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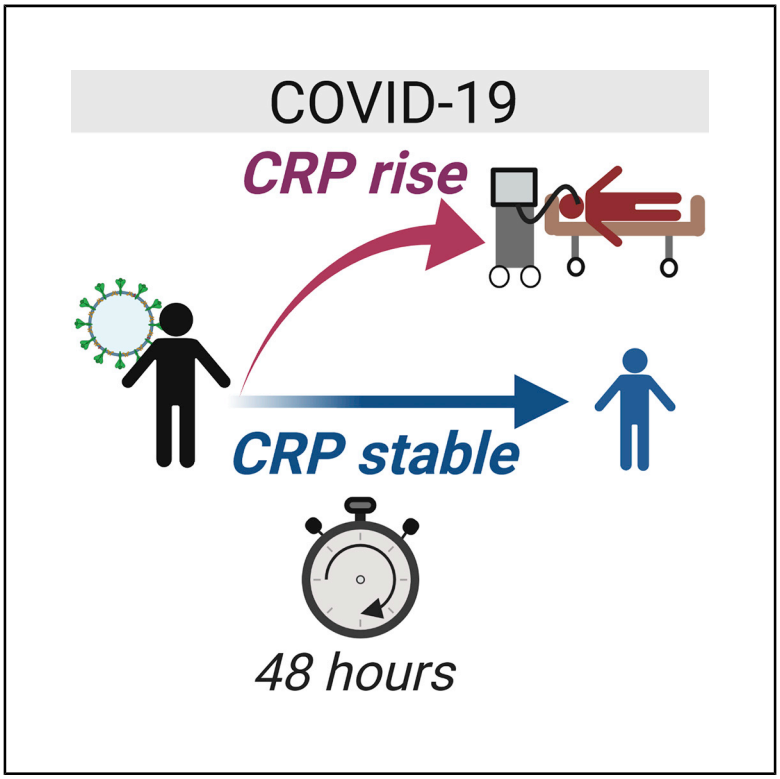
Inflammatory Biomarker Trends Predict Respiratory Decline in COVID-19 Patients

Graphical Abstract



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In Brief

Mueller et al. demonstrate in hospitalized COVID-19 patients that trending C-reactive protein (CRP), an inflammatory biomarker, is a simple and accessible strategy for predicting respiratory deterioration. An early rise in CRP predicts intubation, and CRP levels correlate with IL-6 levels and physiological measures of hypoxemic respiratory failure.

Highlights

- Rising CRP levels predict intubation in COVID-19 inpatients stable at admission
- Early CRP trend outperforms initial CRP level in prediction of respiratory failure
- CRP trend outperforms a physiological index (ROX) in prediction of respiratory failure
- CRP and IL-6 levels correlate with each other and with hypoxemia (P_aO_2/F_iO_2)

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Report

Inflammatory Biomarker Trends Predict Respiratory Decline in COVID-19 Patients

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SUMMARY

In this single-center, retrospective cohort analysis of hospitalized coronavirus disease 2019 (COVID-19) patients, we investigate whether inflammatory biomarker levels predict respiratory decline in patients who initially present with stable disease. Examination of C-reactive protein (CRP) trends reveals that a rapid rise in CRP levels precedes respiratory deterioration and intubation, although CRP levels plateau in patients who remain stable. Increasing CRP during the first 48 h of hospitalization is a better predictor (with higher sensitivity) of respiratory decline than initial CRP levels or ROX indices (a physiological score of respiratory function). CRP, the proinflammatory cytokine interleukin-6 (IL-6), and physiological measures of hypoxemic respiratory failure are correlated, which suggests a mechanistic link. Our work shows that rising CRP predicts subsequent respiratory deterioration in COVID-19 and may suggest mechanistic insight and a potential role for targeted immunomodulation in a subset of patients early during hospitalization.

INTRODUCTION

As of October 2020, coronavirus disease 2019 (COVID-19) has caused more than 1,100,000 global deaths and over 9,500 deaths in Massachusetts (Johns Hopkins University & Medicine. Coronavirus Resource Center: https://coronavirus.jhu.edu. Accessed October 16, 2020). Retrospective studies have associated inflammatory cytokines and biomarkers, including C-reactive protein (CRP), D-dimer, ferritin, and procalcitonin, with severe disease and mortality.[1–9] These studies group patients into the binary categories of non-critical and critical illness. However, among patients who are non-critical and have mild oxygen requirements at hospital admission, it is not known what features distinguish patients who will remain stable from those who will progress to severe respiratory failure requiring intubation, mechanical ventilation, and transfer to the intensive care unit (ICU). Identification of patients who will deteriorate and progress to critical illness could guide risk stratification, the need for close clinical monitoring, and early immunomodulatory intervention.

To address this question, we performed a retrospective cohort study of the first 100 patients admitted to the Brigham and Women's Hospital (BWH) for COVID-19 infection. We hypothesized that inflammatory biomarker profiles would stratify patients into three cohorts: (1) stable and non-intubated throughout their hospital admission ("mild"); (2) initially stable and non-intubated but then had respiratory deterioration requiring intubation or high-flow nasal cannula later in their hospital course ("progressive"); and (3) unstable and required intubation within 12 h of admission ("severe"). Among patients who were stable and did not require intubation at admission, elevated CRP values at admission were associated with progressive respiratory failure later during their hospital course. CRP level at admission correlated with physiological measures of disease severity (sequential organ failure assessment [SOFA] score and PaO_2/FiO_2) and with the inflammatory cytokine interleukin-6 (IL-6). However, the significant overlap in admission CRP values between mild and progressive patient sub-cohorts would limit the practical utility of initial CRP values for clinical care decisions. Remarkably, we found that a rise in CRP values over the first 48–72 h of hospital admission distinguished patients who would develop progressive respiratory failure from patients who would remain stable throughout their hospital course. First, we show that the CRP trend is a clinically predictive tool and can be

Table 1. Demographics and Clinical Features of COVID-19 Patients Classified as "Mild," "Progressive," and "Severe"

Clinical Variables	Mild (n = 54)	Progressive (n = 29)	Severe (n = 17)	p Value
Age, mean (SD), y	59 (16)	67 (13)	68 (14)	0.02
Male, No. (%)	23 (43)	18 (62)	10 (59)	0.19
Race or Ethnicity, No. (%)				
Hispanic or Latino	16 (30)	11 (38)	1 (6)	0.05
Black or African American	13 (24)	5 (17)	2 (12)	0.59
White	17 (31)	6 (21)	5 (29)	0.63
Asian	4 (7)	0	2 (12)	0.20
Cape Verdean	1 (2)	0	1 (6)	0.39
Unknown	3 (6)	7 (24)	6 (35)	0.003
Past Medical History, No. (%)				
Chronic lung disease or asthma	13 (24)	7 (24)	3 (18)	0.90
Severe cardiac disease	13 (24)	2 (7)	4 (24)	0.12
Diabetes	14 (26)	12 (41)	5 (29)	0.45
Cancer	15 (28)	7 (24)	2 (12)	0.43
Chronic kidney disease	7 (13)	6 (21)	5 (29)	0.24
Smoking, No. (%)[a]	25 (46)	11 (38)	3 (18)	0.09
Current immunosuppressive use or chemotherapy, No. (%)[b]	11 (20)	9 (31)	0	0.03
Symptom onset to admission, median (IQR), d	7 (5–8)	5 (3–7)	7 (4–10)	0.15
Admission to intubation, median (IQR), h		45 (29–84)	1 (0–4)	<0.001
P/F ratio on admission, median (IQR)	362 (288–455)	283 (201–410)	188 (108–246)	<0.001
Oxygen Requirement upon Admission, No. (%)				
Room air	32 (59)	7 (24)	0	<0.001
Nasal cannula or mask	22 (41)	22 (76)	4 (24)	<0.001
Intubation	0	0	13 (76)	<0.001
Vasopressors use, No. (%)	0	28 (97)	17 (100)	<0.001
Treatment Received, No. (%)				
Hydroxychloroquine	8 (15)	18 (62)	9 (53)	<0.001
Remdesivir trial[c]	29 (54)	12 (41)	9 (53)	0.58
Tocilizumab	1 (2)	8 (28)	6 (35)	<0.001
Length of stay, median (IQR), d	6 (4–9)	19 (10–31)	20 (12–25)	<0.001
Mortality, No. (%)	2 (4)	12 (41)	10 (59)	<0.001

SOFA, sequential organ failure assessment.
[a]Current or prior history of smoking.
[b]Use of immunosuppressives or immunomodulators of any dosing.
[c]Patients were either given remdesivir or placebo as part of a clinical trial.

superior to a physiological index, such as the ROX index. Second, because CRP is downstream to several immune pathways, including IL-6, our results suggest that these pathways are dynamic early in hospital admission and precede respiratory deterioration. Our work suggests that close, serial monitoring of early CRP values may aid clinical prognostication and consideration of immunomodulatory therapy in COVID-19 patients.

RESULTS

Demographic and Clinical Features

We reviewed the first 111 consecutive cases admitted to BWH who tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the course of their hospitalization (Figure S1). 11 patients (10%) were excluded for the following reasons: SARS-CoV-2 testing was later deemed as false positive (n = 3); medical issues unrelated to COVID-19 drove hospital admission with SARS-CoV-2 positivity as a later incidental finding (n = 3); and the patient was an inpatient for more than 48 h before transfer to BWH (n = 5). Thereafter, 100 patients remained for evaluation. Prior studies typically stratify COVID-19 patients into either "non-critical" or "critical" categories and do not distinguish patients who were critically ill at admission versus those patients who initially had non-critical illness and then deteriorated. Given the clinical importance of predicting which non-critically patients would remain stable and which non-critically patients would later deteriorate, we divided our cohort into three categories. (1) Mild patients were stable patients who remained on room air or supplemental oxygen (via low-flow nasal cannula or face mask) throughout their hospital course and never required intubation or high-flow nasal cannula (HFNC). (2) Progressive patients were initially stable on room air, low-flow nasal cannula, or face mask but then deteriorated and required intubation and mechanical ventilation or HFNC. (3) Severe patients required intubation or HFNC within 12 h of admission.

These hospitalized COVID-19 patients were evaluated and classified as mild (54; 54%), progressive (29; 29%), or severe (17; 17%; Figure S1; Table 1). Patients with progressive and severe disease were older than patients with mild disease (68 ± 14 [severe] versus 67 ± 13 [progressive] versus 59 ± 16 [mild] years; p = 0.02; Table 1). Notably, other demographic characteristics, co-morbidities, or social history did not show significant differences between mild, progressive, and severe cases (Table 1).

Patients in all groups presented to the hospital approximately 1 week after symptom onset (Table 1). Those with mild cases spent 6 (interquartile range [IQR]: 4–9) days in the hospital, although progressive and severe patients were discharged or deceased within 19 (10–31) and 20 (12–25) days of admission, respectively (Table 1). At the time, the hospital's institutional guidelines (Brigham and Women's Hospital COVID-19 Clinical Guidelines: https://www.covidprotocols.org) advised against the use of HFNC in SARS-CoV-2-positive patients, so only 1 patient was on HFNC and 45 were intubated and on mechanical ventilation at some point during their hospitalization. Among the 46 patients who were classified as progressive or severe, 45 (98%) required vasopressors (Table 1). Treatment strategies for patients included administration of hydroxychloroquine, remdesivir versus placebo as part of a clinical trial, or tocilizumab (Table 1). All cases were followed until the hospital discharge. Mortality rate among 100 patients was 24 (24%) overall, with a

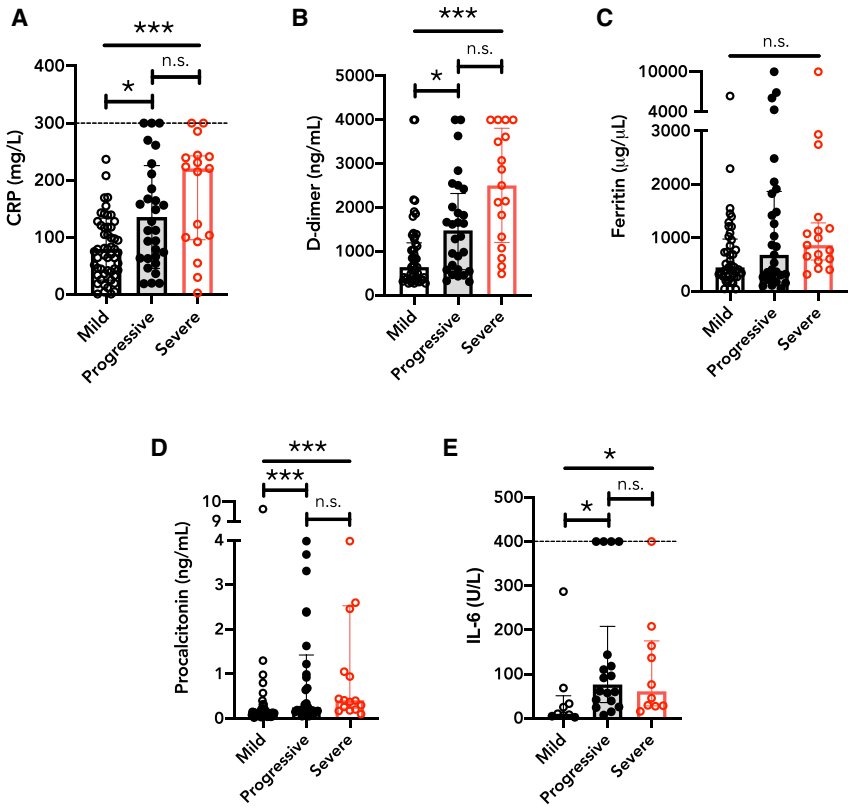

Figure 1. Initial CRP, D-Dimer, Procalcitonin, and IL-6 Levels Are Correlated with Mild, Progressive, and Severe Respiratory Failure

Initial levels of (A) CRP, (B) D-dimer, (C) ferritin, (D) procalcitonin, and (E) IL-6 are shown for patients grouped into "mild" (n = 54), "progressive" (n = 29), and "severe" (n = 17) cohorts. Broken lines indicate the upper limit of the assay. Data are represented as median and IQR. Kruskal-Wallis and Dunn's multiple comparison tests were performed. *p < 0.05; ***p < 0.001. CRP, C-reactive protein; n.s., non-significant.

significant difference (p < 0.001) in mild (mortality rate 4%), progressive (41%), and severe (59%; Table 1). Although our primary analyses were focused on the comparison of patients in the three aforementioned categories, we also evaluated patients with a second model based on treatment location that is commonly used in other studies. In this model, patients are deemed as "floor" versus "ICU," whereby patients designated as floor cases receive care solely on the general medical floors in the non-ICU setting and ICU patients are those who required care in the ICU at some point during their hospitalization (Figure S1; Table S1).

Inflammatory Biomarkers Distinguish Mild from Progressive COVID-19

Similar to prior studies, we first compared patients who only required non-ICU care during their hospital course (floor) with patients who required ICU-level care at any point during their hospitalization (ICU). Initial levels of several inflammatory biomarkers, including CRP, D-dimer, and procalcitonin, were more elevated in patients requiring ICU-level care compared to patients who only required care on the floor (141.8 [66.7–229.3] versus 69.6 [37.7–105.1] ng/mL, p < 0.001; 1,646 [683–2,622] versus 564 [351–1,142] ng/mL, p < 0.001; and 0.31 [0.15–1.63] versus 0.12 [0.07–0.19] U/L, p < 0.001; respectively; Figure S2). Ferritin also trended upward in ICU patients but did not reach statistical significance (693 [317–1,483] versus 492 [312–994] μg/μL; p = 0.16; Figure S2C). Notably, IL-6 was markedly elevated in patients who required ICU level care at any point during their hospitalization, compared to non-ICU patients (61.3 [28.9–154.0] versus 9.0 [5.2–57.9] U/L; p = 0.007).

The binary categories of floor versus ICU masks one key aspect of the natural history of COVID-19 illness during hospital admission—inpatients who are initially stable on the floor who later decline and require ICU-level care. The factors that distinguish patients who remain stable from patients who are initially stable but then deteriorate are poorly characterized. We classified COVID-19 inpatients into three cohorts according to the stability and severity of their respiratory failure: (1) mild (remained on room air or supplemental oxygen); (2) progressive (initially on room air or supplemental oxygen and then later required intubation or high-flow nasal cannula); or (3) severe (required intubation within 12 h of admission; Figure S1; Table 1). Initial levels of CRP, D-dimer, procalcitonin, and IL-6 were elevated in patients with progressive disease compared with mild disease (112.5 [63.4–198.0] versus 73.6 [38.5–118.3] mg/L, p = 0.03; 1,476 [580–2,321] versus 639 [359–1,196] ng/mL, p = 0.02; 0.25 [0.15–1.43] versus 0.12 [0.07–0.19] ng/mL, p < 0.001; and 76.4 [35.8–208.0] versus 10.0 [5.6–51.0] U/L, p = 0.03; respectively; Figures 1A, 1B, 1D, and 1E). Ferritin levels trended upward in progressive cases compared with mild cases, though did not reach statistical significance (682 [268–1,870] versus 449 [279–978] μg/μL; p = 0.66; Figure 1C).

CRP Levels Correlate with Physiological Measures of Disease Severity and Hypoxemic Respiratory Failure

There are a number of tools used to assess clinical condition. Perhaps the most commonly used is the SOFA score, which is a measure of organ dysfunction with higher scores representing worsening organ damage. Respiratory function is assessed as a subscore of this system and is based on the ratio of the arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2 or P/F ratio).[10] Lower P/F ratios indicate worsening hypoxemia. Patients with progressive disease presented with a higher SOFA score than those with mild disease, although there was significant overlap (3 [2–3.5] versus 1 [0.8–2]; p = 0.01; Figure 2A). Additionally, as expected given our classification criteria, admission respiratory SOFA score (Figure 2B) and P/F ratios (Table 1) were not significantly different between progressive patients and mild

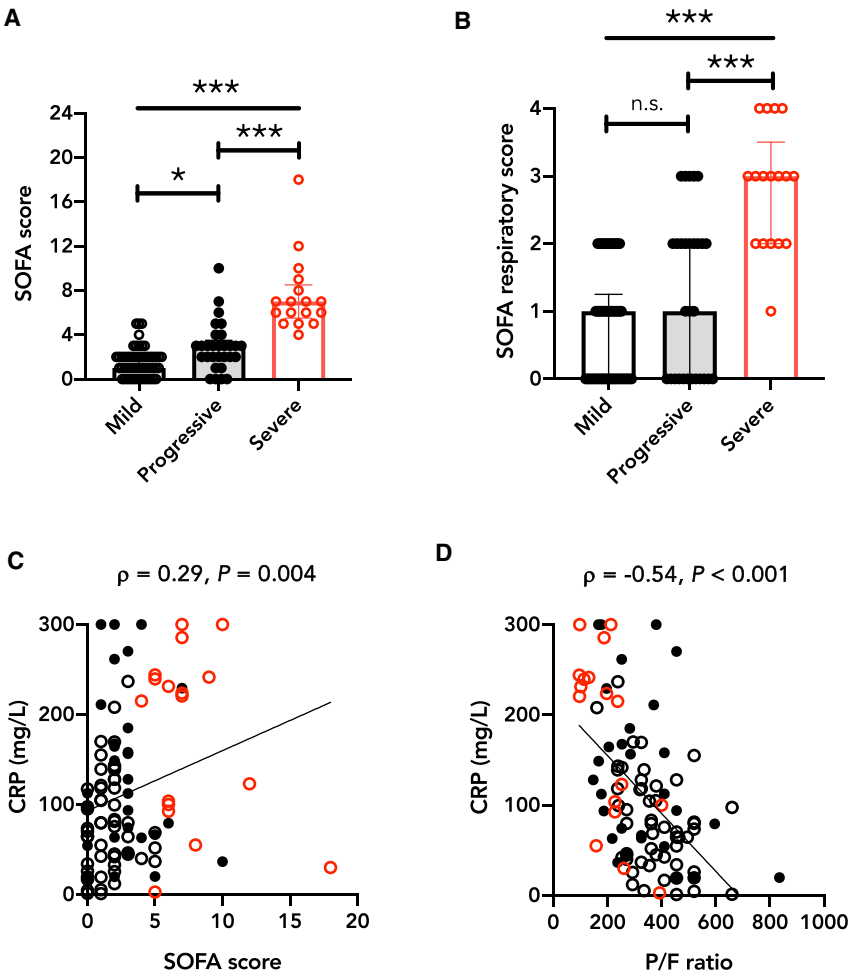

Figure 2. CRP Levels Are Associated with Physiological Measures of Disease Severity and Hypoxemic Respiratory Failure

COVID-19 inpatients are grouped into mild, progressive, or severe cohorts defined by respiratory failure.

(A) SOFA scores on admission.

(B) SOFA respiratory scores on admission.

(C) Correlation of SOFA scores to initial CRP.

(D) Correlation of P/F ratios to initial CRP. Open black circles, mild; filled black circles, progressive; open red circles, severe.

Data in (A) and (B) are represented as median and IQR. Kruskal-Wallis and Dunn's multiple comparison tests were performed for (A) and (B); Spearman rank correlation was performed for (C) and (D). *p < 0.05; ***p < 0.001. P/F, PaO_2/FiO_2; SOFA, sequential organ failure assessment.

patients. At admission, CRP and D-dimer were clinically relevant, as they were correlated with measures of organ and respiratory function. Both CRP levels and D-dimer levels showed a strong positive association with SOFA score ($\rho = 0.41$, $p < 0.001$ and $\rho = 0.47$, $p < 0.001$, respectively; Figures 2C and S3A). Moreover, these levels were inversely correlated to P/F ratio on admission ($\rho = -0.54$, $p < 0.001$ and $\rho = -0.23$, $p = 0.02$, respectively), demonstrating an association of these markers with the severity of acute hypoxemic respiratory failure (Figures 2D and S3B). Among patients who were intubated, CRP values at the time of intubation showed some correlation with the P/F ratio at the time of intubation, though it was not statistically significant (Figure S3C).

The association of CRP to respiratory deterioration and physiological measures of disease severity was particularly intriguing, as CRP levels can have mechanistic implications. CRP levels are tracked in a wide range of inflammatory diseases and are linked to IL-6 signaling, which has been a therapeutic target in COVID-19. Indeed, IL-6 levels did show a striking correlation to CRP (Figure S3D) and P/F ratio (Figure S3E). However, the number of patients with IL-6 levels were limited, as this institution's clinical guidelines did not endorse routine clinical measurement of IL-6 because results took over 48 h to return. In many institutions, CRP levels result within several hours and can capture rapidly evolving clinical courses that cytokine assays, which take more than 1 to 2 days, cannot. These results supported the further investigation of CRP as a biomarker with mechanistic implications and potential practical clinical utility.

Early Rise in CRP Has a Clinically Significant Association with Progressive Respiratory Failure

Initial CRP and ferritin values showed modest elevations in progressive disease compared with mild disease. However, maximum CRP levels were highly correlated (300.0 [237.4–300.0] versus 116.8 [64.0–158.1] mg/L; $p < 0.001$; Figure 3A) and suggested that CRP values were dynamic during COVID-19 illness. To explore this finding, we tracked CRP longitudinally throughout hospitalization (Figures 3B and 3C; Tables S3 and S4). In all patients, CRP levels peaked early within approximately 10 days of symptom onset (Figure 3C). The longitudinal CRP trend of progressive patients closely resembled that of severe patients, with a sharp, early rise in CRP (Figure 3B). In contrast, mild patients (who remained non-critically ill) had a lower plateau and then a steady decline in CRP (Figure 3B). Further quantification revealed that the change in CRP over the time course less than 72 h of admission was significantly different between mild and progressive patients ($p = 0.009$), whereas it was similar between progressive and severe patients ($p = 0.81$; Figure 3D). This indicates that progressive patients when compared to mild patients had a more rapid rise in CRP levels drawn at 24–48 h and 48–72 h after admission (182.0 ± 101 versus 97.6 ± 72 mg/L, $p = 0.006$ and 190.1 ± 99 versus 90.2 ± 64 mg/L, $p < 0.001$, respectively; Figure 3D). The odds ratio of requiring advanced respiratory support was 16.9 (95% confidence interval [CI]: 1.96–145.3; $p = 0.01$) when CRP value of greater than 300 mg/L (upper limit of the assay at our institution) was achieved within 72 h of admission.

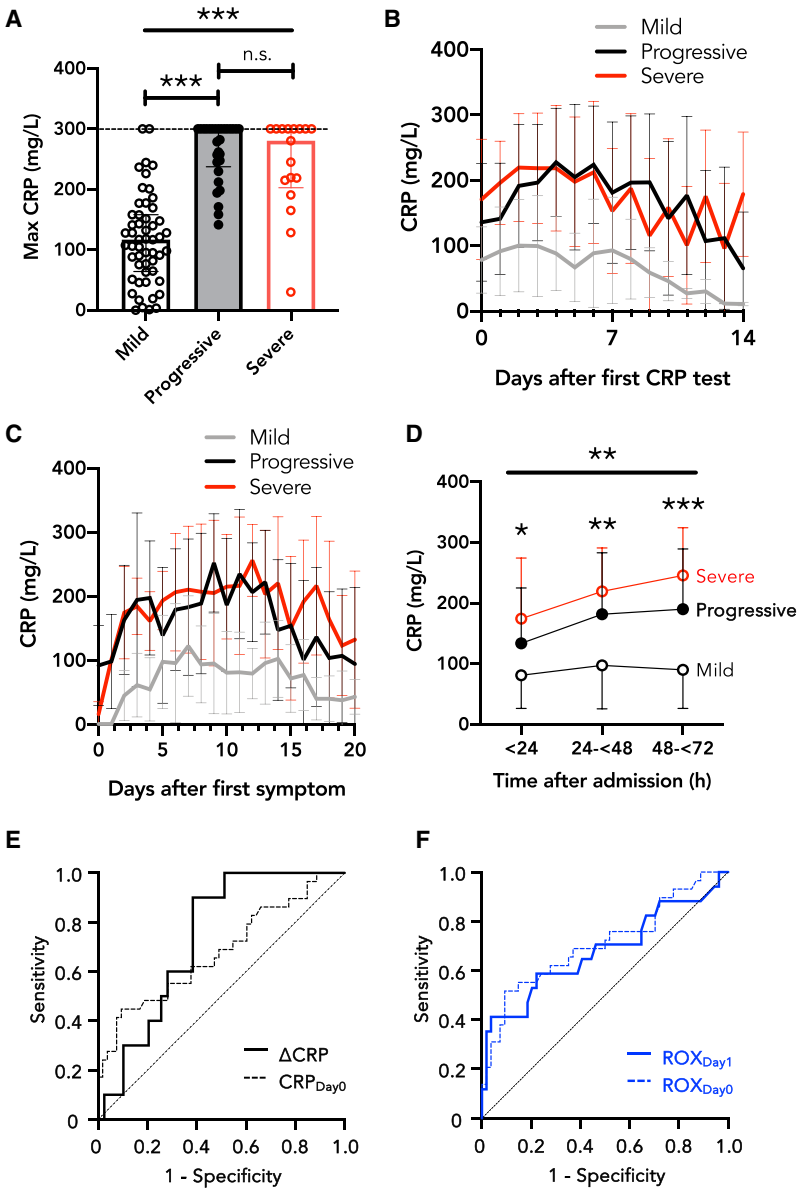

Figure 3. Rise in CRP Predicts Respiratory Deterioration Requiring Intubation or HFNC

COVID-19 inpatients are grouped into mild, progressive, or severe cohorts defined by respiratory failure.

(A) Maximum CRP value during hospital course. The broken line indicates the upper limit of the assay.

(B and C) Mean CRP values are shown as a function of (B) days after first recorded CRP level and (C) days after onset of first symptom.

(D) CRP values taken 0–<24, 24–<48, and 48–<72 h after admission.

(E) ROC analyses using day 0 (<24 h after admission) CRP was performed in all progressive and mild patients whose day 0 CRP value was available. ΔCRP value was defined as change in CRP values obtained between day 0 and day 1 (24–<48 h of admission). Similarly, the ROC analysis using ΔCRP included all mild patients and progressive patients in whom the second CRP was measured prior to intubation.

(F) ROC analyses were performed using the ROX index on day 0 and day 1. ROX index was calculated using the following formula: ROX index = SpO_2 (%)/FiO_2/respiratory rate (/min).

(G) Area under the curve (AUC) with 95% confidence interval (CI) and the cutoff value with sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV).

For (A)–(D): open black circles, mild; filled black circles, progressive; open red circles, severe. (A) Median and interquartile range are plotted; (B–D) mean and standard deviation are plotted. Kruskal-Wallis and Dunn's multiple comparison tests were performed for (A); a mixed effect model was used for (D). *p < 0.05; **p < 0.01; ***p < 0.001. Max, maximum. See also Tables S3 and S4.

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	AUC [95% CI]	P value	Cutoff value	Sn	Sp	PPV	NPV
ΔCRP, mg/L	0.74 [0.59-0.88]	0.02	13.1	0.90	0.62	0.73	0.86
Day 0 CRP, mg/L	0.68 [0.55-0.80]	0.008	146.1	0.45	0.91	0.59	0.73
Day 1 ROX index	0.68 [0.51-0.85]	0.03	10.2	0.41	0.96	0.78	0.84
Day 0 ROX index	0.71 [0.58-0.83]	0.002	14.5	0.52	0.91	0.68	0.77

Rise in CRP and ROX Index Is Predictive of Respiratory Deterioration during Hospitalization

We tested the prognostic utility of CRP levels in determining the need for advanced respiratory support in COVID-19 patients, using receiver operating characteristic (ROC) curve analyses incorporating patients with mild versus progressive disease. In particular, we first compared with predictive value of admission CRP levels (day 0 CRP) and change in CRP from day 0 to day 1 (ΔCRP). ΔCRP and admission CRP were both predictive of a need of advanced respiratory support, though the area under the curve (AUC) for ΔCRP was greater than for admission CRP (AUC 0.74 [0.59–0.88] and 0.68 [0.55–0.80], respectively; Figures 3E and 3G). Next, we compared the molecular approach (measuring a biomarker like CRP) with a physiological approach (calculating the ROX index, an established clinical scoring system for predicting intubation).[11] The ROX index incorporates oxygenation and respiratory rate, specifically oxygen saturation divided by fraction of inspired oxygen divided by respiratory rate (i.e., [SpO_2/FiO_2]/RR). Thus, the ROX index integrates hypoxemia and "work of breathing." The AUC for ΔCRP was greater than that for the ROX indices on day 0 and day 1 (0.68 [0.51–

0.85] and 0.71 [0.58–0.83], respectively; Figures 3F and 3G). ΔCRP and day 0 CRP were independently associated with a need for an advanced respiratory support (Table S2). We demonstrated the predictive value of both CRP levels and ROX index to predict respiratory deterioration during hospitalization. Further, we showed that the change in CRP had superior predictive value to either initial CRP value alone or the ROX index.

DISCUSSION

Dynamic Trends in a Single Molecular Biomarker Are Predictive of Respiratory Failure

In this retrospective observational study, we analyzed clinical features and biomarkers in inpatients with COVID-19 at a single institution. Consistent with previous reports,[9,12] patients requiring ICU-level care at any point during their hospital course had elevated CRP, D-dimer, procalcitonin, and IL-6 levels, compared to patients who remained on the non-ICU medical floor throughout their hospitalization. To study the natural history of COVID-19 in hospitalized patients, we categorized patients as mild (stable on room air or supplemental oxygen), progressive (initially room air or supplemental oxygen then progression to respiratory failure requiring intubation or high-flow nasal cannula), and severe (intubation on hospital admission). CRP, D-dimer, and procalcitonin levels at admission were increased in the progressive cohort, compared to mild (i.e., stable). At the same time, we found that CRP did have a remarkably close association with the degree of respiratory failure as the correlation of CRP to P/F ratio was highly significant. Although significantly different, these tests would have limited prognostic utility for frontline clinicians, as there was a high degree of overlap between mild and progressive cohorts that precluded a simple threshold value. To address this clinical challenge, we noted that maximum CRP distinguished stably non-critical (mild cohort) patients from those with progression of respiratory failure. This finding suggested that CRP values are dynamic in COVID-19 patients that develop later respiratory failure. Similar results were reported, indicating the utility of maximal CRP for the need of mechanical ventilation.[13] However, the maximal CRP is not useful as a clinical decision-making tool because the determination of whether the CRP value is at the maximum is only made retrospectively. We did find that a rapid rise in CRP preceded and was associated with respiratory deterioration among patients that were stable at admission. By tracking CRP values longitudinally during hospitalization, we found that CRP levels rose more precipitously in the first 3 days after hospital admission in the progressive cohort compared to the mild cohort, with an appreciable elevation detectable as early as 24–48 h after admission. Thus, the rate of change of CRP, rather than the absolute value of admission values, was more closely associated with clinical deterioration. Initial absolute values of CRP were similar between mild and progressive patients, but the dynamic trends of CRP were similar between progressive and severe patients. Another study also found limited prognostic utility for admission CRP values and required a 10 variable risk score to predict clinical deterioration.[14] Our study suggests that examination of dynamic trends, rather that absolute value at admission, can lead to strong associations with prognosis despite only using a single laboratory value. We confirmed that change in CRP had clinical utility in predicting intubation as shown in our ROC analyses where utilizing change in CRP resulted in a higher AUC than the ROX index, a clinically validated index used to predict intubation. Similar to our results, a high CRP cutoff for a single-value CRP was reported.[15] This high CRP cutoff selects for an extremely ill patient population and leads to high specificity and low sensitivity, which make it unhelpful as clinical predictors. In contrast, although the AUC value for ΔCRP was not dramatically higher than that of ROX or admission CRP, the high sensitivity for respiratory decompensation makes it a much more valuable screening test. Our study suggests that trending CRP, a highly accessible tool for frontline clinicians compared to complicated scoring systems, has predictive value for respiratory failure among initially non-critically ill patients on the general medical floor.

Hyper-acute Activation of IL-6 Pathway Associates with Prognosis of Respiratory Failure

Although our study has implications for clinical prognostication, our result also may suggest underlying pathological mechanisms and possible strategies for therapeutic intervention. As in SARS-CoV and MERS-CoV infection,[16,17] several proinflammatory cytokines (e.g., IL-6, IL-10, IL-2, and interferon [IFN]-gamma) are increased in COVID-19.[12,18–21] We demonstrate a correlation between the CRP and D-dimer inflammatory biomarkers with disease severity and a particularly close association of CRP with hypoxemic respiratory failure (P/F ratio). Our study highlights the potential role of IL-6, which is upstream of increased CRP. In our cohort analysis, IL-6 levels showed a positive correlation with CRP in patients who had IL-6 levels drawn, and patients treated with tocilizumab, an IL-6 receptor monoclonal antibody, had rapid and sustained decrease in CRP levels (n = 15; Figure S4). Our study suggests that increased CRP rise, and by virtue presumed elevation of IL-6, in the first 24–48 h may be of critical importance to disease progression; no other study is focused only on this hyper-acute period. Furthermore, in many studies, COVID-19 patients are simply categorized as non-critically ill (floor) or critically ill (ICU),[8] as in a longitudinal study of lymphocyte subsets and cytokines[8] or single-cell RNA sequencing of bronchoalveolar lavage.[22] Our results may suggest that "critically ill" COVID-19 patients should be sub-divided into two sub-cohorts as patients who developed a requirement for advanced oxygen support later in their hospital course (progressive) had a distinct inflammatory biomarker profile than patients who required immediate intubation on hospital admission (severe). Multiple randomized control trials are examining tocilizumab in COVID-19 infection,[23–27] and preliminary results from recent phase III studies have been mixed. The COVACTA trial evaluating tocilizumab (F. Hoffmann-La Roche, press release: https://www.roche.com/investors/updates/inv-update-2020-07-29.htm) and another trial centered on sarilumab (Sanofi-Aventis U.S., press release: https://www.sanofi.com/en/media-room/press-releases/2020/2020-07-02-22-30-00) did not meet primary endpoints. However, the EMPACTA trial (F. Hoffmann-La Roche, press release: https://www.roche.com/media/releases/med-cor-2020-09-18.htm) did show that tocilizumab reduced likelihood of progression to mechanical ventilation. Our work may suggest that there is value in delineating the particular progressive patients whose uptrend in CRP may suggest that they could be particularly poised to benefit from this type of IL-6-directed therapy.

Recent studies have suggested that acute respiratory distress syndrome (ARDS) related to COVID-19 is not more inflammatory than ARDS unrelated to COVID-19, with similar levels for plasma IL-6 in COVID-19 and non-COVID-19 ARDS.[28,29] However, it is clear from this study and others that severe COVID-19 is more inflammatory than milder COVID-19. This study highlights that the dynamic nature of the inflammation in COVID-19 is key and directly associated to physiological parameters. The key limitations of this work are its single-center and retrospective design. Future prospective studies could study a wider range of cytokines and chemokines along with the interaction of CRP rise and immunomodulatory treatment. In conclusion, we suggest that closely tracking the levels of CRP in the hyper-acute phase of admission for COVID-19 patients is a valuable tool to stratify the risk that a patient will have progressive hypoxemic respiratory failure requiring intubation. This metric is feasible for frontline clinicians in the emergency department observation units or medical floor inpatient wards. Second, longitudinal CRP profile may distinguish unique phenotypes of patients with critical illness from COVID-19. Finally, these findings suggest that clinical trials of IL-6 receptor monoclonal antibodies should pay particular attention to intervention in the first 48 h of the hospital course.

Limitations of Study

Limitations of this study include its single-centered retrospective nature and small sample size, and future efforts focused on the prospective analyses will strengthen our understanding of the prognostic utility of CRP. The size of the study was a consequence of balancing the need for more immediate analysis for frontline physicians. It should be noted that, in our analyses, the ROX index was adapted for patients on supplemental oxygen using an imputed FiO_2.[30] Additionally, in the comparison of progressive and severe cases, one cannot definitively exclude the possibility of lead time bias, though our analyses were mainly focused on the comparison of mild and progressive cases where lead time bias is not clearly evident.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.xcrm.2020.100144.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.Y.K., A.A.M., and T.T.; Formal Analysis, T.T. and A.A.M.; Investigation, A.A.M., E.H.P., T.T., J.L.J., H.H., J.R.D., and C.P.C.; Resources, E.Y.K. and A.F.M.; Data Curation, T.T. and A.A.M.; Writing – Original Draft, A.A.M., T.T., J.L.J., and E.H.P.; Writing – Review and Editing, A.A.M., T.T., and E.Y.K.; Visualization, T.T. and A.A.M.; Supervision, E.Y.K.; Project Administration, G.K., A.F.M., and E.Y.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
Prism version 8.4.1	GraphPad	https://www.graphpad.com/scientific-software/prism/
R version 3.6.1	The R Project	https://www.r-project.org

RESOURCE AVAILABILITY

Lead Contact

Further information and requests should be directed to and fulfilled by Lead Contact, Dr. Edy Kim (ekim11@bwh.harvard.edu), Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Materials Availability

No new reagents or materials were generated as part of this study.

Data and Code Availability

Patient data reviewed in this study is not publicly available due to restrictions on patient privacy and data sharing. There was no new code developed as part of this study.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

This investigation was approved by the Partners Healthcare Institutional Review Board (Protocol 2020P001139). Opt-out consent was designated for the study. Demographic information including age and gender are provided in Tables 1 and S1.

METHOD DETAILS

Study Design and Population

This was a single-center retrospective cohort study. We included patients who were admitted to BWH between March 12, 2020 and April 9, 2020 and had laboratory-confirmed COVID-19 infection. A confirmed case of COVID-19 was defined by a positive result on a reverse transcription polymerase chain reaction (RT-PCR) assay of SARS-CoV-2. Patients were excluded if they were not initially admitted due to COVID-19, deemed to have had a false positive result, or transferred from a non-affiliated hospital later than 48 hours after their initial admission (Figure S1). Patient charts were last reviewed to the end of the hospitalization, allowing follow-up until hospital discharge for all patients. Any information from subsequent clinic visits or hospitalizations was not incorporated into the chart review. This study was approved by the Partners Healthcare Institutional Review Board (Protocol 2020P001139). Opt-out consent was selected in this study.

Patients were classified using 2 different classifications. In the first classification scheme, patients were designated either as "Floor" patients (requiring only floor-level care throughout their hospitalization) or "ICU" patients (required care in the ICU at any point during hospitalization). With a specific interest in COVID-19 patients whose respiratory conditions would deteriorate after admission, inpatients were divided according to a second classification system: "mild, progressive, and severe." Those with "Mild" disease maintained adequate oxygenation with room air or oxygen supplementation with nasal cannula or face mask throughout the entire course of their hospitalization. On the other end, the "Severe" group included patients who required advanced respiratory support, including mechanical ventilation or high-flow nasal cannula, within 12 hours of admission, with the admission time being defined as the time that the first vitals were recorded for the patient. Patients categorized as having "Progressive" disease were initially admitted to the hospital on room air or oxygen supplementation with nasal cannula or face mask but required advanced respiratory support due to respiratory deterioration later during the hospital course at least 12 hours after admission.

Data Collection

Data were retrospectively collected by reviewing electronic medical records. Patient demographic data included age, gender, race or ethnicity, past medical history, use of immunosuppressive medication or chemotherapy, and history of smoking. Clinical information

included date of first symptom, first positive SARS-CoV-2 result, admission, intubation, extubation, and discharge; admission ward (ICU or non-critically ill medical floor); requirement for oxygen, vasopressors, and intubation or high-flow nasal cannula; respiratory rate; amount of oxygen requirement at admission; the maximum amount of oxygen requirement; sequential organ failure assessment (SOFA) score upon admission; respiratory rate and oxygen saturation (SpO_2) on the day of admission (day 0) and day 1; administration of hydroxychloroquine, tocilizumab, or clinical trial agent composed of remdesivir or placebo; and survival status. Partial pressure of arterial oxygen (PaO_2) or oxygen saturation (SpO_2) and the fraction of inspired oxygen (FiO_2) upon admission as well as immediately following intubation was obtained. For patients in whom an arterial gas analysis was not tested and was not intubated upon admission, PaO_2 value was imputed using a non-linear method,[30] and FiO_2 was calculated using a formula of FiO_2 = 0.20 + oxygen flow rate (L/min) * 0.04. Then, PaO_2/FiO_2 (P/F) ratio was calculated using these values. ROX index is a prediction tool developed to identify the need for mechanical ventilation in pneumonia patients treated with high-flow nasal cannula.[31] We calculated ROX index in COVID-19 patients independent of the high-flow nasal cannula treatment. ROX index on Day 0 (the day of admission) and day 1 were calculated using a formula of ROX Index = SpO_2 / FiO_2 / respiratory rate (breaths/min). Laboratory test results collected upon admission included: C-reactive protein, serum ferritin, D-dimer, and procalcitonin. Additionally, maximum serum ferritin value during the hospital course, C-reactive protein values during admission, and the first three interleukin (IL)-6 levels drawn after admission were obtained.

General Treatment of COVID-19 Patients

Inpatient treatment was conducted based on institutional COVID-19 clinical guidelines that are in continual development and shared with the public (Brigham and Women's Hospital COVID-19 Clinical Guidelines: https://www.covidprotocols.org). Regarding local practices at Brigham and Women's Hospital, guidelines allowed patients to remain on the floor while on room air, nasal cannula, high-flow nasal cannula and Venturi face masks up to 60% FiO2. Ideally, if it was anticipated that a patient may require intubation, that patient was transferred to the ICU, and any patient requiring pressure support was required to be in the ICU unless it was part of a nightly CPAP or BiPAP regimen that they had used at home. In general, during the time of this study, the institution recommended against high-flow nasal cannula use in COVID-19 patients due to concern regarding potential transmission with aerosolization. Instead, patients who were unstable on regular nasal cannula were transitioned to Venturi mask or intubated.

QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive statistics were reported as mean ± SD or median with IQR, and frequencies with percentages for continuous and categorical variables, respectively, as noted in the results section. Student's t test or Mann-Whitney U test was used for two-group comparison of continuous variables. Analysis of variance with Tukey's multiple comparison or Kruskal-Wallis test with Dunn's multiple comparison was used for the comparison of continuous variables among the 3 groups. The chi-square test or Fisher exact test was used for comparisons of binary variables. Spearman rank correlation coefficient was calculated to determine the correlation between the two variables. The mixed effects model with Sidak's multiple comparison was used for the analysis of repeated-measures. The tests used are designated in the figure legends.

Receiver operating characteristic (ROC) curve analysis was used to estimate the prognostic performance of the following parameters to predict the need for aggressive respiratory support including intubation and high-flow nasal cannula: day 0 CRP, defined as the first CRP drawn less than 24 hours after admission; ΔCRP, defined as the change in CRP from less than 24 hours after admission to the CRP 24-48 hours after admission; day 0 ROX index, which was the ROX index using the first vital signs after admission; and day 1 ROX index, which was the ROX index using the morning vital signs on day 1. CRP values exceeding the upper limit of assay (300 mg/L) were imputed as the maximum value of the assay (300 mg/L) in the ROC analysis of day 0 CRP. In the ROC analysis for ΔCRP, only patients in whom the ΔCRP values before intubation could be calculated were included. In particular, patients who were intubated before the second CRP measurement or whose CRP values exceeded the upper limit of assay were excluded. For the ROC analyses of ROX indices, all patients in whom the ROX index was able to be calculated were included. The area under the curve (AUC) with 95% confidence interval and the optimal cutoff value determined by Youden's J static with corresponding sensitivity and specificity calculated. A multivariate regression model was used to assess the association between day 0 CPR and ΔCRP and the need for advanced respiratory support. Variables that showed P value of less than 0.1 with univariate regression analysis were selected as covariates for the adjustment in the multivariate regression model. Of the potential confounders including age, chronic lung disease, severe cardiac disease, cancer history, and smoking history, the covariates included in the multivariate regression model were age and severe cardiac condition. A *P value* of < 0.05 was considered as statistically significant. Analyses were conducted using GraphPad Prism version 8.4.1 and R version 3.6.1.