Although it is now widely accepted that host inflammatory responses contribute to lung injury, the pathways that drive severity and distinguish coronavirus disease 2019 (COVID-19) from other viral lung diseases remain poorly characterized. We analyzed plasma samples from 471 hospitalized patients recruited through the prospective multicenter ISARIC4C study and 39 outpatients with mild disease, enabling extensive characterization of responses across a full spectrum of COVID-19 severity. Progressive elevation of levels of numerous inflammatory cytokines and chemokines [including interleukin-6 (IL-6), CXCL10, and granulocyte-macrophage colony-stimulating factor (GM-CSF)] was associated with severity and accompanied by elevated markers of endothelial injury and thrombosis. Principal component and network analyses demonstrated central roles for IL-6 and GM-CSF in COVID-19 pathogenesis. Comparing these profiles with archived samples from patients with fatal influenza, IL-6 was equally elevated in both conditions, whereas GM-CSF was prominent only in COVID-19. These findings further identify the key inflammatory, thrombotic, and vascular factors that characterize and distinguish severe and fatal COVID-19.

INTRODUCTION

Fatal coronavirus disease 2019 (COVID-19) is associated with acute respiratory distress syndrome (ARDS) and elevated markers of systemic inflammation including interleukin-6 (IL-6) and C-reactive protein (CRP), often accompanied by peripheral blood neutrophilia and lymphopenia (1). However, IL-6 concentrations are typically 10-fold lower than those reported in ARDS and sepsis, and other mediators also have major roles in pathogenesis (2–5). The beneficial effect of corticosteroids (6, 7) and IL-6 receptor antagonists (8) in severe COVID-19 indicates that immune inhibition can be beneficial at advanced stages of disease and that inflammation is a modifiable component of COVID-19 pathogenesis. With many biologic therapies to choose from, it is important to establish which additional pathways and mediators should now be prioritized in clinical trials. Identification of inflammatory mediator profiles reflective of processes that are associated with disease severity may define “treatable traits” (9), allowing both stratification of patients likely to benefit from therapies such as dexamethasone and targeted biological anti-cytokine therapies and design of novel therapeutics targeting causative pathways.

An influx of monocytes/macrophages into the pulmonary parenchyma and a myeloid pulmonary artery vasculitis has been reported in autopsy studies of COVID-19. In addition, critical illness in COVID-19 is associated with genotype-inferred CCR2 expression in the lung and there is strong evidence that myeloid cells contribute to immunopathology (10–14). In addition to macrovascular thrombosis (15), pulmonary microthrombosis is a frequent autopsy finding with additional evidence of endothelial injury and endotheliitis, implicating endothelial activation and coagulation in respiratory failure (10, 11, 13, 16). The virus-induced inflammatory state has laboratory features that resemble secondary hemophagocytic lymphohistiocytosis (sHLH) (17–19), but the exact pattern and severity of inflammatory responses have only been partially characterized. Other host factors also influence COVID-19 severity, with polymorphisms in interferon (IFN) pathway genes IFNAR2 and OAS1/2/3 associating with variations in disease severity (14).

Early clinical studies of COVID-19 showed elevated neutrophil counts and lymphopenia in peripheral blood (1, 20), especially in late-stage disease, although neutrophilia is commonly seen in other severe respiratory viral (21) and bacterial (22) infections. Elevated levels of D-dimer, a product of fibrin degradation associated with thrombosis and inflammation, have also been observed in COVID-19 (20), consistent with systemic inflammation and the high frequency of intravascular thrombotic complications (13, 23). Thromboses and pulmonary microthrombi are common in fatal COVID-19 and are associated with endothelial responses distinct from those that occur during fatal influenza A virus infection (10–12, 24). However, the thrombotic aspects of life-threatening COVID-19 have hitherto...
been described in relatively small groups of cases, from single-center studies, or with a narrow range of disease severities.

The exceptional scale and range of the ISARIC4C study allows us to examine a wide range of responses that reflect the spectrum of COVID-19 disease severity. We also analyzed and compared selected samples from patients with severe influenza from the 2009–2010 A/H1N1 pandemic, enabling comparative analysis and the identification of unique aspects of COVID-19 pathogenesis that may be amenable to therapeutic manipulation.

RESULTS
Routine clinical data did not completely distinguish COVID-19 severities

We obtained clinical data and plasma samples from 471 patients hospitalized with COVID-19 within the ISARIC4C study (25, 26) using a publicly available protocol as a prepositioned pandemic preparedness study (26, 27). Patients were stratified into five clinical groups based on their peak illness severity according to the World Health Organization (WHO) COVID-19 ordinal scale (28) (table S1): (i) no oxygen requirement (severity 3, n = 132), (ii) patients requiring oxygen by face mask or nasal prongs (severity 4, n = 106), (iii) patients requiring high-flow nasal cannulae oxygen or noninvasive ventilation (severity 5, n = 79), (iv) patients requiring invasive mechanical ventilation (severity 6/7, n = 85), and (v) fatal COVID-19 (severity 8, n = 69).

The median duration of symptoms before recruitment and sample collection was 9 days, but differences were evident between severity groups: severity 3, 7 days; severity 4, 10 days; severity 5, 11 days; severity 6/7, 12 days; and severity 8, 11 days. These differences were significant between groups 3 and 5 (P = 0.0003) and groups 3 and 6/7 (P = 0.0030) (fig. S1A), possibly reflecting the recruitment of patients with mild symptoms that were hospitalized for monitoring or isolation early in the pandemic. The median duration between admission and study completion (discharge or death) was 9 days (interquartile range, 6 to 18; range, 1 to 89).

Some differences in routinely performed clinical hematology and biochemistry measures were evident between clinical groups at enrollment: Lymphopenia was present in groups 6/7 and 8 relative to 3, and neutrophilia in groups 6/7 and 8 relative to 3 and 4 (fig. S1, B and C, respectively). No differences between groups were observed in ferritin levels (elevated in most patients), although lactate dehydrogenase (LDH) was elevated in groups 5, 6/7, and 8 relative to 3 and 4 (fig. S1, D and E, respectively). Procalcitonin levels were elevated in group 8 relative to groups 3, 4, and 5 (all P < 0.05; fig. S1F). Partial HSCOREs (29) were calculated [fever, cytopenia, ferritin, triglycerides, and aspartate transaminase (AST)], but the only significant difference between groups was between severities 6/7 and 4, indicating that sHLH-like disease is unlikely to be the predominant pathophysiological mechanism in life-threatening COVID-19 (fig. S1G). The ISARIC4C mortality scores (30) for these patients demonstrated a stepwise increase with each increment in severity and, as expected, were highest in patients who would progress to fatal disease (group 8) relative to all other groups (fig. S1H).

A broad inflammatory response scaled with COVID-19 severity

To determine the relationship between levels of plasma markers and disease severity, we used immunoassays to measure 33 mediators chosen to represent putative mechanistic pathways of disease (20, 23). Data were hierarchically clustered and annotated with the severity, age, disease duration at the time of sampling (“Onset”), and sex of each patient. This analysis identified three clusters of patients with distinct patterns of mediators, which fell into five hierarchical clusters (Fig. 1).

The three clusters of patients had different clinical characteristics (table S2). Cluster A (n = 59) had milder illness (with no deaths and 76.3% of patients from severity group 3), were more commonly female (55.9%), had a lower median age (54.7 years), and had lower rates of diabetes mellitus (15.3%) than other clusters. Cluster A was also associated with lower levels of most mediators (Fig. 1). Patients in cluster C (n = 174) had a substantially greater severity of illness (32.2% fatal; 33.9% mechanical ventilation), higher routine inflammatory markers (neutrophils and CRP) and temperature, and lower lymphocyte counts (median, 0.8 × 10⁹/liter). These patients were also older (median, 64.1 years), predominantly male (74.1%), and more likely to have diabetes mellitus (32.6%). This cluster was associated with the highest levels of many mediators including tumor necrosis factor-α (TNF-α) and IL-6. Patients in cluster B (n = 238) had an intermediate clinical phenotype relative to clusters A and C and a more mixed profile of immune mediators.

Principal component analysis (PCA) was used to determine the main drivers in the variance between individuals, again using only plasma mediator data but annotating the PCA plot with patient disease severity. This analysis demonstrated considerable overlap between disease severity groups but some distinction between milder and more severe COVID-19, largely determined by PC1 (fig. S2A). The top five drivers of this variance (determined by PC1 loading values) were IL-6 (0.256), CXCL10 (0.254), GDF-15 (0.246), GM-CSF (granulocyte-macrophage colony-stimulating factor) (0.241), and CCL2 (0.239) (fig. S2B).

Therefore, at the time of enrollment, different COVID-19 outcome groups were identifiable based on distinct patterns of inflammatory mediators; IL-6, CXCL10, and GM-CSF were key determinants of cluster assignment.

Myeloid and vascular inflammatory markers distinguished hospital- and community-managed COVID-19

To further explore the relationship between mediator levels and severity, we analyzed plasma from 15 healthy controls (HCs; 7 males; median age, 55; range, 45 to 71) and 39 individuals recruited 7 days after a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR) test who did not require hospitalization (15 males; median age, 43; range, 27 to 62; termed group, “1/2” per the WHO scale (31)) and related these to hospitalized patients. Numerous differences were evident between hospitalized COVID-19 patients, outpatients, and HCs, along with many differences across the clinical groups in hospitalized patients (Fig. 2 and fig. S3). In contrast to other reports (32), we found no association between IFN-α levels and disease severity (Fig. 2A) when analyzed in a random subset of patient samples. IFN-γ was elevated in hospitalized COVID-19 patients relative to HC and group 1/2 (Fig. 2B) and was elevated in the most severe outcome groups relative to lower severity grades. The IFN-induced chemokine CXCL10 was also substantially elevated in all hospitalized COVID-19 cases relative to the control groups, with the most pronounced increases in groups 6/7 and 8 (Fig. 2C). These results contrasted with decreased IFN-stimulated gene expression in peripheral blood samples from patients with severe COVID-19 (32). These differences...
led us to speculate that the abundance of IFN-γ and CXCL10 resulted from release at the site of disease rather than from circulating cells, although anti-IFN autoantibodies (33) and polymorphisms in IFN signaling (14) may have influenced this pathway.

The fibrin degradation product D-dimer is elevated in severe COVID-19 (20), implicating thrombosis in disease severity, consistent with autopsy findings (10, 11, 24). In agreement with these reports, D-dimer was elevated in all hospitalized groups, with stepwise increases between severity groups (Fig. 2D). Given reports of the association between COVID-19 mortality and pulmonary vasculitis (10), we hypothesized that endothelial injury may be a feature of COVID-19, potentially triggering coagulation and the thrombotic complications common in severe disease (23, 34). Levels of angiopoietin-2, a marker of endothelial injury, were elevated in all hospitalized patients relative to both control groups (Fig. 2E), with levels 5.6-fold higher in the mildest hospitalized patients (severity 3, median = 1983 pg/ml) than HCs (median = 352 pg/ml). Angiopoietin-2 levels were also significantly elevated in groups 6/7 and 8 relative to all other hospitalized COVID-19 outcome groups (Fig. 2E). As both angiopoietin-2 and von Willebrand factor (vWF)–A2 can enter the blood plasma through exocytosis of endothelial cell Weibel-Palade bodies (35), we also quantified vWF-A2 and endothelin-1, which were similarly elevated in patients with severe COVID-19 (Fig. 2F and fig. S3, respectively). Elevations in these prothrombotic mediators were not counteracted by the inhibitors angiopoietin-1 or soluble Tie2, which were equivalent between groups (fig. S3). These results suggest that endothelial injury and coagulation are common features of patients hospitalized with COVID-19 and that these are most pronounced in severe and fatal COVID-19.

In line with other reports (1, 3), we found that IL-6 was significantly elevated in most hospitalized groups relative to controls (Fig. 2G), with a stepwise increase in levels with escalating severity. IL-6 levels in groups 6/7 and 8 were significantly elevated above all other groups (all P < 0.0001; Fig. 2G). GM-CSF was similarly elevated in all hospitalized groups relative to controls and was most pronounced in groups 6/7 and 8 (Fig. 2H). Numerous other inflammatory mediators were also significantly elevated in COVID-19 patients relative to controls, including IL-2, TNF-α, IL-8, IL-10, IL-18, G-CSF, IL-4, IL-17, GM-CSF, and IL-13. These findings are consistent with the broad exaggerated immune response observed in COVID-19 patients, as demonstrated by the clustered heatmap in Fig. 1.
cytokines and chemokines showed similar results including TNF-α, IL-2, GDF-15, G-CSF, and VEGF-D (vascular endothelial growth factor–D) (fig. S3). EN-RAGE/S100A12 has previously been characterized as a biomarker of inflammation in ARDS (36) and was elevated in groups 6/7 and 8 relative to most others (Fig. 2I). The neutrophil chemokine IL-8 (CXCL8) was similarly elevated in severe disease, as was the neutrophil gelatinase–associated lipocalin (LCN-2/NGAL) (fig. S3), in line with the reported association between blood neutrophilia and severity (20) also seen in this cohort (fig. S1C).

In agreement with the PCA, CCL2 and GDF-15 were increased in groups 6/7 and 8, whereas other immunological mediators (IL-6Rα, IL-13, and IL-17) were not significantly different between groups (fig. S3), indicating that only limited aspects of the immune repertoire were active in COVID-19. IL-4 levels were lower in the moderate/nonsevere disease outcome groups (3, 4, and 5) relative to both control groups and severe disease groups (fig. S3), indicating that suppression of normal type II cytokines levels may be associated with milder COVID-19, and that this is lost in severe disease. These data partially recapitulate the association of type 2 mediators

**Fig. 2. Antiviral, coagulation, and inflammation-associated mediators distinguished severity groups early in disease.** Plasma samples from the time of study enrollment were analyzed for levels of the antiviral cytokines (A) IFN-α, (B) IFN-γ, and (C) the IFN-induced chemokine CXCL10 in healthy control (HC, n = 15), patients with COVID-19 not requiring hospitalization (“1/2”; n = 39), and hospitalized patients with COVID-19 that would not require oxygen support (“3”; IFN-α, n = 32; other mediators, n = 132), require an oxygen face mask (“4”; IFN-α, n = 23; other mediators, n = 106), require noninvasive ventilation or high-flow nasal cannulae (“5”; IFN-α, n = 19; other mediators, n = 79), require invasive mechanical ventilation (“6/7”; IFN-α, n = 19; other mediators, n = 85), or progress to fatal disease (“8”; IFN-α, n = 14; other mediators, n = 69). Mediators associated with coagulation and endothelial injury were also quantified in these plasma samples: (D) D-dimer, (E) angiopoietin-2, and (F) vWF-A2. Similarly, mediators associated with inflammation were quantified: (G) IL-6, (H) GM-CSF, and (I) EN-RAGE/S100A12. Violin plots display medians (solid lines) and interquartile ranges (dashed lines). Data were analyzed for statistical significance using Kruskal-Wallis tests with Dunn’s tests for multiple comparisons between all groups. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
with COVID-19 severity (37), although levels are typically low and require careful interpretation relative to control samples. Similarly, IL-12p70, commonly released by antigen-presenting cells (APCs) (38), was decreased in all hospitalized cases relative to the HCs and group 1/2 (fig. S3). These results demonstrated that numerous mediators distinguished COVID-19 disease severity groups, yet many mediators remained equivalent between COVID-19 patients and controls, indicating that a broad, yet specific, inflammatory response contributes to immunopathogenesis.

The inflammatory response in COVID-19 was coordinated around IL-6 and GM-CSF

PCA demonstrated that some specific inflammatory mediators (e.g., IL-6, CXCL10, GDF-15, GM-CSF, and CCL2) were the strongest determinants of the variance apparent between COVID-19 patients (fig. S2). To determine the strength of the relationships between these plasma mediators, we performed a hierarchical correlation matrix analysis of mediator data. This identified a strongly correlated group of inflammatory mediators (including GM-CSF, CXCL10, D-dimer, vWF-A2, and IL-6; Fig. 3A), increases of which were associated with the most severe COVID-19 outcome groups (Fig. 2 and fig. S3). The overlap between these mediators and those identified by PCA indicated a coordinated myeloid and vascular inflammatory response associated with disease severity.

Using network analyses structured on the correlation values between mediator pairs, several inflammatory mediators (including GM-CSF, IL-2, and IL-6) grouped together and closely correlated with a wider group of inflammatory mediators (Fig. 3B and interactive 3D visualization: https://isaric4c.net/networks/). The antiviral mediators IFN-\(\gamma\) and CXCL10 were associated with this inflammatory mediator group, and there were close associations between many inflammatory mediators and markers of vascular and thrombotic responses (particularly D-dimer and vWF-A2). Network analyses therefore indicated a close association between the inflammatory and thrombotic elements of the immune response during COVID-19.

Age, but not sex, influenced plasma mediator levels

Given the strong association between age and COVID-19 severity (26) and reports of increased inflammatory responses in males with COVID-19 (39), we investigated the influence of age and sex on plasma mediator levels. As the major effect in our cluster analysis was severity (Fig. 1), we further stratified each of these severity groups by age (\(\geq 70\) or \(< 70\) years of age) and sex.

After adjustment for multiple testing, no mediator was found to be statistically different between males and females within each severity group (Fig. S4). By contrast, several differences were evident between those aged \(\geq 70\) and \(< 70\) years within individual severity groups, with elevated levels of CXCL10, D-dimer, GM-CSF, IL-1\(\alpha\), IL-6, IL-8, LCN-2, and TNF-\(\alpha\) in those aged \(\geq 70\) years (Fig. 3C and fig. S4); by contrast, IFN-\(\gamma\) in severity group 4 was the only mediator significantly elevated in younger patients (fig. S4).

Early inflammatory mediator elevations in severe COVID-19

We next sought to identify changes in the levels of plasma mediators over the course of disease by relating mediator levels to the patient reported duration of symptoms at the time of sampling. For this exploratory analysis, patients were grouped into “Moderate” (severity 3, 4, and 5; \(n = 317\)) and “Severe” (severities 6/7 and 8; \(n = 154\)) outcome categories. Many mediators apparently remained largely stable over time, including IFN-\(\gamma\), angiopoietin-2, and GM-CSF (Fig. 4, A to C, respectively). By contrast, there was a slight decrease over time of CXCL10 levels (Fig. 4D), an increase over time in D-dimer (Fig. 4E), and an increase over time in S100A12/EN-RAGE in the Severe but not the Moderate category (Fig. 4F).

Most other tested mediators were largely stable over time (fig. S5).

Given the stability over time of those mediators most closely associated with disease severity (including IL-6 and GM-CSF), we hypothesized that differences in plasma mediator levels between patients with severe and moderate COVID-19 would be apparent early in the course of disease. Within the first 4 days of symptoms, several mediators were significantly elevated in the severe group, relative to those with moderate disease, including IL-2, IL-6, and GM-CSF (\(P < 0.0001\), \(P < 0.0001\), and \(P < 0.006\); Fig. 4, G to I, respectively), indicating a pronounced inflammatory response early in severe disease. Similarly, many markers of coagulation and endothelial injury were elevated in severe disease, relative to moderate, including D-dimer and vWF-A2 (\(P < 0.001\); Fig. 4, J and K, respectively), in addition to angiopoietin-2 and IL-1\(\alpha\) [which can be activated by thrombin (40)] (fig. S6). By comparison, the lung damage–associated marker EN-RAGE (36) was not significantly different between the severe and moderate groups in the first 4 days of symptoms (\(P = 0.098\); fig. S6), although time course data indicated that this mediator may be elevated in the later stages of severe disease (Fig. 4F).

GM-CSF and IL-1\(\alpha\) distinguished fatal COVID-19 from fatal influenza

To compare the inflammatory response seen during fatal COVID-19 and influenza, plasma samples from fatal pH1N1 influenza infections (\(n = 20\); table S3), collected during the 2009–2011 pandemic, were analyzed on our immunoassay panels. Z scores were determined between fatal COVID-19, fatal influenza, and HCs, with the mean \(z\) score of each group for each mediator ordered according to the hierarchical clustering (see in Fig. 1). This comparison demonstrated that many of the mediators elevated in fatal COVID-19 were also greatly raised in fatal influenza (Fig. 5A and fig. S7), including IL-1\(\beta\), thrombomodulin, and vWF-A2 (Fig. 5B). By contrast IL-6 levels were raised in both groups of patients as shown in other studies (5, 41). However, we found that IL-1\(\alpha\) and GM-CSF were significantly elevated in fatal COVID-19 but not in fatal influenza (Fig. 5B), GM-CSF especially distinguishing COVID-19 patients from cases of influenza (fig. S2B).

Given these findings, we sought to determine whether demographic differences between patients with fatal COVID-19 (\(n = 69\); table S1) and influenza (\(n = 20\); table S3) could account for differences in GM-CSF levels using multiple linear regression. This showed that age and sex were not associated with high GM-CSF levels, which were strongly associated with COVID-19 (\(P < 0.0001\); table S4). However, chronic cardiac disease (\(P = 0.0397\)) was independently associated with lower GM-CSF levels, likely reflecting the contribution of this risk factor to COVID-19 severity (26, 30) aside from the influence of the inflammatory response. In addition, diabetes mellitus was independently associated with elevated GM-CSF levels (\(P = 0.0220\) (table S4), in agreement with a previous report (42).

To ensure that these differences in GM-CSF levels were not storage artifacts, we reanalyzed historic data on GM-CSF levels quantified in matched serum samples from these patients, relative to a contemporaneous HC cohort (\(n = 36\), median age = 30.5, 56% male),
that we previously made publicly available (21). This confirmed that GM-CSF was not significantly elevated in fatal influenza, although a trend was apparent (\(P = 0.063\); fig. S8). This difference represented a median 1.4-fold increase in GM-CSF relative to HCs (medians: HC, 1.06 pg/ml; influenza, 1.46 pg/ml), whereas analysis of plasma samples demonstrated equal medians between HCs and fatal influenza (both 7.92 pg/ml) but a 9.7-fold elevation in fatal COVID-19 relative to HCs (medians: HC, 7.92 pg/ml; COVID-19, 76.86 pg/ml).

Together, these data support a prominent role for GM-CSF in immunopathology during COVID-19 but not in influenza.

**DISCUSSION**

We demonstrated that severe COVID-19 was associated with elevated levels of numerous plasma mediators indicative of coagulation, endothelial activation, and a broad inflammatory response including CXCL10, GM-CSF, and IL-6. Among these, GM-CSF and IL-1\(\alpha\) stood out as being characteristic of COVID-19 and were not found in samples from fatal influenza. Raised levels of many mediators, including GM-CSF, were apparent within the first days of symptoms, potentially indicating a pathologic role for pathways associated with these mediators early in disease.

Although markers of fibrinolysis have previously been associated with disease severity (20) and thrombosis is common in severe and fatal COVID-19 (10, 11, 24), the causes of this feature of severe disease are not known. The elevation of angiopoietin-2, thrombomodulin, endothelin-1, and vWF-A2 in fatal COVID-19 cases provides evidence for the involvement of endothelial injury in COVID-19. Endothelial injury after inflammatory damage, including the increasingly recognized pulmonary artery vasculitis (10, 24), may result in the initiation of a procoagulant process involving these cells (43). Alternatively, this endothelial injury could be triggered by direct viral infection of vascular cells [although this possibility is uncertain (43, 44), viral replication in nonrespiratory tissues is commonly observed at postmortem (10, 13)] or thrombin-mediated activation of IL-1\(\alpha\) (40). This procoagulant role could lead to the deposition of microthrombi, evident in COVID-19 (10), activation of the clotting cascade, and ultimately elevated D-dimer levels through the degradation of fibrin-rich thrombi (34).

Neutrophilic inflammation may also contribute to endothelial injury, although neutrophilia is predominantly a feature of the later phases of COVID-19 (1), whereas endothelial injury was evident in the first days of symptoms. However, continued thrombotic events in late-stage fatal COVID-19 may result from neutrophil-mediated
coagulation as observed in other settings (45–47) and recently demonstrated in COVID-19 (48). Combined, these results indicate a multiplicity of possible procoagulant triggers that may contribute to pathology at different stages of disease.

We found that the antiviral immune mediator and leukocyte recruitment factor CXCL10 and the myeloid cell growth factor GM-CSF were strikingly elevated in fatal cases of COVID-19. This is supported by the potential utility of CXCL10 as an early prognostic

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...inhibition of IL-6 may be expected to be equally as effective in influenza as in COVID-19 (5, 47). The benefits of IL-6 inhibition in COVID-19 are not seen in some trials (54), but the REMAP-CAP study of critically ill patients with COVID-19 suggests that IL-6 receptor inhibition has a place in those with the most severe forms of COVID-19 (8). Similarly, the RECOVERY consortium recently demonstrated that biologic IL-6 inhibitors decrease mortality and requirement for invasive ventilation in COVID-19 patients already treated with corticosteroids (55).

Our findings support therapeutic targeting of GM-CSF, as previously suggested on theoretical grounds (56). Small-scale studies of anti–GM-CSF have shown promising results (57, 58) but require formal testing in large clinical trials. One such study, Otilimbab in Severe COVID-19 Related Disease (OSCAR; NCT04376684), has recently indicated efficacy in patients >70 years of age (59). Given the role of GM-CSF in myelopoiesis and enhancement of neutrophil survival, alongside the neutrophil activation and dysfunctional myeloid cell populations observed in severe COVID-19 (60, 61), these trials may inform our understanding of the importance of this pathway in COVID-19 immunopathogenesis (56).

Although early studies demonstrated elevated GM-CSF levels in both intensive care unit (ICU)– and non-ICU–treated COVID-19 patients (1), we now demonstrate a positive association with disease severity and outcome, in agreement with reports of elevated frequencies of GM-CSF+ T helper 1 (T(h)1) cells in patients with COVID-19 requiring ICU treatment (62). In addition, a population of IL-17A and GM-CSF expressing clonally expanded tissue-resident memory T cells have been identified in the lungs of patients with COVID-19 (63). These studies indicate that pathogenic T cell populations may contribute to the GM-CSF production in patients with severe COVID-19.

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**Fig. 5. GM-CSF and IL-1α were elevated in fatal COVID-19 relative to influenza.** (A) Median z scores for each mediator between HCs (HC, n = 15) and patients with fatal influenza (n = 20) or fatal COVID-19 (severity 8, n = 69). (B) Levels of IL-6, GM-CSF, IL-1α, IL-1β, thrombomodulin, and vWF-A2 in plasma samples from patients with fatal influenza or COVID-19. Data were analyzed for statistical significance using Mann-Whitney tests with between groups. ***P < 0.001; ****P < 0.0001.
One limitation of our study is the lack of a contemporaneous non–COVID-19 ARDS disease control group. This is particularly important for GM-CSF, where analysis of historical plasma samples indicates that elevated levels of GM-CSF appear relatively specific to severe COVID-19. We considered the possibility that prolonged storage may have resulted in the degradation of mediators, although many other cytokines were elevated in these samples relative to COVID-19 (including IL-1β and vWF-A2). However, serum cytokine measurements made at the time of sample collection suggest that elevated GM-CSF is not prominent in cases of severe influenza. A contemporaneous fatal ARDS disease control group would enable direct comparison between infections, but there are currently very few cases of severe influenza due to the non-pharmaceutical measures taken to control COVID-19 (64).

The multicenter nature of ISARIC4C adds to the ability to interpret and apply these results to other settings. Further studies are needed to determine the prognostic value of the plasma biomarkers that we identify, alongside markers identified using other methods to enable multivariable analyses of biological data alongside clinical and demographic data. This form of analysis may also enable the phenotyping of patients most likely to respond to individual therapies. The clear early distinction between mediators in patients who progress to severe COVID-19 and those who do not indicates that early therapeutic intervention may be crucial to effective disease modification. We hope that the patterns of responses that we describe will enable rational and novel prognostic and therapeutic approaches to be adopted for controlling COVID-19.

MATERIALS AND METHODS

Study design

The ISARIC WHO Clinical Characterization Protocol for Severe Emerging Infections in the UK (CCP-UK) is an ongoing prospective cohort study of hospitalized patients with COVID-19, which is recruiting in 258 hospitals in England, Scotland, and Wales (National Institute for Health Research Clinical Research Network Central Portfolio Management System ID: 14152) (64). The ISARIC4C study aims to comprehensively characterize COVID-19 at the clinical and biological level with the ambition of developing interventions that decrease the morbidity and mortality of COVID-19. Studies so far have defined the clinical risk factors for disease severity and progression (26, 30) and the contribution of host genetics to disease severity (14). Future studies seek to define the contribution of viral variants, environmental factors, and the host immune response to disease severity. The protocol, revision history, case report form, patient information leaflet, consent forms, and details of the Independent Data and Material Access Committee are available online (27). This was a prepositioned pandemic preparedness study with urgent public health research status (65).

Participants

Hospitalized patients with PCR-proven (n = 422, 90%) or high likelihood of SARS-CoV-2 infection (PCR-negative, n = 12, 3%; no PCR data recorded, n = 37, 8%) were recruited, including both patients with community- and hospital-acquired COVID-19. This study analyzed EDTA plasma from blood samples obtained on the day of enrollment to the study following a protocol harmonized with international investigators to allow meaningful comparison of results between studies (25).

Study registration and approvals

The ISARIC WHO CCP-UK study was registered at https://www.isrctn.com/ISRCTN66726260 and designated an Urgent Public Health Research Study by the National Institute for Health Research UK. Ethical approval for the ISARIC WHO CCP-UK and this work was given by the South Central–Oxford C Research Ethics Committee (REC) in England (reference 13/SC/0149), the Scotland A REC (reference 20/SS/0028), and the WHO Ethics Review Committee (RPC571 and RPC572, 25 April 2013). HCs were recruited before December 2019 under approval from the London–Fulham REC (reference 14/LO/1023) or from healthy donors following informed consent from a subcollection of the Imperial College Healthcare NHS Trust National Institute for Health Research Imperial Biomedical Research Centre Tissue Bank. Use of the subcollection was approved by the Tissue Bank Ethics Committee (approval R12023). Samples from community-managed COVID-19 cases were collected through a subproject of Imperial College London Communicable Disease Research Tissue Bank, under approval from the South Central Oxford REC (reference 15/SC/0089). Patients with influenza were recruited between 2009 and 2011, following study approval by the NHS National Research Ethics Service, Outer West London REC (09/H0709/52 and 09/MRE00/67), with contemporaneous HCs recruited following study approval by the Central London 3 REC (09/H0716/41), as previously reported (21).

Clinical data collection

A prespecified case report form was used to collect data on patient characteristics, treatments received in hospital, and outcomes. A modified Charlson comorbidity index was used to define comorbidities, and obesity was clinician-defined. COVID-19 severity was assessed according to the WHO COVID-19 ordinal scale for clinical improvement (28). Data were available to report a patient’s maximum illness severity using this scale. To calculate partial HScores (29), ferritin, triglyceride, and AST measurements from this study were combined with recorded results from case report forms for temperature and routine hemoglobin, white cell counts, and platelet counts.

Immunoassays

IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-6, CXCL8/IL-8, IL-10, IL-12p70, and IL-13 were quantified using MSD (MesoScale Diagnostics, Rockville, MD, USA) V-Plex proinflammatory plates on a SQ120 QuickPlex instrument. IL-1α, IL-1ra, IL-6Ra, angiopoietin-1, angiopoietin-2, coagulation factor XIV, endothelin-1, VEGF-D, D-dimer, thrombomodulin, tissue factor, Tie2, vWF-A2, GDF-15, G-CSF, GM-CSF, S100A12/EN-RAGE, IL-17A, IL-18, LCN-2/NGAL, CXCL10/IP-10, CCL2, CCL3, CCL4, and CCL5 were quantified using a Bio-Plex 200 instrument (Bio-Rad, Hercules, CA, USA) with custom Lumienex panel kits from Bio-Techne (Minneapolis, MN, USA) and MilliporeSigma (Burlington, MA, USA). IFN-α was quantified in a randomly selected subset of samples as an exploratory analysis using Quanterix (Billerica, MA, USA) IFN-α assay kits on the SIMOA platform. All values at or below the lower limit of detection (LLOD) were replaced with the geometric mean of the LLODs across plates for each assay. Quantification of GM-CSF in serum samples from influenza cases and controls was performed using MSD.

Statistical analyses

Statistical analyses used GraphPad Prism v8.3.0 (GraphPad, La Jolla, CA, USA) R version 3.6.1 and Python 3.7.3 with Pandas 1.0.3 and
REFERENCES AND NOTES


Inflammatory profiles across the spectrum of disease reveal a distinct role for GM-CSF in severe COVID-19


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GM-CSF is a targetable marker of COVID-19 severity

Critical respiratory failure in COVID-19 is an inflammatory disease—several proinflammatory factors correlate with disease severity in COVID-19, and anti-inflammatory treatments such as corticosteroids and anti-IL-6 monoclonal antibodies can reduce mortality. There is a need to identify additional "targetable" proinflammatory factors that correlate with disease severity. Here, Thwaites et al. analyzed the plasma of 471 hospitalized COVID-19 patients, finding that IL-6 and GM-CSF were substantially elevated in those with severe disease. GM-CSF was elevated in cases of fatal COVID-19, but not in fatal influenza, whereas IL-6 was elevated in both. Thus, elevated GM-CSF distinguishes severe COVID-19 from severe influenza, providing insights into disease pathogenesis and a theoretical rationale for therapeutic inhibition of GM-CSF in COVID-19. Inhibitor studies are now ongoing in COVID-19.