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Original article

# Better prognosis in females with severe COVID-19 pneumonia: possible role of inflammation as potential mediator

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ABSTRACT

*Objectives:* Sex differences in COVID-19 severity and mortality have been described. Key aims of this analysis were to compare the risk of invasive mechanical ventilation (IMV) and mortality by sex and to explore whether variation in specific biomarkers could mediate this difference.
*Methods:* This was a retrospective, observational cohort study among patients with severe COVID-19 pneumonia. A survival analysis was conducted to compare time to the composite endpoint of IMV or death according to sex. Interaction was formally tested to compare the risk difference by sex in sub-populations. Mediation analysis with a binary endpoint IMV or death (yes/no) by day 28 of follow-up for a number of inflammation/coagulation biomarkers in the context of counterfactual prediction was also conducted.
*Results:* Among 415 patients, 134 were females (32%) and 281 males (67%), median age 66 years (IQR 54–77). At admission, females showed a significantly less severe clinical and respiratory profiles with a higher $PaO_2/FiO_2$ (254 mmHg vs. 191 mmHg; p 0.023). By 28 days from admission, 49.2% (95% CI 39.6–58.9%) of males vs. 31.7% (17.9–45.4%) of females underwent IMV or death (log-rank $p < 0.0001$) and this amounted to a difference in terms of HR of 0.40 (0.26–0.63, p 0.0001). The area under the curve in C-reactive protein (CRP) over the study period appeared to explain 85% of this difference in risk by sex.
*Discussion:* Our analysis confirms a difference in the risk of COVID-19 clinical progression by sex and provides a hypothesis for potential mechanisms leading to this. Specifically, CRP showed a predominant role to mediate the difference in risk by sex. **Cristina Mussini, Clin Microbiol Infect 2021;27:1137**


## Introduction

The clinical spectrum of COVID-19 is heterogeneous, ranging from asymptomatic cases to severe disease [1–3]. Reported mortality rate of COVID-19 ranges between 1.4% and 61.0% [4–6]. Main risk factors for poor prognosis are male sex, ethnicity, age

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>65 years, overweight and pre-existing co-morbidities [5,7–9]. Indeed, women seem to be protected from developing a severe disease [4,10,11], intensive care unit (ICU) admission and mortality [1,5,6,12]. A recent study also showed a significant difference by sex in laboratory parameters abnormalities, suggesting a mediation effect on prognosis [13].

Several mechanisms have been hypothesized. A difference by sex expression/function of angiotensin converting enzyme 2 (ACE2), the host cell receptor of the virus, was found in both animal models and in humans (over-expressed in females). Male mice are more susceptible to severe acute respiratory syndrome-coronavirus-1 (SARS-CoV-1) infection than age-matched females, but this protection is lost after ovariectomy [14], implying a pivotal role of sex hormones [15]. In humans, Asian men showed higher ACE2 expression than women [16].

From an immunological perspective, females mount stronger innate and adaptive immune responses than males, resulting in faster clearance of pathogens parallel to an increased susceptibility to autoimmune diseases [17].

The aim of this analysis is twofold: first to compare the risk of invasive mechanical ventilation (IMV) and mortality by sex and, second, to explore whether variation in specific biomarkers could mediate this difference.

## Materials and methods

This was a retrospective, observational cohort study carried out at the University Hospital of Modena, Italy, among patients admitted to hospital between 21 February and 25 May 2020 for severe COVID-19 pneumonia. The study was approved by the regional ethics committee. All consecutively enrolled adult patients (≥18 years) with severe COVID-19 pneumonia, defined by the presence of at least one of the following criteria: a respiratory rate (RR) ≥30 breaths per minute (bpm), peripheral blood oxygen saturation ($SaO_2$) ≤93%, a $PaO_2/FiO_2$ ratio <300 mmHg in room air and lung infiltrates >50% within 24–48 hr, were included in the study [18,19].

Patients were treated according to standard of care (SoC) consisting of hydroxychloroquine, lopinavir and low molecular weight heparin, and a non-randomly selected subset of patients received tocilizumab treatment in addition to SoC as described elsewhere [20].

### *Outcome measures*

The primary outcome of the study was the composite endpoint of IMV or death; the secondary outcome was all-cause mortality.

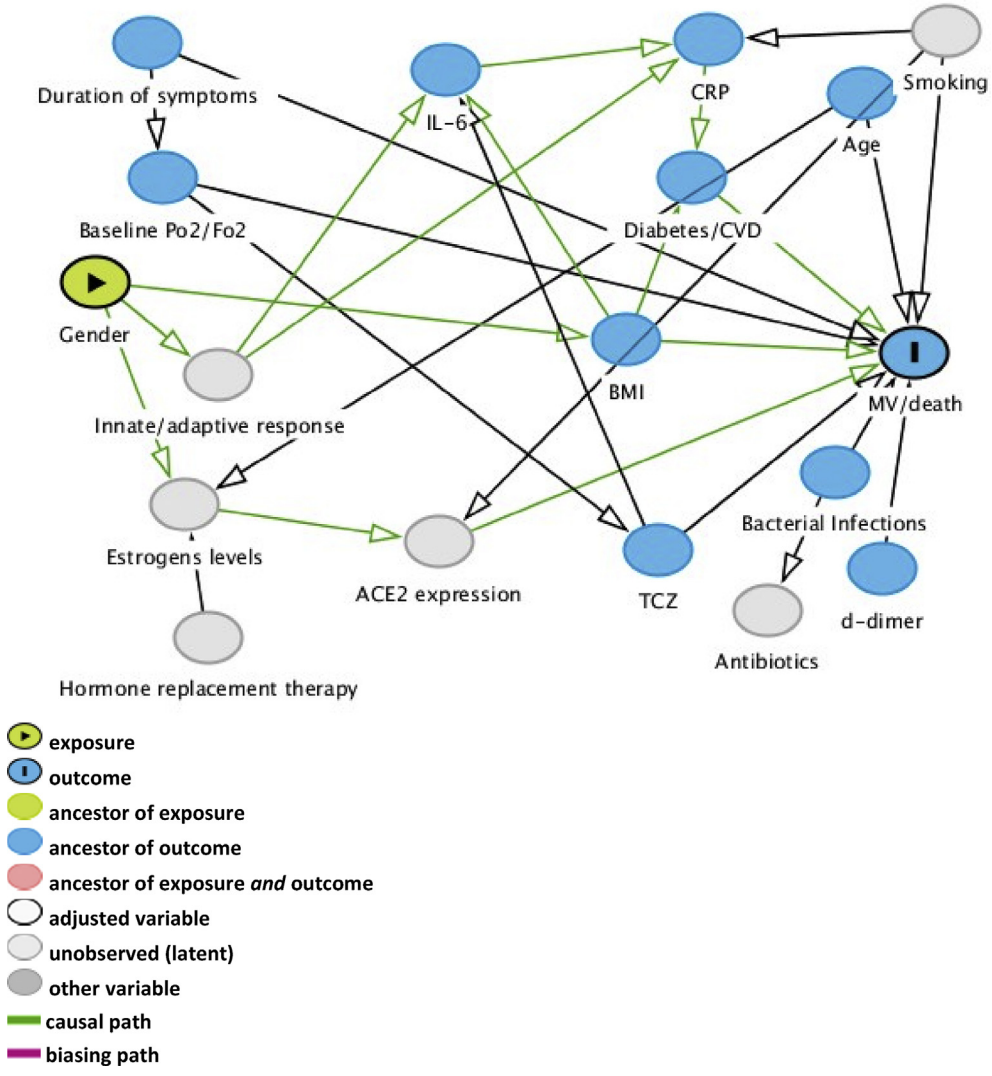



**Fig. 1.** Direct acyclic graph (DAG) of the underlying causal structure of the data.

*Statistical analysis*

Baseline characteristics of the participants were compared according to sex. Continuous variables were expressed as median (IQR) and compared using the Mann–Whitney U test. Categorical variables were expressed as numbers (%) and compared by sex using the chi-squared test or Fisher's exact test.

Standard survival analysis was performed with participants' follow-up accrued from the date of hospital entry until initiation of IMV (see footnote of Fig. 2 for exact defintion) or death by means of unweighted Kaplan–Meier curves and univariable Cox regression analysis. The effect of sex is shown by means of unadjusted hazard ratio (HR) with 95% confidence intervals (CI) from fitting an unadjusted standard Cox regression model. The key assumption was that sex was un-confounded so only univariable analyses were performed. This assumption was tested in the data by fitting a multivariable model adjusting for potential confounding factors and results were similar (Supplementary material Fig. S3 and Table S2). The sex effect was further evaluated in subsets of the study population (stratified by extent of existing co-morbidities, age, post baseline glucocorticoids use, body mass index (BMI) and use of tocilizumab, respectively) and the HRs in the strata were shown by means of a forest plot and formally compared using an interaction test in the Cox regression model.

For the mediation analysis, the time to event framework was simplified to the risk of developing the primary outcome by day 28 from admission (binary endpoint). Because all patients had completed follow-up by day 28 (either experienced the endpoint or were discharged), this amounted to a reasonable approximation.

Sex differences in C-reactive protein (CRP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and serum creatinine were previously found and it was hypothesized that these differences could explain the sex-specific clinical characteristics and outcomes [13]. We considered the same set of biomarkers in this investigation.

The underlying causal structure of the model is described in Fig. 1 through the visual aid of a direct acyclic graph (DAG). The hypothesis is that the total effect of sex is the sum of a direct effect and of an indirect effect mediated through the burden of exposure to biomarkers over follow-up (Fig. 1). The indirect pathway entailed the assumption that males are more likely to be obese and that obesity, in turn, will lead to an increase in CRP levels which will trigger a higher probability of developing diabetes and other complications of COVID-19 leading to the need of invasive ventilation and ultimately death.

The post-baseline biomarker burden was calculated for each participant and each of the biomarkers, using a summary measure for the repeated measures of the markers, as the area under the curve (AUC) in follow-up divided per the exact number of days of follow-up. We also considered the biomarkers' peak over follow-up as an alternative summary measure. This hypothesized model was then in turn separately applied to each of the biomarkers and the percentage of the total effect of sex which was explained by the indirect effect pathway was calculated.

For the markers showing the larger mediation effect, we also described the AUC values and the mean baseline values and slopes over follow-up by sex using a standard mixed linear regression model with random intercept and slope. Unsupervised learning was also used to plot the AUC of the markers on a plane using the first two principal components in a principal component analysis (PCA) in which also individual data points were plotted and labelled by sex.

Symmetric secondary analyses were performed for the endpoint death alone (using a competing risk approach including all deaths).

A two-sided test of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SAS software, version 9.4 (Carey NC, USA) apart from the mediation analysis which was performed using the command 'Medeff' in Stata v16 and unsupervised learning which was done using the Factoshiny GUI of the Factorminer library in R. The DAG was drafted using the R codes in www.dagitty.net.

**Table 1**
Epidemiological and clinical characteristics by sex

| Characteristics | Sex | | | |
|---|---|---|---|---|
| | Female | Male | p value[a] | Total |
| | $n = 134$ | $n = 281$ | | $n = 415$ |
| **Age, years** | | | | |
| Median (IQR) | 69 (54, 79) | 65 (55, 76) | 0.29 | 66 (54, 77) |
| **BMI, kg/m$^2$** | | | | |
| Median (IQR) | 30.1 (25.4, 33.2) | 26.2 (24.5, 29.4) | 0.009 | 26.7 (24.7, 30.9) |
| **Smoking, *n* (%)** | 16/41 (39) | 60/113 (53) | 0.14 | 76/154 (49) |
| **Any comorbidity, *n* (%)** | | | | |
| Yes | 44 (32.8) | 87 (31.0) | 0.70 | 131 (31.6) |
| **Comorbidities, *n* (%)** | | | | |
| Diabetes | 18 (13.4) | 28 (10.0) | 0.29 | 46 (11.1) |
| Hypertension | 39 (29.1) | 79 (28.1) | 0.84 | 118 (28.4) |
| Cardiovascular disease | 7 (5.2) | 32 (11.4) | 0.04 | 39 (9.4) |
| Chronic renal insufficiency | 9 (6.7) | 12 (4.3) | 0.29 | 21 (5.1) |
| Cancer | 6 (4.5) | 7 (2.5) | 0.28 | 13 (3.1) |
| Hepatitis B/C virus | 0 (0.0) | 0 (0.0) | | 0 (0.0) |
| **Disease duration** | | | | |
| Days from symptoms onset to hospitalization, median (IQR) | 7 (3, 13) | 7 (3, 10) | 0.11 | 7 (3, 11) |
| Days from hospitalization to intubation, median (IQR) | 2 (0, 2) | 3 (1, 4) | 0.10 | 3 (1, 4) |
| **Sign and symptoms, *n* (%)** | | | | |
| Fever, median (IQR) | 37 (36, 37) | 36 (36, 37) | 0.54 | 36 (36, 37) |
| Cough | 40 (29.9) | 98 (34.9) | 0.31 | 138 (33.3) |
| Myalgia | 11 (8.2) | 9 (3.2) | 0.03 | 20 (4.8) |
| Sputum | 4 (3.0) | 6 (2.1) | 0.60 | 10 (2.4) |
| Headache | 10 (7.5) | 16 (5.7) | 0.49 | 26 (6.3) |
| Haemoptysis | 0 (0.0) | 3 (1.1) | 0.23 | 3 (0.7) |
| Systolic pressure, mmHg, median (IQR) | 120 (110, 130) | 125 (110, 135) | 0.14 | 123 (110, 135) |

[a] Chi-squared or Mann-Whitney test as appropriate.

## Results

During the study period, 415 patients with severe COVID-19 pneumonia were admitted. Among them, 134 were females (32.3%) and 281 males (67.7%). Epidemiological and respiratory characteristics are shown in Table 1.

Females had significantly higher baseline $PaO_2/FiO_2$ (254 mmHg vs. 191 mmHg; p 0.023), a lower lactate dehydrogenase (LDH = 527 vs. 597, p 0.001) and a lower SOFA score (1 vs. 2; p < 0.019) (Tables 2 and 3). In addition, women had higher platelet counts, but lower haemoglobin, ALT, creatinine phosphokinase, calcium and creatinine. The inflammatory profile at hospital admission was also better among women who showed lower levels of D-dimer, CRP and ferritin (Table 3).

By 14 days from admission, 93 males (38.3%; 95% CI 31.9–44.7%) had undergone IMV or had died; by 28 days this proportion increased to 49.2% (95% CI 39.6–58.9%). The equivalent estimates for females were lower at 16.5% (95% CI 8.9–24.2%) and 31.7% (95% CI 17.9–45.4%), respectively (log-rank test p < 0.0001). The difference by sex at 28 days was less marked for the mortality endpoint: 33.6% (95% CI 25.4–41.8%) in males vs. 20.9% (95% CI 10.1–31.7%) females (log-rank test p 0.06), counting all in-hospital deaths.

Infections were recorded for a subset of 381 participants (92%). There was a total of 48 infections (73% bacterial pneumonias ($n = 35$), 15% bloodstream infections (BSIs, $n = 7$), 10% urinary tract infections (UTIs, $n = 5$) and one (female) with a dental abscess (2%). There was no evidence for an unequal distribution of these events by sex (p 0.37). The proportions in males vs. females were 11% vs. 6% for pneumonia, 1.9% vs. 1.8% for BSI and 1.1% vs. 1.8% for UTI. There were 12 deaths that could be attributable to bacterial pneumonias, 10/267 (3.8%) in males and 2/114 (1.8%) in females (Fisher exact p 0.52).

Figs. 2A,B shows the forest plot of the HR for the composite endpoint comparing females with males in specific subpopulations, with interaction test p values. The overall effect of sex on the risk of IMV or death was HR = 0.40 (95% CI: 0.26–0.63, p 0.0001) and on mortality alone was HR = 0.61 (95% CI: 0.36–1.025,

**Table 2**
Respiratory function/disease severity, treatment and clinical outcomes by sex

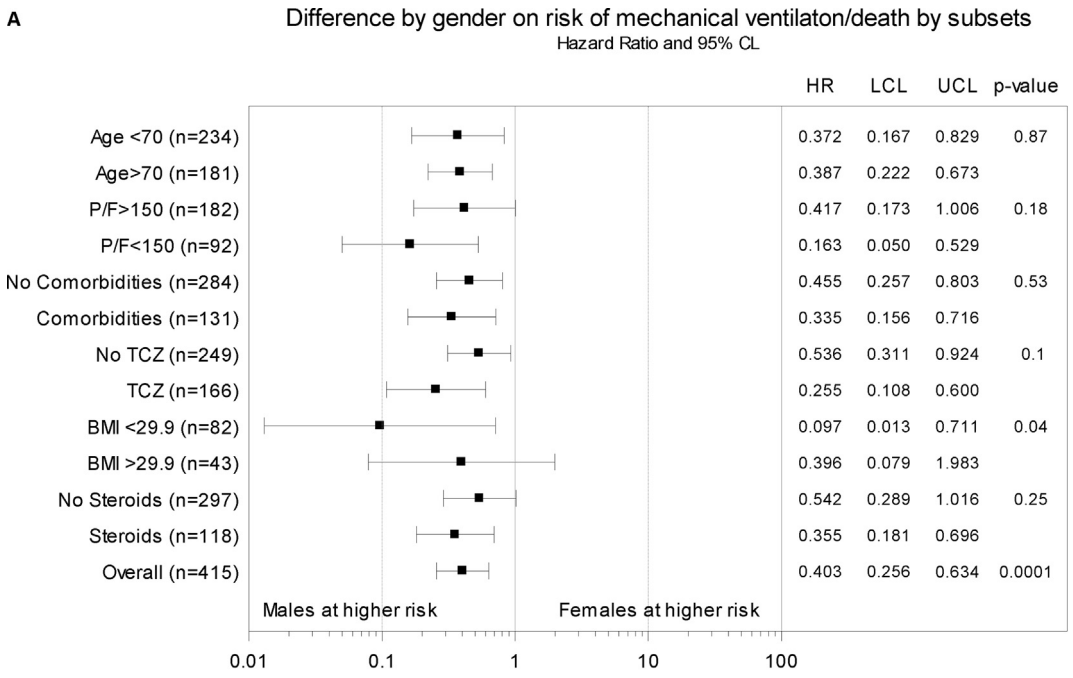
| | Sex | | | |
|---|---|---|---|---|
| Characteristics | Female | Male | p-value[a] | Total |
| **Respiratory function,** median(IQR) | N = 77 | N = 197 | | N = 274 |
| *Baseline $PaO_2/FiO_2$, mmHg* | 254 (140, 336) | 191 (122, 294) | 0.02 | 216 (126, 302) |
| Respiratory rate, % | 22 (18, 28) | 22 (18, 28) | 0.89 | 22 (18, 28) |
| *SOFA Score* | 1 (0, 3) | 2 (0, 3) | 0.02 | 2 (0, 3) |
| ***Intervention**, n(%)* | N = 134 | N = 281 | 0.25 | N = 415 |
| Tocilizumab subcutaneous | 21 (16.8) | 50 (20.8) | | 71 (19.5) |
| Tocilizumab intravenous | 20 (16.0) | 25 (10.4) | | 45 (12.3) |
| SOC | 84 (67.2) | 165 (68.8) | | 249 (68.2) |
| ***Use of antibiotics**, n(%)* | N = 114 | N = 267 | | N = 381 |
| | 10 (8.8) | 35 (13.1) | | 45 (11.8) |
| ***Events**, n(%)* | N = 134 | N = 281 | | N = 415 |
| Invasive echanical ventilation (IMV) | 8 (6.0) | 58 (20.6) | <.001 | 66 (15.9) |
| Death - pre IMV | 15 (11.2) | 42 (14.9) | 0.30 | 57 (13.7) |
| Death - all | 19 (14.2) | 62 (22.1) | 0.06 | 81 (19.5) |

[a] Chi-squared or Mann-Whitney test as appropriate.

**Table 3**
Baseline biomarkers by sex

| Blood tests | Sex | | | | |
|---|---|---|---|---|---|
| | *N* | Female | Male | p value[a] | Total |
| **Markers, Median (IQR)** | | | | | |
| Haemoglobin, g/dL | 380 | 12.0 (10.5, 13.1) | 13.3 (12.1, 14.2) | <0.001 | 12.8 (11.4, 14.0) |
| White cells, $mm^3$ | 380 | 5760 (4540, 8400) | 6340 (4930, 8520) | 0.27 | 6110 (4810, 8480) |
| Total lymphocytes, *N* | 264 | 1368 (730.0, 2420) | 1584 (870.0, 2390) | 0.43 | 1545 (857.5, 2405) |
| Total lymphocytes, % | 289 | 20.2 (7.2, 37.5) | 25.4 (11.0, 34.7) | 0.53 | 22.9 (9.1, 35.3) |
| Alanine amino-transferase, U/L | 225 | 31.0 (21.0, 44.0) | 39.0 (27.0, 58.0) | 0.002 | 35.0 (25.0, 54.0) |
| Bilirubin, mg/dL | 370 | 0.4 (0.3, 0.6) | 0.6 (0.5, 0.8) | <0.001 | 0.6 (0.4, 0.8) |
| Calcium, mg/dL | 356 | 8.7 (8.3, 9.1) | 8.6 (8.2, 8.9) | 0.02 | 8.6 (8.2, 9.0) |
| Creatine Kinase, U/L | 375 | 51.5 (33.0, 108.0) | 132.0 (57.0, 282.0) | <0.001 | 88.0 (44.0, 221.0) |
| Chloride, mmol/L | 353 | 100.0 (98.0, 102.0) | 100.0 (98.0, 103.0) | 0.58 | 100.0 (98.0, 103.0) |
| Creatinine, mg/dL | 380 | 0.7 (0.6, 0.9) | 0.9 (0.8, 1.2) | <0.001 | 0.9 (0.7, 1.1) |
| D-dimer, ng/mL | 375 | 1015 (640.0, 2070) | 1160 (640.0, 2210) | 0.59 | 1150 (640.0, 2170) |
| Lactate dehydrogenase, U/L | 379 | 527.0 (397.0, 651.0) | 597.0 (461.5, 767.0) | 0.001 | 563.0 (446.0, 726.0) |
| C-reactive protein, mg/dL | 380 | 5.8 (2.0, 14.4) | 7.5 (4.1, 17.5) | <0.001 | 7.0 (3.2, 16.4) |
| Platelets, $10^9$/L | 380 | 229.0 (166.0, 292.0) | 199.0 (149.0, 269.0) | 0.04 | 207.5 (155.0, 274.5) |
| Potassium, mmol/l | 369 | 3.8 (3.5, 4.2) | 3.8 (3.5, 4.1) | 0.68 | 3.8 (3.5, 4.1) |
| Sodium, mmol/L | 369 | 137.0 (135.0, 139.0) | 137.0 (134.0, 139.0) | 0.56 | 137.0 (135.0, 139.0) |
| IL-6, pg/mL | 262 | 189.7 (67.4, 366.0) | 193.4 (77.2, 399.9) | 0.46 | 191.8 (71.0, 399.9) |
| Ferritin, ng/ml | 244 | 433.5 (207.0, 720.0) | 906.5 (535.0, 1492) | <0.001 | 650.0 (368.0, 1251) |

[a] Mann-Whitney test.



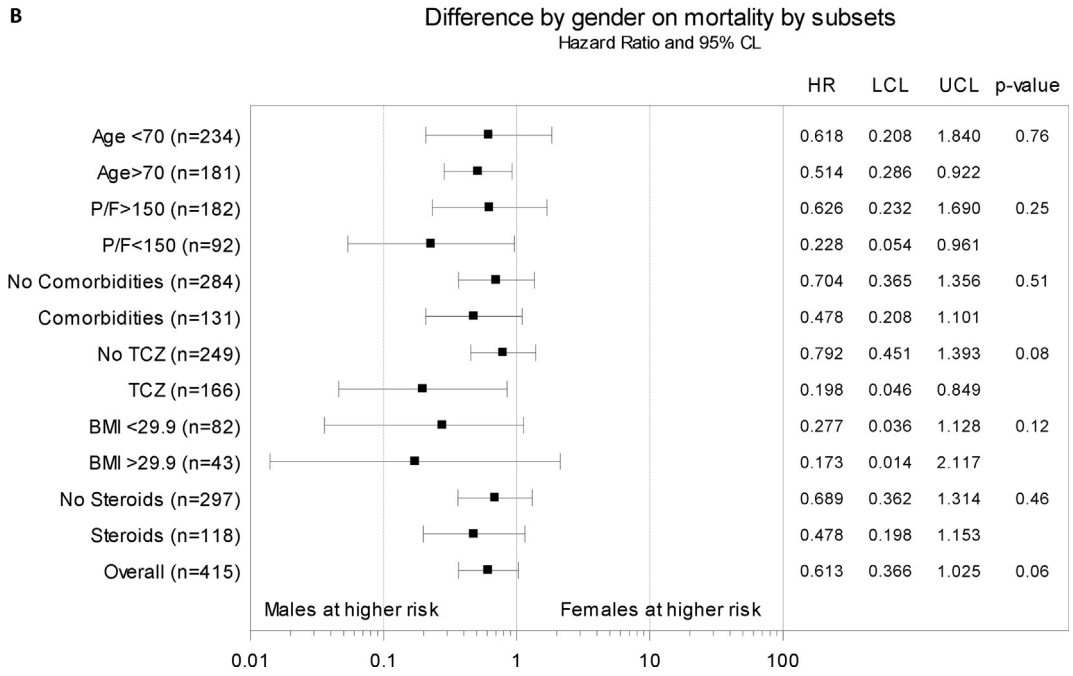




**Fig. 2.** (A) HR of invasive mechanical ventilation (IMV)[a]/death associated with sex overall and in subsets from fitting a standard unadjusted Cox regression model. [a]Definition: Indication for IMV were neurologic failure (i.e. altered consciousness with a Glasgow Coma Scale score <10), cardiovascular failure (i.e. vasopressor requirement or major ECG changes including arrhythmia or changes in repolarization phase) and respiratory failure defined by the presence of at least two of the following criteria: respiratory rate >30 bpm, respiratory distress with activation of accessory respiratory muscles, need for $FiO_2$ at 80% or more to maintain an $SaO_2$ level at 90%, or a $PaO_2/FiO_2$ <100 mm [23]. (B) HR of death associated with sex overall and in subsets from fitting a standard unadjusted Cox regression model.

p 0.060). There was no evidence that these HR varied in specific subpopulations with perhaps the exception of BMI for risk of IMV/death (HR = 0.097, 95% CI 0.01 − 0.71 when comparing females with males with a BMI <29.9, interaction p 0.04, Fig. 2A).

Difference in plasma CRP values at hospital admisison between males vs. females was 0.32 log10 mg/dL (p 0.65), as estimated by fitting a linear mixed regression model (Table S1 and Fig. S1).

AUC-CRP over the study period (log10 scale) was 0.51 mg/dL (SD 0.55) in males vs. 0.18 (SD 0.55) mg/dL in females (t-test $p < 0.0001$). A similar difference was observed comparing peaks in CRP by sex: 1.02 mg/dL in males vs. 0.75 in females ($p < 0.0001$). The burden over follow-up of other inflammatory and biochemical markers analysed was higher in males vs. females, namely the AUC of interleukin (IL)-6 (2.35 vs. 2.09 pg/mL

p 0.006), LDH (2.78 vs. 2.70 U/L, p 0.0008), ferritin (2.89 vs. 2.57 ng/mL, p < 0.0001) and to a lower extent ALT (1.57 vs. 1.47 U/L, p 0.02). Unsupervised learning indicated that 39% of the variables cloud total variability was explained by the first two dimensions. A cluster in the first dimension was characterized by high values for the post baseline AUC of haemoglobin, CRP, platelets, ferritin, ALT, IL-6 and LDH which matched the location of male gender (Fig. S2A/B).

We finally tested the underlying model depicted by the DAG in Fig. 1 by means of a formal mediation analysis (Tables 4 and 5).

Table 4 shows that, the risk of IMV and death would decrease, on average, by only 2% in females vs. males, under the hypothetical scenario in which all participants would keep the CRP AUC level that was seen in males (direct effect). Of note, this is an estimate of the sex effect on risk of IMV/death which is not explained by post-baseline variation in CRP, since this was held fixed and it appears to be small. In contrast, the estimate of the average indirect effect is much larger and indicates that, regardless of sex, the risk of IMV/death is likely to decrease by 10% per 1 mg/dL/day difference in CRP-AUC. When expressed as a relative proportion, the CRP mediation effect on sex towards the risk of IMV/death was 85% when considering the summary measure AUC/day and 53% when alternatively considering the peak.

The AUC mediation effect for other biomarkers, using the same underlying causal structure hypothesised for CRP in Fig. 1, was 47% for ferritin and 43% for LDH, while for all other biomarkers the impact was much lower and below 30% (Table 4).

Similar results were observed considering the death end point. Mediation effect was the highest for CRP AUC (78% when using AUC, and 74% when using CRP peak), while 64% was the mediation effect for LDH and 62% for ferritin (Table 5).

## Discussion

This study explores the impact of sex on risk of unfavourable COVID-19 outcomes. In a cohort of hospitalized patients with severe COVID-19 pneumonia, we observed a significant lower risk of IMV or death in women (60% risk reduction). This signal was also observed with regards to the death end point, although data were more compatible with the null hypothesis (HR = 0.61, p 0.060), probably due to low number of deaths. Our results are consistent with those shown in previously published study on sex in COVID-19 (Fig. 2) [13].

Levels of oestrogens may affect ACE2 expression [21] potentially reducing virus entry and progression of COVID-19 disease, suggesting that sex difference could be attenuated in the elderly population. Nevertheless, sex difference was similar in women aged 70+ (or 50+ in a sensitivity analysis).

BMI appeared to be an effect modifier for the association between sex and the risk of experiencing the composite endpoint. Indeed, females with a BMI <29.9 kg/m$^2$, were more protected than males with similar BMI from the risk of IMV and death compared to participants with BM>29.9 kg/m$^2$. Explanation for this interaction is unclear, it is possible that non-obese men were more likely to be hospitalized because they had more important risk factors for death than obesity, so the results are due to collider bias.

Regarding the impact of specific biomarkers, high levels of CRP both at baseline and during follow-up were explaining most of the difference seen by sex.

Our key assumption was that sex is randomly allocated at birth, and herefore it is virtually an un-confounded variable, an assumption which was confirmed by supplementary analyses (Fig. S3 and Table S2).

**Table 4**
Mediation analysis. Risk of invasive mechanical ventilation/death for female vs. male

| | Direct effect | | Indirect effect | | Percentage of total effect mediated |
|---|---|---|---|---|---|
| | % | 95% CI | % | 95% CI | |
| Biomarker (per log10 higher) | | | | | |
| Total lymphocytes, AUC | −11 | −21%; 0% | 0 | −2%; +2% | 0.5 |
| Alanine amino-transferase, AUC | −15 | −24%; −5% | +2 | 0%; +5% | −18 |
| D-dimer, AUC | −13 | −22%; −3% | −0.4 | −1%; 0% | 3 |
| Lactate dehydrogenase, AUC | −7 | −16%; +3% | −5 | −10%;−1% | 43 |
| C-reactive protein, AUC | −2 | −11%; +8% | −10 | −16%; −6% | 85 |
| C-reactive protein, peak | −7 | −16%; +3% | −7 | −12%; −4% | 53 |
| Platelets, AUC | −12 | −21%; −2% | −2 | −5%; −1% | 16 |
| IL-6, AUC | −16 | −26%; −5% | −4 | −8%; 0% | 18 |
| Ferritin, AUC | −4 | −16%; +8% | −5 | −10%; 0% | 47 |
| Haemoglobin, AUC | −19 | −27%; −1% | +1 | 0%; +5% | −10 |
| Use of steroids, yes/no | −9 | −18%; 0% | −3 | −8%; 0% | 27 |

**Table 5**
Mediation analysis. Risk of death for female vs. male

| | Direct effect | | Indirect effect | | Percentage of total effect mediated |
|---|---|---|---|---|---|
| | % | 95% CI | % | 95% CI | |
| Biomarker (per log10 higher) | | | | | |
| Total lymphocytes, AUC | −6 | −16%; +5% | 0 | −1%; +2% | 0.5 |
| Alanine amino-transferase, AUC | −4 | −15%; +8% | −2 | −4%; 0% | 19 |
| D-dimer, AUC | −9 | −16%; 0% | −1 | −0.3%; 0% | 14 |
| Lactate dehydrogenase, AUC | −3 | −11%; +6% | −6 | −10%;−2% | 64 |
| C-reactive protein, AUC | −2 | −10%; +6% | −7 | −12%; −4% | 78 |
| C-reactive protein, peak | −3 | −10%; +5% | −9 | −13%; −5% | 74 |
| Platelets, AUC | −8 | −16%; 0% | 0 | −3%; −1% | 10 |
| IL-6, AUC | −11 | −20%; 0% | −4 | −5%; 0% | 16 |
| Ferritin, AUC | 0 | −10%; +12% | −4 | −10%; −1% | 62 |
| Haemoglobin, AUC | −11 | −19%; −2% | +2 | 0%; +5% | −28 |
| Use of steroids, yes/no | −7 | −15%; 0% | −1 | −3%; 0% | 12 |

Indeed, our data support the hypothesis that the difference in risk of outcome by sex is only marginally explained by our presumed direct pathway, acting through oestrogen-dependent blockage of virus entry through the ACE2 receptor.

We postulated other possible pathways, including one which assumes a mediation effect of inflammatory biomarkers. At the basis of this alternative pathway is the innate immune response, which leads to the hypothesis of a mediating effect of CRP. Indeed, our data support that the CRP inflammation pathway is likely to be the leading mediation effect of the sex-difference observed (the AUC of CRP post baseline explains 85% of the difference in clinical progression between sex).

Of interest, mediation effects explaining >50% of the total sex effect were found also for ferritin and LDH markers that were both clustering with CRP and male sex by unsupervised learning analysis. In contrast, we did not find, as hypothesized in the DAG, any significant mediation effect of D-dimer which accounted only for a 3% of the sex specific effect. Taken all this together, we argue that the majority of difference by sex seen in COVID-19 outcomes is mediated by the effect of CRP.

CRP is a well-known marker of inflammation and cardiovascular risk mainly synthesized by hepatocytes after stimulation of IL-6 and to a lesser extent of IL-1beta [22,23]. Moreover, it is an established predictor of COVID-19 complications because it acts as a direct mediator of inflammatory reactions and the innate immune response and it has a tight connection with incident diabetes [24]. Both these causal links are explicitly indicated in the DAG as the pathways underlying the mechanisms of the predominant mediating role of CRP.

Our study presents several limitations. First, it is an observational study and, despite our assumption of no confounding being present, unmeasured confounding cannot be excluded. Second, the magnitude of the effects and the interpretation of the results heavily rely on the causal structure hypothesised in the DAG and on our simple linear model without interactions. Third, despite accurate data collection, BMI and $PiO_2/FiO_2$ data were missing for a number of participants and this could have introduced residual confounding and collider bias.

Nevertheless, several points of strengths should also be considered. This is the largest study conducted so far, specifically evaluating the impact of sex on COVID-19 prognosis. Data have been collected in real time during the first wave of COVID-19 pandemic in Italy, resulting in a complete dataset for most variables used in the analysis (e.g., socio-demographics, treatment and outcome). Second, the underlying causal structure has been transparently depicted using a DAG. Third, to our knowledge, this is the first analysis attempting to gain insights into the mechanism that underlies the effect of sex on COVID-19 prognosis.

In conclusion, our study not only confirms a difference in risk of COVID-19 clinical outcome by sex, but also highlights the predominant role of CRP to mediate the observed difference by sex. Further studies are needed to confirm our observation.

## Transparency declaration

Conflict of interest: None to declare. This study was not funded.

## Author contributions

C.M., A.C.L., Mar.Men., Ma.Mass., E.F. and G.G. conceptualized and designed the study. C.M., A.C.L., Mar.Men., Ma.Mass., J.M. and G.G. wrote and revised the manuscript. C.M., A.C.L., Mar.Men., Ma.Mass., J.M. and G.G. did the supervision of the final version of the manuscript. ACL did the statistical analysis. All the authors contributed to data collection, clinical management of the patients and data interpretation.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.12.010.

## References

[1] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507–13.
[2] Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. JAMA Cardiol 2020;5:802–10.
[3] Fogarty H, Townsend L, Ni Cheallaigh C, Bergin C, Martin-Loeches I, Browne P, et al. More on COVID-19 coagulopathy in Caucasian patients. Br J Haematol 2020;189:1060–1.
[4] Guan W, Ni Z, Hu Y, Liang W, Ou Q, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708–20.
[5] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506.
[6] Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475–81.
[7] Zhang J, Wang X, Jia X, Li J, Hu K, Chen G, et al. Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. Clin Microbiol Infect 2020;26:767–72.
[8] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020;180:934–43.
[9] Fogarty H, Townsend L, Ni Cheallaigh C, Bergin C, Martinloeches I, Browne P, et al. COVID-19 coagulopathy in Caucasian patients. Br J Haematol 2020;189:1044–9.
[10] Sharma G, Volgman AS, Michos ED. Sex differences in mortality from COVID-19 pandemic: are men vulnerable and women protected? JACC Case Rep 2020;2:1407–10.
[11] Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol 2020;146:110–8.
[12] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–9.
[13] Meng Y, Wu P, Lu W, Liu K, Ma K, Huang L, et al. Sex-specific clinical characteristics and prognosis of coronavirus disease-19 infection in Wuhan, China: a retrospective study of 168 severe patients. PLoS Pathog 2020;16:e1008520.
[14] Channappanavar R, Fett C, Mack M, Ten Eyck PP, Meyerholz DK, Perlman S. Sex-based differences in susceptibility to severe acute respiratory syndrome coronavirus Infection. J Immunol 2017;198:4046–53.
[15] La Vignera S, Cannarella R, Condorelli RA, Torre F, Aversa A, Calogero AE. Sex-specific SARS-CoV-2 mortality: among hormone-modulated ACE2 expression, risk of venous thromboembolism and hypovitaminosis D. Int J Mol Sci 2020;21:2948.
[16] Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. bioRxiv 2020:919985.
[17] Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016;16:626–38.
[18] Zhou F, Yu T, Du R, Fan G, Liu Y, Li Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;6736:1–9.

[19] National Health Commission of the People's Republic of China. Chinese management guideline for COVID-19 (version 6.0). Feb 19 2020. Available at: http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfeb1bc54639af227f922bf6b817. pdf. [Accessed 6 April 2020] (in Chinese).
[20] Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol 2020;2:e474–84.
[21] Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Version 2. Impact of sex and gender on COVID-19 outcomes in Europe. Biol Sex Differ 2020;11:29.
[22] Ji SR, Ma L, Bai CJ, Shi JM, Li HY, Potempa LA, et al. Monomeric C-reactive protein activates endothelial cells via interaction with lipid raft microdomains. FASEB J 2009;23:1806–16.
[23] Zouki C, Haas B, Chan JS, Potempa LA, Filep JG. Loss of pentameric symmetry of C-reactive protein is associated with promotion of neutrophil-endothelial cell adhesion. J Immunol 2001;167:5355–61.
[24] Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, et al. Race and sex differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464–9.