Understanding the link between obesity and severe COVID-19 outcomes: Causal mediation by systemic inflammatory response

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Abstract

**Background:** Obesity is an established risk factor for severe COVID-19 outcomes. The mechanistic underpinnings of this association are not well-understood.

**Objective:** To evaluate the mediating role of systemic inflammation in obesity-associated COVID-19 outcomes.

**Design:** Hospital-based, observational.

**Setting:** Massachusetts General Hospital (MGH) or Columbia University Irving Medical Center/New York-Presbyterian Hospital (CUIMC/NYP).

**Patients or Other Participants:** N=3828 SARS-CoV-2-infected patients hospitalized February to May 2020.

**Main Outcome Measures:** Mediation analysis is used to evaluate whether peak inflammatory biomarkers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), D-dimer, ferritin, white blood cell count and interleukin-6] are in the causal pathway between obesity (BMI ≥ 30) and mechanical ventilation or death within 28 days of presentation to care.

**Results:** In the MGH cohort (n=1202), obesity was associated with greater likelihood of ventilation or death [OR=1.73, 95% CI=(1.25, 2.41), p=0.001] and higher peak CRP (p<0.001) compared to non-obese patients. The estimated proportion of the association between obesity and ventilation or death mediated by CRP was 0.49 (p<0.001). Evidence of mediation was more pronounced in patients <65 years [proportion mediated=0.52 (p<0.001) versus 0.44 (p=0.180)]. Findings were more moderate but consistent for peak ESR. Mediation by other inflammatory markers was not supported. Results were replicated in CUIMC/NYP cohort (n=2626).
Conclusions: Findings support systemic inflammatory pathways in obesity-associated severe COVID-19 disease, particularly in patients <65 years, captured by CRP and ESR. Contextualized in clinical trials findings, these results reveal therapeutic opportunity to target systemic inflammatory pathways and monitor interventions in high-risk subgroups and particularly obese patients.

Keywords: Biomarkers, COVID-19, inflammation, obesity, SARS-CoV-2, severe disease
Introduction

Obesity is one of the strongest risk factors for critical illness among severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected individuals (1-4). Moreover, emerging evidence suggests that obesity-associated severe disease is more pronounced in younger populations compared to older populations (5-8). At the same time, studies of multiple biomarkers of pathophysiological processes, reveal elevated levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), D-dimer, ferritin, interleukin-6 (IL-6) and white blood cell count (WBC) and its fractions contribute to severe COVID-19 outcomes (4, 9). Indeed, emerging immunophenotyping data suggest that a marked dysregulation of innate and adaptive immunity is a hallmark of poor outcomes in COVID-19 (10-12).

As the relationship between obesity and inflammatory markers is well-established in population and clinical based epidemiologic studies of non-SARS-CoV-2-infected individuals (13-15), several commentaries have postulated on complex mechanisms linking obesity, inflammatory pathways, age and race/ethnicity to severe disease in COVID-19 (16, 17). However, the specific role of inflammation in the causal pathways between obesity and severe COVID-19 outcomes remains unknown and the translational clinical utility of disease biomarkers in COVID-19 remains to be fully developed and exploited. In this work, we hypothesize that biomarkers of obesity-associated systemic inflammation are mediators of severe outcomes in COVID-19. Through a rigorous investigation of causal mediation by systemic inflammatory responses in the link between obesity and severe COVID-19 outcomes, this research is designed to advance clinical translational opportunities for targeted interventions.
Materials/Subjects and Methods

We addressed our hypothesis using data derived from 3,828 SARS-CoV-2-infected patients hospitalized at two independent quaternary care facilities, Massachusetts General Hospital (MGH) and Columbia University Irving Medical Center / New York Presbyterian Hospital (CUIMC/NYP).

MGH COVID-19 Patient Registry

The MGH COVID-19 Patient Registry includes n=1202 confirmed SARS-CoV-2-infected patients hospitalized at MGH between March 11, 2020 and May 31, 2020 (18, 19). Data includes demographic information, comorbid conditions, medications, laboratory tests and clinical outcomes at index hospitalization. The current analysis considered the composite outcome defined as invasive ventilation or death within 28 days of presentation to care (PTC), defined as first contact with a health care provider due to COVID-19 related symptoms. Obesity was defined at time of hospitalization as body mass index (BMI) ≥30. CRP, ESR, D-dimer, ferritin, IL-6 and WBC were chronicled daily during hospitalization for up to 28 days until the time of death or discharge and the first measurement per day used in analysis. Admission biomarkers were defined as the first available within 3 days of hospitalization and peak values as the maximum during the observation period. In the primary causal mediation analysis, the peak value was defined as the maximum observed within 28 days of PTC and prior to ventilation.

CUIMC/NYP COVID-19 Cohort

A replication analysis was performed using retrospective data on n=2,626 individuals from the CUIMC/NYP COVID-19 cohort(20). This cohort includes adult patients (≥18 years of age) hospitalized between February 1, 2020 and May 12, 2020 and positive for SARS-CoV-2. Patients who were admitted for <24 hours were excluded from analysis and patients were followed until discharge or death, or for ≥30 days of hospitalization. Patient data were identified in the EHR by using the institution’s clinical data warehouse, which included information on individuals who receive care at
CUIMC/NYP. No manual abstraction was performed. Analysis was based on index hospitalization. The primary outcome was a composite of use of invasive ventilation or death. Peak biomarker values were defined over the duration of hospitalization. We chose this pragmatic replication despite some differences in exposure definitions between MGH and CUIMC/NYP cohorts because successful replication would suggest a robust result likely to be generalizable across a wide range of geographic and clinical settings.

Statistical Methods

Baseline characteristics were summarized overall and by obesity for the MGH and CUIMC/NYP cohorts. Unadjusted two-sided tests of proportions (or means) were reported to characterize differences between obese and non-obese patients. We evaluated the mediating role of six inflammatory markers – CRP, ESR, D-dimer, ferritin, IL-6 and WBC – in the association between obesity and severe disease (Figure 1). Peak laboratory measurements were natural log transformed and standardized prior to model fitting.

To begin, we fitted the following generalized linear multivariable models for each biomarker:

1) A Total Effect Model using a logit link with mechanical ventilation/death (Y) as the outcome and obesity (T) as a predictor variable, where the biomarker (M) is not included in the model, f(Y|T); 2) A Mediator Model using an identify link with peak biomarker as the outcome and obesity as a predictor, f(M|T); and 3) An Outcome Model using a logit link with mechanical ventilation/death as the outcome and both obesity and peak biomarker as predictor variables, f(Y|T, M). All models were initially conditioned on age, sex, race/ethnicity and the number of biomarker measurements contributing to determination of peak value. The reported odds ratios (ORs) are computed in the same way as using standard statistical model fitting procedures.

We applied the causal mediation analysis approach described in (21) which uses the results of the model fitting described above to determine the proportion of the association between obesity and severe outcomes that is mediated by the biomarker. Conceptually, the analysis estimates a
decomposition of the total effect (TE) of obesity (T) on severe disease status (Y) into an average natural direct effect (NDE) and natural indirect effect (NIE) through inflammatory markers (M), i.e. 

\[ \text{TE} = \text{NDE} + \text{NIE} \] (22, 23). The mediation analysis draws model parameters based on their sampling distributions and simulates what are referred to as “counterfactual” biomarker values for each patient under the obese and non-obese conditions. The regression coefficients obtained from the generalized linear multivariable models fit previously are then used to simulate “counterfactual” ICU/death outcomes under obese and non-obese conditions with counterfactual biomarker values.

The NDE and NIE are estimated from contrasting average counterfactual outcomes:

\[ \text{NDE} = E[Y(1,M(0)) - Y(0,M(0))] \]

\[ \text{NIE} = E[Y(1,M(1)) - Y(1,M(0))] \]

where \( Y(t,m) \) denotes the counterfactual outcome had a patient had obesity status \( T=t \) and biomarker value \( M=m \), \( M(t) \) denotes the counterfactual mediator value under obesity status \( T=t \) and \( E[.] \) denotes statistical expectation. The proportion mediated is given by the ratio of NIE to TE.

The average proportion mediated and corresponding p-value were reported for each biomarker. Models were fitted overall and within age subgroups (<65 or \( \geq \) 65 years) based on data from adult patients (≥18 years of age) with at least one recorded biomarker measure prior to ventilation. A complete case analysis was performed assuming data were missing completely at random. Summary level data on characteristics of patients with and without missing data are provided in Supplement Table S3 (24). Primary analysis was based on data derived from the MGH cohort. Replication analysis was based on the CUIMC/NYP cohort. Additional analyses using the MGH cohort were performed to check the consistency of our conclusions when adjusting for additional potential confounders (type 2 diabetes mellitus, hypertension, dyslipidemia and pulmonary disease) in the multivariable models or including CRP measurements after mechanical ventilation in the calculation of peak CRP. All analyses were completed using R version 3.5.0.

Mediation analysis was performed using the R package ‘mediation’.
IRB approval

The Partners HealthCare Institutional Review Board (IRB) (#2020P000829) approved collection of curated data based on comprehensive manual chart reviews and data extractions from electronic health records (EHR) on patients who receive care through the Mass General Brigham (MGB, formerly Partners) system. The CUIMC IRB approved this study (#AAAS9835) and waived the requirement for obtaining informed consent.

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Results

Demographic and clinical characteristics of the MGH cohort

Table 1 and Supplement Table S1 (24) provide demographic, clinical and laboratory characteristics overall and by obesity (BMI ≥30 or BMI <30) for the MGH cohort. At PTC, obese patients were more likely to be female (48% vs 41%, p=0.020), were younger (56.0 vs 65.0 median age, p<0.001), less likely to have ever smoked (33% vs. 42%, p=0.001) and more likely to be Hispanic (42% vs 32%, p<0.001) compared to non-obese patients. They were also more likely to experience a longer time from start of symptoms to PTC, experience more symptoms at PTC, have a reported history of type 2 diabetes mellitus (T2DM) and be admitted directly to the ICU than non-obese patients. At 28 days after PTC, obese patients had more complications, including acute respiratory distress syndrome (ARDS), and were more likely to be mechanically ventilated.
CRP trajectories over time are illustrated in Figure 2 stratified by obesity. The individual level trajectories are presented as a function of the number of days before or after peak CRP. Medians each day by mechanical ventilation/death status are provided. This exploratory figure suggests: 1) a shift upwards in CRP within both obesity strata for patients with more severe disease; and 2) a higher median peak CRP for obese compared to non-obese patients. In unadjusted analysis, median peak biomarker values in obese patients compared to non-obese patients were greater for CRP and ESR.

**Primary analysis of CRP in the MGH cohort**

Table 2 provides results of the causal mediation analysis of peak CRP overall and stratified by age (<65 or ≥65 years) in the MGH cohort. In the adjusted Total Effect Model (Supplement Table S2) [24], obesity was a significant predictor of ventilation or death [OR = 1.73, 95% CI = (1.25, 2.41)] and in the adjusted Mediator Model obesity of was significant predictor of peak CRP (estimate = 0.261, p < 0.001). After additional adjustment for peak CRP in the adjusted Outcome Model, the effect of obesity on ventilation or death was partially attenuated (OR = 1.49, 95% CI = (1.03, 2.16)). A statistically significant positive interaction between peak CRP and age on severe outcome was also observed, suggesting a greater effect of peak CRP on COVID-19 outcomes with increasing age. There was no observed interaction between peak CRP and obesity on severe outcome. Summary data on patients with and without complete data are in Supplement Table S3 [24]. The average proportion of the effect of obesity on ventilation/death that was mediated by peak CRP is 0.49 (p<0.001). In stratified analysis, the proportion mediated was 0.44 (p=0.180) in patients ≥ 65 years of age and 0.52 (p<0.001) in patients <65 years of age.
**Analysis of additional inflammatory biomarkers in the MGH cohort**

The model results for five additional inflammatory biomarkers are provided in Table 2 and Supplement Figure S1 (24). In all cases, a statistically significant positive association was observed between peak biomarker value and the probability of ventilation or death (Outcome Model). Strikingly, with the exception of ESR, there was no detectable association between obesity and the peak values for any of these additional biomarkers, overall nor within age strata (Mediator Model). This result suggests that peak D-dimer, ferritin, WBC and IL-6 are unlikely to be in the causal pathway between obesity and severe outcomes in this population. Similar to CRP though to a lesser extent, peak levels of ESR also mediated the effect of obesity on ventilation/death and, like CRP, this was more pronounced in the younger age group (<65 years old) though this was not statistically significant.

**Replication analysis based on the CUIMC/NYP cohort**

Replication analysis was performed using the CUIMC/NYP COVID-19 cohort (N= 2,626). Supplement Table S4 (24) provides characteristics of this cohort overall and by obesity. Similar to MGH, in the CUIMC/NYP cohort, obese patients were more likely to be female (51% vs 37%, p<0.001) and were younger (60 vs 69 median age, p<0.001) compared to non-obese patients. Obese patients were also more likely to be Black (14% vs 11%, p=0.027). Despite younger age, a history of hypertension and T2DM were numerically greater in obese patients compared to non-obese patients, although not statistically significantly different in unadjusted analysis. Summary data on patients with and without complete data are in Supplement Table S3 (24).

**Table 3** presents the results of causal mediation analysis of CRP in the CUIMC/NYP cohort. The overall estimated average proportion mediated was 0.67 (p=0.002). In stratified analysis, the proportion mediated was 0.63 (p=0.230) in patients ≥65 years of age and 0.68 (p<0.001) in patients <65 years of age. Consistent also with the MGH registry data, an increase in peak values was associated with an increased odds of ventilator or death for ESR, WBC, D-dimer, ferritin, and IL-6.
There was no association, however, between obesity and peak values of D-dimer, WBC and IL-6, while obesity was negatively associated with peak ferritin in patients ≥65 (Supplement Table S5) (24). Similar to MGH data, there was evidence of modest mediation effect captured by elevated ESR that was more pronounced in the younger age strata (<65 years of age).

**Alternative strategies using the MGH cohort**

Analysis based on a more fully adjusted model that included additional potential confounders type 2 diabetes mellitus, hypertension, dyslipidemia and pulmonary disease yielded similar results of a proportion mediated equal to 0.57 (p<0.001) overall, 0.51 (p=0.220) in patients ≥ 65 years of age and 0.53 (p=0.002) in patients <65 years of age. The results of using all available CRP measurements before and after ventilation in the calculation of peak CRP are reported in Supplement Table S6 (24). Here, the estimated average proportion mediated was 0.63 (p<0.001) overall, 0.76 (p=0.260) in patients ≥ 65 years of age, and 0.47 (p=0.004) in patients <65 years of age.

**Discussion**

We find compelling and reproducible evidence for mediation by CRP and ESR-related inflammation, but not other biomarkers of outcomes, in obesity-associated severe disease in 3,828 COVID-19 patients at two independent quaternary care centers.

Obesity is one of the strongest risk factors for critical illness in COVID-19 (1-4) while dysregulation of innate and adaptive immunity is a hallmark of patients with poor COVID-19 outcomes (10-12). A direct causal role of obesity, especially by immuno-metabolically abnormal adipose tissue, in an exaggerated inflammatory response to SARS-CoV-2 infection could underlie the convergence of obesity, severe systemic inflammation and poor outcomes. Experimental mouse models and clinical studies have shown immune cell infiltration in obese adipose (25) and obesity converges with low-grade, systemic inflammation in increased risk for cardiometabolic diseases (26-28). Previous work has shown that obesity is also associated with inflammatory responses and worse
outcomes in sepsis and ARDS (29). Here, we show that obesity-associated systemic inflammation, as reflected in peak circulating CRP, is indeed a mediator of severe outcomes in COVID-19, especially in younger obese patients, with greater mediation in patients < 65 relative to > 65 years of age.

It is notable that the mediation effect was specific to peak CRP and ESR but not peak levels of other interrogated biomarkers. This is explained in part by the interesting result that peak CRP during hospitalization had a much stronger relationship with obesity than peak levels of other biomarkers (e.g., IL-6) despite their known strong correlation with obesity under resting conditions. This finding emphasizes the difference in the performance of biomarkers for obesity-related inflammation under acute inflammatory stress due to COVID-19 compared to their performance under chronic resting conditions. Because of the weaker associations between obesity and peak levels of D-dimer, Ferritin, WBC and IL-6, these biomarkers have less potential to be significant mediators of severe COVID-19 outcomes.

The findings herein are consistent with clinical trials data. For example, the recent success of trials of systemic corticosteroids in reducing all-cause mortality in patients with severe COVID-19 (30-32) provides clear evidence of large clinical benefits by targeting activation of innate immunity, which is captured by peak CRP levels. Indeed, there is an established link between corticosteroid benefit and reductions in CRP and ESR levels in non-COVID-19 inflammatory disorders (33). Whether reductions in CRP and ESR track with therapeutic benefit of corticosteroids in COVID-19 remains to be determined. In contrast, the apparent failure of early trials targeting IL-6 signaling in COVID-19 (34, 35) is consistent with our finding that IL-6 was not a significant mediator of obesity on severe COVID-19 outcomes. Moving forward, elevated CRP levels may be useful for stratifying COVID-19 patients into subgroups in clinical trials of anti-inflammatory strategies as well as to monitor therapeutic efficacy of novel interventions in COVID-19. Failure to design statistically powered COVID-19 clinical trials to target or detect effects in such subgroups may contribute to apparent lack of therapeutic efficacy (36) and limit progress due to false negative trial data.
Methodological strengths of our work include the novel application of mediation analysis as well as robust and independent replication. Our work also has limitations. First, there are inherent biases in use of observational data to draw causal inference. The causal mediation framework assumes sequential ignorability (21) which relies on methodical consideration of potential confounding variables and assumes no unmeasured confounding. Second, missing data and measurement error are ubiquitous in observational studies that rely on EHR data and this can result in biased findings (37). An alternative to our complete case analysis strategy is to multiply impute missing values assuming missing at random. Additionally, patients enter the hospital at different stages of disease severity and therefore the biomarker trajectories are not fully observed. Moreover, Hispanics and Blacks have disparity in availability and comorbidity reporting in EHR data and a greater propensity than whites to obesity at younger ages (38). Third, despite robust replication across MGH and CUIMC/NYP, these two observational studies have differences in design. It is critical moving forward to test anti-inflammatory therapies in prospective randomized controlled trials that are designed for targeted subgroup analyses, e.g., using obesity and CRP-based stratification. Fourth, given that central obesity measures are stronger predictors than BMI of outcomes in cardiometabolic diseases (39, 40), future studies should determine if CRP-related inflammation plays an even more powerful role in central obesity effects on outcomes in COVID-19 (36, 41). This may be particularly important given the impact of central obesity on respiratory-related COVID-19 outcomes, including impaired mechanics of the respiratory system, the inability to pronate patients, and difficulties with intubation procedures. Fifth, a latent pre-diabetic state, e.g., glucose intolerance or insulin resistance, could correlate with obesity and be the cause of severe outcomes. Testing this hypothesis is not yet feasible given the available clinical laboratory data and in the setting of acute illness. Finally, given the complex relationships among obesity and severe COVID-19 outcomes, additional work is required to define optimal clinical trial and therapeutic stratification for high-risk patients with obesity.
In conclusion, our results elucidate the mediating role of systemic inflammation in obesity associated severe COVID-19 outcomes. The highly consistent pattern for all biomarkers and by age strata in both MGH and CUIMC/NYP cohorts supports generalizability of a specific mediating role captured by peak CRP (and to a lesser extent ESR) levels in the pathways between obesity and severe disease. Stronger evidence of mediation in younger obese patients highlights clinical importance and potential therapeutic opportunity to target systemic inflammatory pathways and to monitor therapeutic interventions in high-risk, obese subgroups with elevated CRP levels.
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Competing interests

The authors declare no competing financial interests.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.
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Figure Legends

**Figure 1.** Causal mediation model.

The aim of this study is to evaluate the extent to which the association between obesity (BMI ≥ 30) and severe disease (mechanical ventilation or death) among confirmed SARS-CoV-2-infected PCR-positive hospitalized patients was mediated by an increase in inflammation, as measured by CRP level, prior to mechanical ventilation, discharge or death. Potential confounders accounted for in the primary analysis included age, sex, race/ethnicity and number of CRP measurements. Fully adjusted models also included history of type 2 diabetes mellitus, hypertension, dyslipidemia and pulmonary disease. The proportion mediated is given by the Natural Indirect Effect (NIE) divided by the Total Effect = NIE + Natural Direct Effect (NDE).

**Figure 2.** CRP trajectory over time by obesity status for MGH cohort.

Subject level CRP trajectories are plotted against the number of days before and after the corresponding individual’s peak value (day = 0) for obese (left-hand panel) and non-obese (right-hand panel) patients. Patients who died or were mechanically ventilated 28 days of PTC are represented by gold lines, and those patients who survived and were not mechanically ventilated are represented by blue lines. Larger filled circles represent medians at the corresponding day from peak within patients who died or were mechanically ventilated (gold) or survived and were not mechanically ventilated (blue). The primary causal mediation analysis included the subset of CRP values up to and including the day of mechanical ventilation, death or discharge.
### Table 1. Clinical characteristics overall and by obesity status for MGH cohort

|                  | Overall (N=1202) | Obese (*) (N=499) | Not Obese (*) (N=592) | P-value (†) |
|------------------|-------------------|--------------------|-----------------------|-------------|
| **Presentation to Care (PTC)** |                   |                    |                       |             |
| Female sex– count/total (%) | 514/1202 (0.43) | 239/499 (0.48) | 241/592 (0.41) | 0.02        |
| Age in years (Median [IQR]) | 60.1 (46.4,73.7) | 56 (42.3,66.6) | 65 (52.2,77.9) | <0.001      |
| Age ≥ 65 years | 478/1201 (0.40) | 137/499 (0.27) | 296/592 (0.5) | <0.001      |
| Smoker (ever) | 443/1195 (0.37) | 161/494 (0.33) | 250/590 (0.42) | 0.001       |
| BMI (Median [IQR]) | 29.2 (25.7,33.9) | 34.4 (32.3,38.5) | 26.0 (23.7,27.7) | <0.001      |
| **Co-morbidities (‡)** |                   |                    |                       |             |
| CAD/MI | 178/1202 (0.15) | 62/499 (0.12) | 108/592 (0.18) | 0.011       |
| Hypertension | 616/1202 (0.51) | 247/499 (0.49) | 321/592 (0.54) | 0.135       |
| Dyslipidemia | 450/1202 (0.37) | 183/499 (0.37) | 238/592 (0.40) | 0.258       |
| Pulmonary disease history | 359/1198 (0.30) | 164/497 (0.33) | 163/591 (0.28) | 0.061       |
| Type 2 Diabetes Mellitus | 402/1202 (0.33) | 183/499 (0.37) | 181/592 (0.31) | 0.039       |
| **Outcomes at 28 days (§)** |                   |                    |                       |             |
| Mechanical ventilation or death | 390/1201 (0.32) | 171/499 (0.34) | 173/591 (0.29) | 0.089       |
| Mechanical ventilation (||) | 305/1202 (0.25) | 147/499 (0.29) | 122/592 (0.21) | 0.001       |
| Death | 161/1201 (0.13) | 54/499 (0.11) | 87/591 (0.15) | 0.069       |
| Ordinal Outcome Score = 1-3 | 825/1186 (0.70) | 341/494 (0.69) | 419/584 (0.72) | 0.364       |
| Ordinal Outcome Score = 4-5 | 116/1186 (0.10) | 57/494 (0.12) | 49/584 (0.08) | 0.104       |
| Ordinal Outcome Score = 6-8 | 245/1186 (0.21) | 96/494 (0.19) | 116/584 (0.20) | 0.92        |

(⁎)Obesity was defined as BMI ≤ 30 and is missing for 111 patients; (†)P-values correspond to a two-sample test of proportions (for categorical variables) or Wilcoxon rank sum tests for (numeric variables) comparing corresponding characteristic of obese versus non-obese patients; (‡)Co-morbidities – coronary artery disease (CAD), myocardial infarction (MI), hypertension, dyslipidemia, pulmonary disease history and type 2 diabetes mellitus (T2DM) – were manually extracted based on admission notes, problem lists from past medical history and history of present illness; (§)Score: 1 – not hospitalized, no limitations on activities; 2 – not hospitalized, limitation on activities and/or requiring home oxygen; 3 – hospitalized, not requiring oxygen, no longer requiring ongoing medical care; 4 – hospitalized, not requiring supplemental oxygen, requiring ongoing medical care; 5 – hospitalized requiring supplemental oxygen; 6 – hospitalized, on non-invasive ventilation or high flow oxygen; 7 – hospitalized, on invasive mechanical ventilation or ECMO; 8 – death; (||)N=85 patients died without being mechanically ventilated.
|                | Total Effect Model(†) | Mediator Model(†) | Outcome model(†) | Proportion        |
|----------------|-----------------------|-------------------|------------------|-------------------|
| **Outcome:**   |                       |                   |                  |                   |
| vent/death     |                       |                   |                  |                   |
| **Model:**     |                       |                   |                  |                   |
| CRP            |                       |                   |                  |                   |
| All ages (n=996) | 1.73 (p=0.001)        | 0.261 (p<0.001)   | 1.49 (p=0.035)   | 0.49 (p<0.001)    |
| ≥ 65 yrs (n=407) | 1.45 (p=0.145)        | 0.151 (p=0.146)   | 1.28 (p=0.399)   | 0.44 (p=0.180)    |
| < 65 yrs (n=589) | 1.99 (p=0.003)        | 0.325 (p<0.001)   | 1.57 (p=0.091)   | 0.52 (p<0.001)    |
| ESR            |                       |                   |                  |                   |
| All ages (n=904) | 1.38 (p=0.047)        | 0.184 (p=0.004)   | 1.32 (p=0.086)   | 0.14 (p=0.056)    |
| ≥ 65 yrs (n=357) | 1.10 (p=0.698)        | 0.199 (p=0.048)   | 1.05 (p=0.833)   | 0.08 (p=0.730)    |
| < 65 yrs (n=547) | 1.61 (p=0.030)        | 0.174 (p=0.037)   | 1.56 (p=0.044)   | 0.09 (p=0.076)    |
| D-dimer        |                       |                   |                  |                   |
| (n=662)        | 1.68 (p=0.003)        | 0.049 (p=0.419)   | 1.63 (p=0.006)   | 0.05 (p=0.456)    |
| ≥ 65 yrs (n=384) | 1.32 (p=0.298)        | -0.055 (p=0.593)  | 1.34 (p=0.275)   | <0.01 (p=0.800)   |
| < 65 yrs (n=578) | 2.14 (p=0.002)        | 0.129 (p=0.089)   | 1.97 (p=0.007)   | 0.11 (p=0.076)    |
| Ferritin       |                       |                   |                  |                   |
| (n=1005)       | 1.74 (p<0.001)        | 0.018 (p=0.766)   | 1.83 (p<0.001)   | 0.03 (p=0.770)    |
| ≥ 65 yrs (n=408) | 1.48 (p=0.125)        | -0.078 (p=0.446)  | 1.76 (p=0.041)   | <0.01 (p=0.496)   |
| < 65 yrs (n=597) | 1.98 (p=0.003)        | 0.042 (p=0.568)   | 1.98 (p<0.001)   | 0.07 (p=0.586)    |
| WBC            |                       |                   |                  |                   |
| (n=1039)       | 1.57 (p=0.008)        | -0.019 (p=0.768)  | 1.59 (p=0.008)   | 1.49 (p<0.001)    |
| ≥ 65 yrs (n=422) | 1.46 (p=0.156)        | -0.080 (p=0.438)  | 1.47 (p=0.153)   | <0.01 (p=0.600)   |
| < 65 yrs (n=617) | 1.58 (p=0.052)        | 0.042 (p=0.575)   | 1.58 (p=0.055)   | 0.02 (p=0.584)    |
| IL-6           |                       |                   |                  |                   |
| (n=307)        | 1.71 (p=0.046)        | 0.087 (p=0.459)   | 1.84 (p=0.047)   | 3.87 (p<0.001)    |
| ≥ 65 yrs (n=116) | 0.825 (p=0.645)       | -0.137 (p=0.484)  | 0.96 (p=0.922)   | 3.78 (p<0.001)    |
| < 65 yrs (n=191) | 3.56 (p=0.002)        | 0.217 (p=0.139)   | 3.81 (p=0.005)   | 5.40 (p<0.001)    |

(*) Peak biomarkers prior to mechanical ventilation, death or hospital discharge were used. All values were natural log transformed and standardized for analysis. (†) All models included terms for obesity and were adjusted for age, sex, race/ethnicity and number of biomarker measurements. Additional adjustments for type 2 diabetes mellitus, hypertension, dyslipidemia and pulmonary disease are included in sensitivity analysis. The outcome model included both obesity and the biomarker as predictor variables. (‡) The outcome model had a significant CRP by age interaction and therefore the main effect of CRP was not reported here. See Supplement Table S2 for more detailed results of this model fit.
### Table 3. Replication analysis for CRP\(^{(\ast)}\) using the CUIMC/NYP cohort

|                | Total Effect Model\(^{(†)}\) | Mediator Model\(^{(†)}\) | Outcome model\(^{(†)}\) | Proportion Mediated |
|----------------|------------------------------|--------------------------|-------------------------|---------------------|
| **Outcome:**   | OR (obesity, p)              | Est (obesity, p)         | OR (obesity, p)         | OR (biomarker, p)   | (p-value)          |
| **vent/death** | (All ages (n=1,972))         | 1.24 (p=0.037)           | 0.196 (p<0.001)         | 1.17 (p=0.167)      | \(0.67\) (p=0.002) |
|                | (\(\geq\) 65 yrs (n=1,054)) | 1.16 (p=0.318)           | 0.120 (p=0.071)         | 1.07 (p=0.671)      | \(0.63\) (p=0.230) |
|                | (< 65 yrs (n=918))           | 1.35 (p=0.042)           | 0.247 (p<0.001)         | 1.29 (p=0.133)      | \(0.68\) (p<0.001) |
| **Outcome:**   | CRP                          |                          |                         |                     |                    |
| **mediated**   | OR (biomarker, p)             |                          |                         |                     |                    |
| **vent/death** | (All ages (n=1,972))         | \(0.67\) (p=0.002)      |                         |                     |                    |
|                | (\(\geq\) 65 yrs (n=1,054)) |                          |                         |                     |                    |
|                | (< 65 yrs (n=918))           |                          |                         |                     |                    |

\(^{(\ast)}\)Peak CRP level was determined based on all measurements before and after mechanical ventilation. All values were natural log transformed for analysis. \(^{(†)}\)All models included terms for obesity and were adjusted for age, sex, and race/ethnicity. The outcome model included both obesity and the biomarker as predictor variables. \(^{(‡)}\)Outcome model had significant CRP by age interaction and therefore the main effect of CRP was not reported.
Figure 1

Exposure (T) → Biomarker (M) → Outcome (Y)

Natural Direct Effect (NDE)

Obesity

Peak inflammatory response (CRP, ESR, IL-6 etc)

Ventilation or death within 28 days of presentation to care

Natural Indirect Effect (NIE)
Figure 2

Obese

CRP

Not obese

Days from peak CRP

CRP

Mechanical Ventilation and/or Death

- no
- yes