validated the ability of the proposed criteria to identify COVID-CS. The upper rows of table 5 show the initial validation where all the patients were included. The criteria classified 34% of the patients as in COVID-CS (173/513). Next, we applied the new criteria to the 64 patients originally considered in CS by clinical consensus, and 84% of them (54/64) were correctly classified as in COVID-CS. The new criteria had a specificity of 0.73 (CI 0.70 to 0.77) and a sensitivity of 0.84 (CI 0.78 to 0.92). We then performed the validation after excluding the 82 patients who were enrolled in clinical trials (table 5, bottom rows). In this subpopulation of 431 patients, the criteria showed an even higher specificity of 0.79 (CI 0.76 to 0.83) and a sensitivity of 0.85 (CI 0.78 to 0.93), suggesting that these criteria have a strong predictive power in our population of patients with COVID-19.

When we analysed the disaggregated laboratory parameters to determine the length of time patients required to meet the criteria of COVID-CS, we found that among the patients with the clinical consensus of CS, 43% met the criteria on hospital admission, and the rest reached the asymptote by 10 days of hospitalisation (figure 2, blue line). Among the patients who did not reach the clinical consensus of CS, 20% met the COVID-CS criteria with a similar timeline (figure 2 orange line). These results suggest an early and rapid progression in those patients bound to develop COVID-CS, as well as the low likelihood of developing the condition 10 or more days into the admission.

COVID-CS criteria identify severely ill patients

To determine whether our criteria could predict clinical severity, we analysed the hospital length of stay (LoS) and mortality. We found that the group of patients who met COVID-CS criteria had a significantly higher LoS (15.1±13 vs 5.7±6.7) and importantly higher mortality (28.8% vs 6.6%) (table 5). For both LoS and mortality, the p value was <0.0001. Excluding the patients in trials yielded similar results.

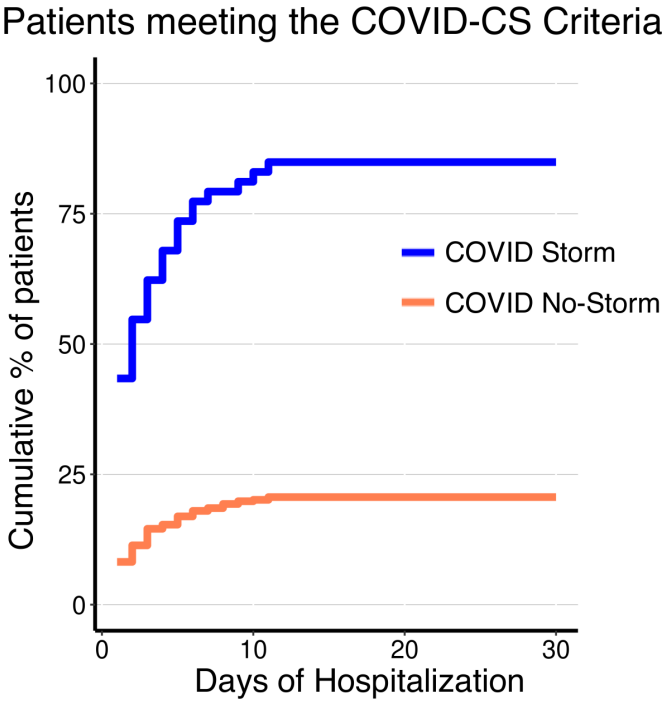

Figure 2 Rapid progression for patients with COVID-19 towards meeting the COVID-CS criteria. The cohort of 431 patients with COVID-19 (no trials) was plotted for the accumulation of the laboratory parameters fulfilling the COVID-CS criteria during hospitalisation. The blue line represents the percentage of patients who received the clinical diagnosis of CS and met the COVID-CS criteria. The orange line represents the percentage of patients who did not receive the clinical diagnosis of CS and met the COVID-CS criteria. COVID-CS, COVID-19 cytokine storm; CS, cytokine storm.

Markers of inflammation and tissue damage in COVID-CS

We analysed the laboratory results in our cohort of patients now divided as fitting or not the COVID-CS criteria (table 6 and online supplemental figure S1). The COVID-CS group had significantly higher levels of ferritin, CRP and triglycerides, and decreased levels of albumin, all signs of systemic inflammation. Ferritin showed an OR of 14, indicating an important role in COVID-CS. Strong inflammation was confirmed by the level of IL-6, which was elevated in most patients with COVID-19 but significantly higher in COVID-CS (35 vs 96 pg/mL). The white blood cells, and especially neutrophils and monocytes, were significantly increased in the COVID-CS group, suggesting an active role of the innate immunity in the storm. The lymphocytes instead were decreased, with averages half of the normal lower limit, suggesting a functional depletion of the adaptive immunity (table 6 and online supplemental figure S1).

We also found that five markers of tissue damage were significantly higher in patients with COVID-CS than in the rest of the patients with COVID-19. The liver enzymes ALT and AST had levels twice as high, indicative of liver damage, while D-dimers had levels more than six times higher, suggesting endothelial damage. The increase in LDH is a sign of cell death, while the moderately elevated levels of troponin I suggest damage to the cardiovascular system (table 6 and online supplemental figure S1).

Laboratory parameters pertaining to the electrolyte metabolism, namely, chloride, potassium and sodium, the first two predictive of COVID-CS, were still in the normal range, while creatinine, BUN and their ratio were all increased compared

Table 6 Laboratory parameters in the cohort of patients with COVID-19 at Temple University associated with the new criteria of COVID-CS

Parameters	Normal range	All	No COVID-CS	COVID-CS	OR	P value	N
Albumin	3.2–4.6 g/dL	2.9±0.6	3.1±0.6	2.6±0.4	0.292	*<0.001*	495
Ferritin	8–388 ng/mL	947±2,754	502±738	1,701±4,319	14.725	*<0.001*	444
C reactive protein	0–0.4 mg/dL	7.0±6.3	5.8±5.8	9.3±6.5	1.781	*<0.001*	457
Triglycerides	<150 mg/dL	178±205	138±72	234±300	3.120	*<0.001*	330
Interleukin-6	<5 pg/mL	69±126	35±35	96±162	3.799	*<0.001*	75
Lymphocytes (%)	20%–40%	18±10	21±10	11±7	0.217	*<0.001*	509
Monocytes Abs	0–0.8 K/mm^3	0.59±0.28	0.56±0.28	0.63±0.27	1.260	*0.01*	509
Neutrophil Abs	1.8–7.8 K/mm^3	6.00±3.6	4.98±3.1	8.01±3.7	2.602	*<0.001*	509
WBC Abs	4–11 K/mm^3	7.88±4.5	6.83±3.1	9.95±5.8	2.818	*<0.001*	508
ALT	16–61 U/L	45±46	35±25	65±66	2.830	*<0.001*	495
AST	15–37 U/L	53±88	40±76	79±101	2.883	*<0.001*	495
D-dimers	0–500 ng/mL	3,119±10,443	1,017±2,138	6,933±16,648	10.409	*<0.001*	456
LDH	84–246 U/L	323±166	249±87	456±191	10.545	*<0.001*	462
Troponin I	0.045–0.1 ng/mL	0.23±2.29	0.05±0.12	0.55±3.8	438.236	*<0.001*	416
Chloride	101–111 mmol/L	104±5.4	103±4.8	105±6.1	1.627	*<0.001*	509
Potassium	3.5–5.2 mmol/L	4.09±0.5	3.99±0.5	4.29±0.5	1.815	*<0.001*	509
Sodium	136–145 mmol/L	137±4.01	136±3.14	138±5.12	1.607	*<0.001*	509
Creatinine	0.6–1.10 mg/dL	1.89±2.8	1.61±2.5	2.43±3.3	1.317	*0.003*	509
BUN	8–20 mg/dL	27±25	21±19	39±32	2.266	*<0.001*	509
BUN:creatinine ratio	10–20 ratio	18.9±8.4	17.1±6.4	22.6±10.5	2.076	*<0.001*	509

Average and SD of the laboratory parameters collected in the first 7 days of hospitalisation in patients with COVID-19 divided according to the new COVID-CS criteria. ORs and p values were calculated by univariate logistic regression. Normal range of values is shown for our laboratory as reference.
Abs, absolute numbers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; COVID-CS, COVID-19 cytokine storm; LDH, lactate dehydrogenase; WBC, white blood cell.

with the upper normal limits in patients fitting the criteria of COVID-CS. These results suggest a prerenal imbalance and renal damage (table 6 and online supplemental figure S1). Together, these results highlight systemic tissue damage affecting many organs in the COVID-CS.

Second validation of the COVID-CS criteria

Finally, we further validated the novel criteria by applying them to a second cohort of 258 patients, 128 women and 130 men, with a mean age of 59 years. Out of the 258 patients, 39 (15%) were considered in CS by the same clinical consensus used in the first cohort. In the new cohort, the novel criteria correctly classified 69% of the patients, with a specificity of 0.73 (CI 0.69 to 0.78) and a sensitivity of 0.69 (CI 0.58 to 0.81), indicating that the criteria can be successfully applied to new cohorts. Similar to the first cohort, patients who met the criteria (33%) had significantly higher LoS (15.5±10.1 vs 4.7±3.7, p<0.001) and mortality (33.7% vs 4.2%, p<0.0001) (online supplemental table S5).

DISCUSSION

Our analyses highlight the unique features of COVID-CS. We found that laboratory parameters indicative of a strong proinflammatory status, systemic cell death and multiorgan tissue damage, and prerenal electrolyte imbalance are predictive of this hyperimmune condition. We found clear differences with other CSs, such as MAS, from which COVID-CS is distinguished for the uncommon thrombocytopenia and the increased number of neutrophils, suggestive of an active innate immune system. Other distinct differences were the increased levels of fibrinogen and the relatively normal levels of triglycerides in the COVID-CS, which, together with the low levels of albumin, suggest a different type of inflammation.

Despite the limitations of missing three HLH criteria and one for the HScore, namely, the hemophagocytosis, the NK-cell activity and the sIL-2R, we propose that the lack of cytopenias, the normal levels of fibrinogen and the only mildly elevated levels of triglycerides indicate that the COVID-CS is very different from HLH and the HScore is not useful.[12]

It was recently reported that LDH, CRP and low lymphocytes are associated with higher mortality in patients with COVID-19.[18 26] Our results are in agreement with these studies. Indeed, our COVID-CS criteria identify a group of patients with longer LoS and increased mortality. Therefore, our criteria predict not only the development of the storm but also clinical severity. Both CD4+ and CD8+ T cells were initially reported to be decreased in severe cases of COVID-19[27 28] and more recently were shown to recover during disease resolution.[29] T cells are pivotal in the elimination of viral infected cells.[30] Moreover, it has been shown in other CSs that the excess of cytokines can be due to a deficient elimination of cytokine-producing innate immune cells, such as inflammatory monocytes and macrophages, by CD8+ T cells.[31] Therefore, low lymphocytes as criterion for COVID-CS highlight the role of deficient T-cell functions in COVID-CS pathogenesis, allowing innate immunity overactivation and uncontrolled viral infection.[32]

The increased levels of cell death markers such as liver enzymes, LDH, D-dimers and troponin I indicate that COVID-CS is characterised by significant systemic tissue damage that in different patients may target the liver, the cardiovascular system and the kidney, as suggested by recent autopsy results.[13–16] High levels of D-dimers have been reported in several cohorts of patients with COVID-19 and correlate with increased mortality.[33] The elevated levels of LDH, D-dimers and troponin, especially early on, could also indicate pulmonary immunothrombosis and secondary pulmonary arterial hypertension, both implicated in the devastating lung damage that COVID-19 inflicts.[34]

Therefore, anticoagulant therapy has been recommended in those with high levels,[35] and our therapeutic approach has changed as well. Compared with the initial cohort, the validation cohort received higher and earlier doses of steroids, and a larger percentage received anticoagulants. These changes might explain the lower sensitivity of the criteria in the validation cohort; nevertheless, they remain very valuable as there is not yet a standard to aggressively or conservatively treat patients with COVID-CS around the world.

Acute respiratory distress syndrome (ARDS) is undeniably one of the most lethal manifestations of COVID-19 infection.[36] The abnormal laboratory work in our criteria could be explained by ARDS in which both hypoxia and hyperaemia could drive elevation of LDH, liver enzymes and renal dysfunction with albumin levels as predictor of ARDS.[37 38] Nevertheless, a significant number of immunoprofiling results point to a systemic inflammatory response with the lung at the epicentre.[32 38 39]

There are limitations to our work. First, in the absence of an established definition of COVID-CS in the literature, the clinical 'gold standard' was defined by the clinical judgement of CS itself. Second, the vast majority of our patients received steroids as part of the early standard of care at Temple University.[40] Third, our investigation was conducted in a single centre and with a specific racial/ethnicity composition. These limitations might make our cohort somewhat different from other centres. Future validations with other cohorts from multiple centres and countries will resolve these limits.

The high levels of cell death markers shed light in COVID-CS pathogenesis. Both necroptosis and pyroptosis can occur during viral infections and are mediated by proinflammatory cytokines such as interferon-gamma and IL-1-beta and by inflammasome.[32 41] A recent longitudinal immune analysis revealed a subgroup of patients who eventually died of COVID-19 with a cytokine profile indicative of CS, inflammasome involvement and tissue damage.[32] In this group, the levels of IL-6 were extremely elevated.[32] We also found elevated IL-6 levels in all patients and more in COVID-CS, although not as high as reported by others [32 39] possibly because we tested early during hospitalisation. Together with the high levels of CRP, which is induced by IL-6,[42] as COVID-CS criterion, these findings demonstrate the validity of our criteria in capturing the impending storm. Several clinical trials testing cytokine inhibitors are presently ongoing in patients with COVID-19 and may soon provide evidence for a role of cytokine-mediated cell death in patients with COVID-CS.

In summary, we provide new criteria to diagnose the COVID-CS at an early stage, which predict longer hospitalisation and increased mortality, therefore requiring specific treatments. While the criteria need further validation, they represent a first step toward early diagnosis and intervention in this lethal pandemic.

Author affiliations
[1]Medicine/Rheumatology, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA
[2]Department of Psychology, University of Milano-Bicocca, Milan, Italy
[3]Radiology, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA
[4]Microbiology and Immunology, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA
[5]Pediatrics, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA
[6]Medicine/Hematology, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA
[7]Thoracic Medicine and Surgery, Lewis Katz School of Medicine at Temple University, Philadelphia, Pennsylvania, USA

Acknowledgements We thank Dr Randy Cron for critically reading the manuscript and the numerous suggestions.

Collaborators Temple University COVID-19 Research Group: Aaron Mishkin; Abbas Abbas; Abhijit S Pathak; Abhinav Rastogi; Adam Diamond; Aditi Satti; Adria Simon; Ahmed Soliman; Alan Braveman; Albert J Mamary; Aloknath Pandya; Amy Goldberg; Amy Kambo; Andrew Gangemi; Anjali Vaidya; Ann Davison; Anuj Basil; Arthur Lau; Arundathi Jayatilleke; Bakhos, Charles T; Bill Cornwell; Brent Lawrence; Brianna Sanguily; Brittany Corso; Carla Grabianowski; Carly Sedlock; Catherine Myers; Charles Bakhos; Chenna Kesava; Reddy Mandapati; Cherie Erkmen; Chethan Gangireddy; Chih-ru Lin; Christopher T Burks; Claire Raab; Crabbe, Deborah; Crystal Chen; Daniel Edmundowicz; Daniel Sacher; Daniel Salerno; Daniele Simon; David Ambrose; David Ciccolella; Debra Gillman; Dolores Fehrle; Dominic Morano; Donnalynn Bassler; Edmund Cronin; Eduardo Dominguez; Ekam Randhawa; Ekamjeet Randhawa; Eman Hamad; Eneida Male; Erin Narewski; Francis Cordova; Frederic Jaffe; Frederich Kueppers; Fusun Dikengil; Galli, Jonathan; Gangemi, Andrew; Garfield, Jamie; Gayle Jones; Gennaro Calendo; Gerard Criner; Gilbert D'Alonzo; Ginny Marmolejos; Gordon, Matthew; Gregory Millio; Gupta, Rohit; Gustavo Fernandez; Hannah Simborio; Harwood Scott; Heidi Shore-Brown; Hernan Alvarado; Ho-Man Yeung; Ibraheem Yousef; Ifeoma Oriaku; Iris Jung-won Lee; Isaac Whitman; James Brown; Jamie L. Garfield; Janpreet Mokha; Jason Gallagher; Jeffrey Stewart; Jenna Murray; Jessica Tang; Jeyssa Gonzalez; Jichuan Wu; Jiji Thomas; Jim Murrett; Joanna Beros; John M. Travaline; Jolly Varghese; Jordan Senchak; Joseph Lambert; Joseph Ramzy; Joshua Cooper;Jun Song; Junad Chowdhury; Justin Levinson; Kaitlin Kennedy; Karim B Ahmed; Karim Loukmane; Karthik Shenoy; Kathleen Brennan; Keith Johnson; Kevin Carney; Kevin Lu; Kraftin Schreyer; Kristin Criner; Kumaran, Maruti; Lauren Miller; Laurie Jameson; Laurie Johnson; Laurie Kilpatrick; Lawrence Brent; Lii-Yoong Criner; Lily Zhang; Lindsay K Mcgann; Llera A Samuels; Marc Diamond; Margaret Kerper; Maria Vega Sanchez; Mariola Marcinkienwicz; Maritza Pedlar; Mark Aksoy; Mark Weir; Marla R. Wolfson; Marla Wolfson; Marron, Robert; Martin Keane; Massa Zantah; Mathew Zheng; Matthew Delfiner; Matthew Gordon; Maulin Patel; Megan Healy; Melinda Darnell; Melissa Navaro; Meredith A. Brisco-Bacik; Michael Bromberg; Michael Gannon; Michael Jacobs; Mira Mandal; Nanzhou Gou; Narewski, Erin; Nathaniel Marchetti; Nathaniel Xander; Navjot Kaur; Neil Nadpara; Nicole Desai; Nicole Mills; Norihisa Shigemura; Ohoud Rehbini; Oisin O'Corragain; Omar Sheriff; Oneida Arosarena; Osheen Abramian; Paige Stanley; Parag Desai; Parth Rali; Patrick Mulhal; IPravin Patil; Priju Varghese; Puja Dubal; Puja Patel; Rachael Blair; Rajagopalan Rengan; Rami Alashram; Randol Hooper; Rebecca A Armbruster; Regina Sheriden; Robert Marron; Rogers Thomas; Rohit Gupta; Rohit Soans; Roman Petrov; Roman Prosniak; Romulo Fajardo; Ruchi Bhutani; Ryan Townsend; Sabrina Islam; Samantha Pettigrew; Samantha Wallace; Sameep Sehgal; Samuel Krachman; Santosh Dhungana; Sarah Hoang; Sean Duffy; Seema Ran; iShapiro William; Sheila Weaver; Shelu Benny; Sheril George; Shuang Sun; Shubhra Srivastava-Malhotra; Stephanie Brictson; Stephanie Spivack; Stephanie Tittaferrante; Stephanie Yerkes; Stephen Priest; Steve Codella; Steven G Kelsen; Steven Houser; Steven Verga; Sudhir Bolla; Sudhir Kotnala; Sunil Karhadkar; Sylvia Johnson; Tahseen Shariff; Tammy Jacobs; Thomas Hooper; Tom Rogers; Tony S. Reed; Tse-Shuen Ku; Uma Sajjan; Victor Kim; Whitney Cabey; Wissam Chatila; Wuyan Li; Zach Dorey-Stein; Zachariah Dorey-Stein; Zachary D Repanshek.

Contributors RC, MC and SG designed, analysed and interpreted the data and drafted the manuscript. RC, CD, DF and XZ acquired and analysed the data. MB, DF and GJC revised the manuscript critically for important intellectual content. RC, MC, SG, RC, CD, XZ. MB, DF and GJC approved the final version of the manuscript.

Funding This work was supported by the NIH grant R56 AR072115-01 (to RC), Lupus Research Alliance (RC).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study was approved by Temple University local institutional review board (IRB) and informed consent was waived based on retrospective evaluation of deidentified data (IRB protocol number 27120).

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Roberto Caricchio http://orcid.org/0000-0002-1379-1118
Marcello Gallucci http://orcid.org/0000-0003-3546-0093
Stefania Gallucci http://orcid.org/0000-0003-4737-8003

REFERENCES

1 Guan W-J, Ni Z-Y, Hu Y, *et al*. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;382:1708–20.
2 Henderson LA, Canna SW, Schulert GS, *et al*. On the alert for cytokine storm: immunopathology in COVID-19. *Arthritis Rheumatol* 2020;72:1059–63.
3 Petrosillo N, Viceconte G, Ergonul O, *et al*. COVID-19, SARS and MERS: are they closely related? *Clin Microbiol Infect* 2020;26:729–34.
4 La Rosée P, Horne A, Hines M, *et al*. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood* 2019;133:2465–77.
5 Ravelli A, Minoia F, Davì S, *et al*. 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: a European League against Rheumatism/American College of Rheumatology/Paediatric rheumatology international trials organisation collaborative initiative. *Ann Rheum Dis* 2016;75:481–9.
6 Zachariah P, Johnson CL, Halabi KC, *et al*. Epidemiology, clinical features, and disease severity in patients with coronavirus disease 2019 (COVID-19) in a children's hospital in New York City, New York. *JAMA Pediatr* 2020:e202430.
7 Adhikari SP, Meng S, Wu Y-J, *et al*. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. *Infect Dis Poverty* 2020;9:29.
8 Mehta P, McAuley DF, Brown M, *et al*. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 2020;395:1033–4.
9 Ruscitti P, Berardicurti O, Iagnocco A, *et al*. Cytokine storm syndrome in severe COVID-19. *Autoimmun Rev* 2020;19:102562.
10 Alunno A, Carubbi F, Rodríguez-Carrio J. Storm, typhoon, cyclone or Hurricane in patients with COVID-19? beware of the same storm that has a different origin. *RMD Open* 2020;6:e001295.
11 McGonagle D, Sharif K, O'Regan A, *et al*. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* 2020;19:102537.
12 Leverenz DL, Tarrant TK. Is the HScore useful in COVID-19? *Lancet* 2020;395:e83.
13 Liu Q, Wang RS, Qu GQ, *et al*. Gross examination report of a COVID-19 death autopsy. *Fa Yi Xue Za Zhi* 2020;36:21–3.
14 Su H, Yang M, Wan C, *et al*. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int* 2020;98:219–27.
15 Wichmann D, Sperhake J-P, Lütgehetmann M, *et al*. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med* 2020;173:268–77.
16 Xu Z, Shi L, Wang Y, *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;8:420–2.
17 Gupta A, Madhavan MV, Sehgal K, *et al*. Extrapulmonary manifestations of COVID-19. *Nat Med* 2020;26:1017–32.
18 Yan L, Zhang H-T, Goncalves J, *et al*. An interpretable mortality prediction model for COVID-19 patients. *Nat Mach Intell* 2020;2:283–8.
19 Caricchio R, Criner GJ. Rheumatologists and Pulmonologists at temple university weather the COVID-19 storm together. *J Rheumatol* 2020. doi:10.3899/jrheum.200740. [Epub ahead of print: 08 Jun 2020].
20 Wang Z, Yang B, Li Q, *et al*. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis* 2020;71:769–77.
21 Kucirka LM, Lauer SA, Laeyendecker O, *et al*. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. *Ann Intern Med* 2020;173:262–7.
22 Luque G, Alab E. *Parallel genetic algorithms theory and real world application*. Spain: Springer-Verlag Berlin Heidelberg, 2011.
23 Scrucca L. Ga: a package for genetic algorithms in R. *Journal of Statistical Software* 2013;53:37.
24 Fardet L, Galicier L, Lambotte O, *et al*. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheumatol* 2014;66:2613–20.
25 Qin C, Zhou L, Hu Z, *et al*. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020;71:762–8.
26 Liu X, Shi S, Xiao J, *et al*. Prediction of the severity of corona virus disease 2019 and its adverse clinical outcomes. *Jpn J Infect Dis* 2020. doi:10.7883/yoken.JJID.2020.194. [Epub ahead of print: 29 May 2020].
27 Sun Y, Dong Y, Wang L, *et al*. Characteristics and prognostic factors of disease severity in patients with COVID-19: the Beijing experience. *J Autoimmun* 2020;112:102473.
28 Wang F, Nie J, Wang H, *et al*. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis* 2020;221:1762–9.
29 Cavalli G, De Luca G, Campochiaro C, *et al*. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol* 2020;2:e325–31.
30 Vardhana SA, Wolchok JD. The many faces of the anti-COVID immune response. *J Exp Med* 2020;217. doi:10.1084/jem.20200678
31 Grom AA, Horne A, De Benedetti F. Macrophage activation syndrome in the era of biologic therapy. *Nat Rev Rheumatol* 2016;12:259–68.
32 Lucas C, Wong P, Klein J, *et al*. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 2020;584:463–9.
33 Cummings MJ, Baldwin MR, Abrams D, *et al*. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *medRxiv* 2020.
34 Parry AH, Wani AH, Yaseen M, *et al*. Demystifying pulmonary vascular complications in severe coronavirus disease-19 pneumonia (COVID-19) in the light of clinico-radiologic-pathologic correlation. *Thromb Res* 2020. doi:10.1016/j.thromres.2020.06.043. [Epub ahead of print: 27 Jun 2020].
35 Al-Samkari H, Karp Leaf RS, Dzik WH, *et al*. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood* 2020;136:489–500.
36 Hasan SS, Capstick T, Ahmed R, *et al*. Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis. *Expert Rev Respir Med* 2020. doi:10.1080/17476348.2020.1804365. [Epub ahead of print: 31 Jul 2020].
37 Maccora I, Calabri GB, Favilli S, *et al*. Long-Term follow-up of coronary artery lesions in children in Kawasaki syndrome. *Eur J Pediatr* 2020. doi:10.1007/s00431-020-03712-6. [Epub ahead of print: 15 Jun 2020].
38 Hoeboer SH, Oudemans-van Straaten HM, Groeneveld ABJ. Albumin rather than C-reactive protein may be valuable in predicting and monitoring the severity and course of acute respiratory distress syndrome in critically ill patients with or at risk for the syndrome after new onset fever. *BMC Pulm Med* 2015;15:22.
39 Hadjadj J, Yatim N, Barnabei L, *et al*. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* 2020;369:718–24.
40 RECOVERY Collaborative Group, Horby P, Lim WS, *et al*. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. *N Engl J Med* 2020. doi:10.1056/NEJMoa2021436. [Epub ahead of print: 17 Jul 2020].
41 Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. *Nat Rev Immunol* 2017;17:151–64.
42 Nehring SM, Goyal A, Bansal P. *C reactive protein (CRP)*. Treasure Island, FL: StatPearls, 2020.