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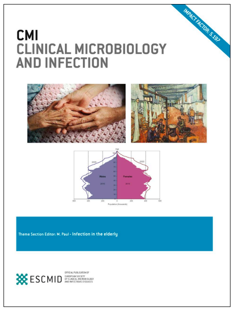

Better prognosis in females with severe COVID-19 pneumonia: possible role of inflammation as potential mediator

Cristina Mussini, MD, Prof., Alessandro Cozzi-Lepri, PhD, Prof., Marianna Menozzi, MD, Marianna Meschiari, MD, Erica Franceschini, MD, PhD, Carlotta Rogati, MD, Gianluca Cuomo, Andrea Bedini, Vittorio Iadisernia, MD, Sara Volpi, MD, Jovana Milic, MD, Roberto Tonelli, MD, Lucio Brugioni, MD, Antonello Pietrangelo, MD, Prof., Massimo Girardis, MD, Andrea Cossarizza, MD, PhD, Prof., Enrico Clini, MD, Prof., Giovanni Guaraldi, MD, Prof., Modena Covid-19 Working Group (MoCo19), Office of information and communication technologies of Policlinico di Modena

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

Prof. Cristina Mussini, MD[1,2*], Prof. Alessandro Cozzi-Lepri, PhD[3*], Marianna Menozzi, MD [1], Marianna Meschiari, MD [1], Erica Franceschini, MD, PhD [1], Carlotta Rogati, MD[2], Gianluca Cuomo [1], Andrea Bedini [1], Vittorio Iadisernia, MD[2], Sara Volpi, MD[2], Jovana Milic, MD [2,4], Roberto Tonelli, MD [4,5], Lucio Brugioni, MD[6], Prof. Antonello Pietrangelo [7], MD, Massimo Girardis, MD[2,8], Prof. Andrea Cossarizza, MD, PhD[7], Prof. Enrico Clini, MD[5,7], Prof. Giovanni Guaraldi, MD[1,2]

*** These authors share first authorship.**

1 Department of Infectious Diseases, Azienda Ospedaliero-Universitaria Policlinico of Modena, Modena, Italy

2 Department of Surgical, Medical, Dental and Morphological Sciences University of Modena and Reggio Emilia, Italy

3 Centre for Clinical Research, Epidemiology, Modelling and Evaluation (CREME), Institute for Global Health, UCL, London, UK

4 Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy

5 Respiratory Diseases Unit, Azienda Ospedaliero-Universitaria Policlinico of Modena, Modena, Italy

6 Internal Medicine Department, Azienda Ospedaliero-Universitaria Policlinico of Modena, Modena, Italy

7 Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Italy

8 Department of Anaesthesia and Intensive Care Unit, Azienda Ospedaliero-Universitaria Policlinico of Modena, Modena, Italy

Correspondence:

Prof. Cristina Mussini, MD

Full Professor

Department of Surgical, Medical, Dental and Morphological Sciences

University of Modena and Reggio Emilia

Largo del Pozzo, 71

41124 Modena, Italy

T: +39 059 4222466

E-mail: cristina.mussini@unimore.it

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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 by sex. Interaction was formally tested to compare the risk difference by sex in subsets. Mediation analysis with a binary endpoint IMV or death (yes/no) by end 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-value<0.0001) and this amounted to a difference in HR of 0.40 (0.26-0.63, p=0.0001). The AUC in C-reactive protein (CRP) over the study period appeared to explain 85% of this difference in risk by sex.

Conclusions: 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. CRP showed a predominant role to mediate the difference in risk by sex.

INTRODUCTION

Clinical spectrum of COVID-19 is heterogeneous ranging from asymptomatic cases to severe diseases (1-3). Reported mortality rate of COVID-19 ranges between 1.4% to 61% (4-6). Main risk factors for poor prognosis are: male sex, ethnicity, age >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, playing a mediation effect on prognosis (13).

Several mechanisms have been hypothesised. 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. 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 two-fold: 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.

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 *ethical committee*. All consecutively enrolled adult patients (≥18 years) with severe COVID-19 pneumonia, defined by the presence of at least one of the following: 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 hours 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.

Statistical analysis

Baseline characteristics of the participants were compared according to sex. Continuous variables were expressed as median (IQR) and compared by Mann-Whitney U test. Categorical variables were expressed as numbers (%) and compared by χ^2 test or Fisher's exact test by sex.

Standard survival analysis was performed with participants' follow-up accrued from the date of hospital entry until initiation of IMV 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 are shown as Supplemental Material (Supplementary Figure 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, BMI and use of tocilizumab, respectively) and the HRs in the strata were shown in 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 should amount 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 this difference 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 Figure 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 (Figure 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. This hypothesized model was then in turn separately applied for 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 with endpoints 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 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.

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) (Table 2 and 3). In addition, women had a higher platelet counts, but lower haemoglobin, alanine aminotransferase, creatinine phosphokinase, calcium and creatinine. The inflammatory profile 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%)] underwent IMV or death while by 28 days this proportion increased to 49.2% (95% CI: 39.6-58.9%). The equivalent estimates for females were 16.5% (95% CI:8.9-24.2%) and 31.7% (95% CI:17.9-45.4%), respectively (log-rank test p-value<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-value=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), 4% bloodstream infections (BSI, n=2), 4% urinary tract infections (UTI, n=2) and one female with a dental abscess (0.2%). 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).

Figures 2A/B show the forest plot of the HR for the composite endpoint comparing females with males in specific sub-populations, with interaction test p-values. The overall effect of sex on the risk of invasive ventilation or death was HR=0.40 (95% 0.26-0.63, p=0.0001) and on mortality alone was HR=0.61 (95% 0.36-1.025, p=0.060). There was no evidence that these HR varied in specific subpopulations with perhaps the exception of BMI for risk of ventilation/death (HR=0.097, CI 0.013, 0.711 comparing females with males with a BMI<29.9, interaction p-value =0.04, Figure 2A).

Difference in plasma CRP values between males vs. females was 0.32 (p=0.65), as depicted by linear mixed regression model (Supplementary Table 1 and Figure 1).

AUC-CRP over the study period (log10 scale) was 0.51 mg/ml (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 were higher in males vs. females, namely the AUC of IL-6 (2.35 vs. 2.09 p=0.006), LDH (2.78 vs. 2.70, p=0.0008), ferritin (2.89 vs. 2.57, p<0.0001) and to a lower extent ALT (1.57 vs. 1.47, p=0.02). Unsupervised learning indicated that 38.7% 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 hemoglobin, CRP, platelets, ferritin, ALT, IL6 and LDH which matched the location of male individuals (Supplementary Figures 2 A/B).

We finally tested the underlying model depicted by the DAG in Figure 1 by means of a formal mediation analysis (Table 4A/B).

Table 4A shows that for the direct effect, 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 level that was seen in males. Therefore, 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 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 one 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 AUC/day and 53% when considering the peak.

The AUC mediation effect for other biomarkers, using the same underlying causal structure hypothesised for CRP in Figure 1, were 47% for ferritin and 43% for LDH, with much less impact of the other biomarkers for which the DAG was much less supported (Table 4A).

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 4B).

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 (Figure 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 resulted an effect modifier of 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. Explanation for this interaction is unclear, it is possible that non-obese men were more likely to be hospitalized because they had greater 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 so it is a virtually an un-confounded variable, an assumption which was confirmed by supplementary analyses (Supplementary Figure S3 and Table S2).

Indeed, the hypothesis that the difference in risk of outcome by sex is only marginally explained by our presumed direct pathway acting through oestrogen dependant block of virus entry by using 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 mediation effect of both IL-6 and CRP. Our data support that this pathway is likely to be the leading mediation effect of the sex-difference observed. In detail, 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 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 hypothesised 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 IL-1beta (22,23). Moreover, it is a well -known 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 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 our simple linear model without interactions. Third, despite accurate data collection, BMI and PiO_2/FiO_2 data were missing for some participants and this could have introduced residual confounding and collider bias.

Nevertheless, several points of strengths may 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 resulting in a complete dataset for most variables used in the analysis. 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.

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Authors' contribution

CM, ACL, MarMen, MaMass, EF and GG conceptualized and designed the study. CM, ACL, MarMen, MaMass, JM and GG wrote and revised the manuscript. CM, ACL, MarMen, MaMass, JM and GG 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.

Conflict of interest

None to declare.

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Table 1 Epidemiological and clinical characteristics by sex

	Sex			
Characteristics	**Female**	**Male**	**p-value***	**Total**
	N= 134	N= 281		N= 415
Age, years				
Median (IQR)	69 (54, 79)	65 (55, 76)	0.292	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%)	0.701	131 (31.6%)
Comorbidities, n(%)				
Diabetes	18 (13.4%)	28 (10.0%)	0.293	46 (11.1%)
Hypertension	39 (29.1%)	79 (28.1%)	0.835	118 (28.4%)
Cardiovascular Disease	7 (5.2%)	32 (11.4%)	0.044	39 (9.4%)
Chronic Renal Insufficiency	9 (6.7%)	12 (4.3%)	0.288	21 (5.1%)
Cancer	6 (4.5%)	7 (2.5%)	0.278	13 (3.1%)
Hepatitis B/C virus	0 (0.0%)	0 (0.0%)		0 (0.0%)
Disease Duration				
Days from symptoms onset to hospitalisation, median(IQR)	7 (3, 13)	7 (3, 10)	0.113	7 (3, 11)
Days from hospitalisation to intubation, median(IQR)	2 (0, 2)	3 (1, 4)	0.104	3 (1, 4)
Sign and symptoms, n(%)				
Fever, median(IQR)	37 (36, 37)	36 (36, 37)	0.544	36 (36, 37)
Cough	40 (29.9%)	98 (34.9%)	0.310	138 (33.3%)
Myalgia	11 (8.2%)	9 (3.2%)	0.026	20 (4.8%)
Sputum	4 (3.0%)	6 (2.1%)	0.598	10 (2.4%)
Headache	10 (7.5%)	16 (5.7%)	0.487	26 (6.3%)
Haemoptysis	0 (0.0%)	3 (1.1%)	0.231	3 (0.7%)
Systolic pressure, mmHg median(IQR)	120 (110, 130)	125 (110, 135)	0.142	123 (110, 135)

*Chi-square or Kruskal-Wallis test as appropriate

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

	Sex			
Characteristics	**Female**	**Male**	**p-value***	**Total**
Respiratory function, median(IQR)				
Baseline PaO_2/FiO_2, mmHg	254 (140, 336)	191 (122, 294)	0.023	216 (126, 302)
Respiratory rate, %	22 (18, 28)	22 (18, 28)	0.893	22 (18, 28)
SOFA Score, median(IQR)	1 (0, 3)	2 (0, 3)	0.019	2 (0, 3)
Intervention, n(%)			0.248	
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	10/114 (9%)	35/267 (13%)	0.23	45/381 (11.8%)
Events, n (%)				
Mechanical ventilation	8 (6.0%)	58 (20.6%)	<.001	66 (15.9%)
Death - pre MV	15 (11.2%)	42 (14.9%)	0.300	57 (13.7%)
Death - all	19 (14.2%)	62 (22.1%)	0.058	81 (19.5%)

*Chi-square or Kruskal-Wallis test as appropriate

Table 3 Baseline biomarkers by sex

	Sex				
Blood tests	**N**	**Female**	**Male**	**p-value***	**Total**
		N= 123	N= 257		N= 380
Markers, Median (IQR)					
Haemoglobin, g/dl	380	12.0 (10.5, 13.1)	13.3 (12.1, 14.2)	<.001	12.8 (11.4, 14.0)
White cells, mm^3	380	5760 (4540, 8400)	6340 (4930, 8520)	0.274	6110 (4810, 8480)
Total lymphocytes, N	264	1368 (730.0, 2420)	1584 (870.0, 2390)	0.428	1545 (857.5, 2405)
Total lymphocytes, %	289	20.2 (7.2, 37.5)	25.4 (11.0, 34.7)	0.525	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)	<.001	0.6 (0.4, 0.8)
Calcium, mg/dl	356	8.7 (8.3, 9.1)	8.6 (8.2, 8.9)	0.019	8.6 (8.2, 9.0)
Creatine Kinase, U/l	375	51.5 (33.0, 108.0)	132.0 (57.0, 282.0)	<.001	88.0 (44.0, 221.0)
Chloride, mmol/l	353	100.0 (98.0, 102.0)	100.0 (98.0, 103.0)	0.575	100.0 (98.0, 103.0)
Creatinine, mg/dl	380	0.7 (0.6, 0.9)	0.9 (0.8, 1.2)	<.001	0.9 (0.7, 1.1)
D-dimer, ng/ml	375	1015 (640.0, 2070)	1160 (640.0, 2210)	0.591	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/ml	380	5.8 (2.0, 14.4)	7.5 (4.1, 17.5)	<.001	7.0 (3.2, 16.4)
Platelets, 10^9/l	380	229.0 (166.0, 292.0)	199.0 (149.0, 269.0)	0.044	207.5 (155.0, 274.5)
Potassium, mmol/l	369	3.8 (3.5, 4.2)	3.8 (3.5, 4.1)	0.678	3.8 (3.5, 4.1)
Sodium, mmol/l	369	137.0 (135.0, 139.0)	137.0 (134.0, 139.0)	0.556	137.0 (135.0, 139.0)
IL-6, pg/ml	262	189.7 (67.4, 366.0)	193.4 (77.2, 399.9)	0.457	191.8 (71.0, 399.9)
Ferritin, ng/ml	244	433.5 (207.0, 720.0)	906.5 (535.0, 1492)	<.001	650.0 (368.0, 1251)

*Kruskal-Wallis test

Figure 1 – Direct acyclic graph (DAG) of the underlying causal structure of the data

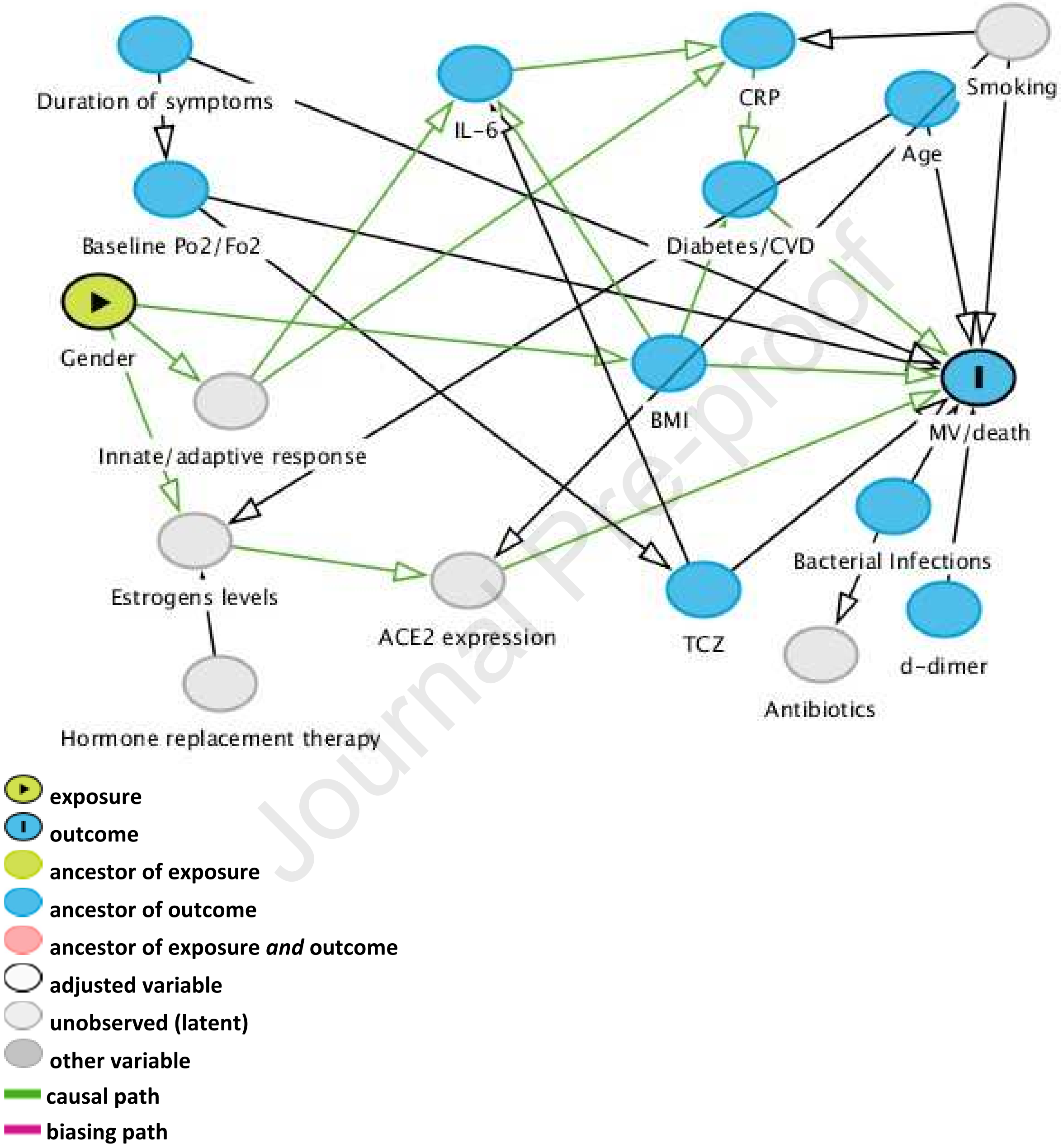
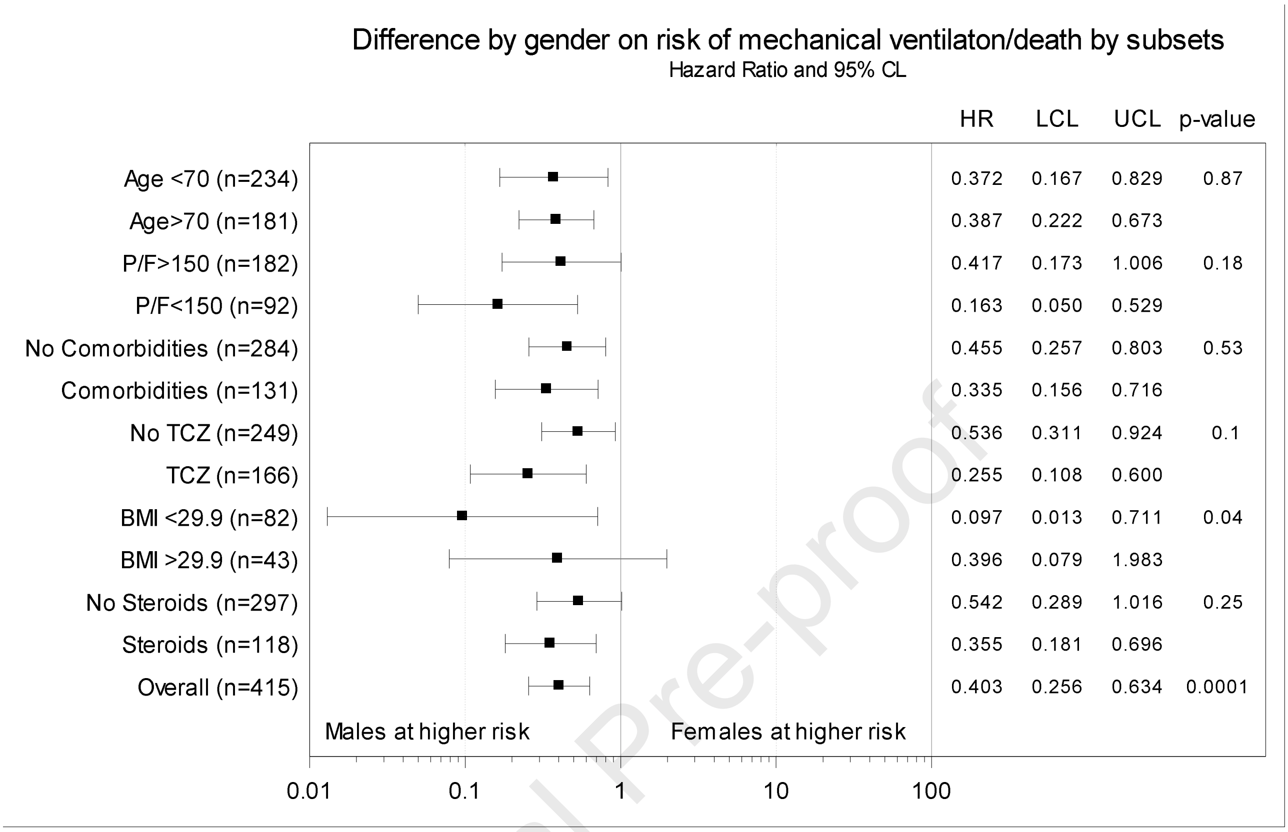


Figure 2A - HR of mechanical ventilation*/death associated with Sex overall and in subsets from fitting a standard unadjusted Cox regression model



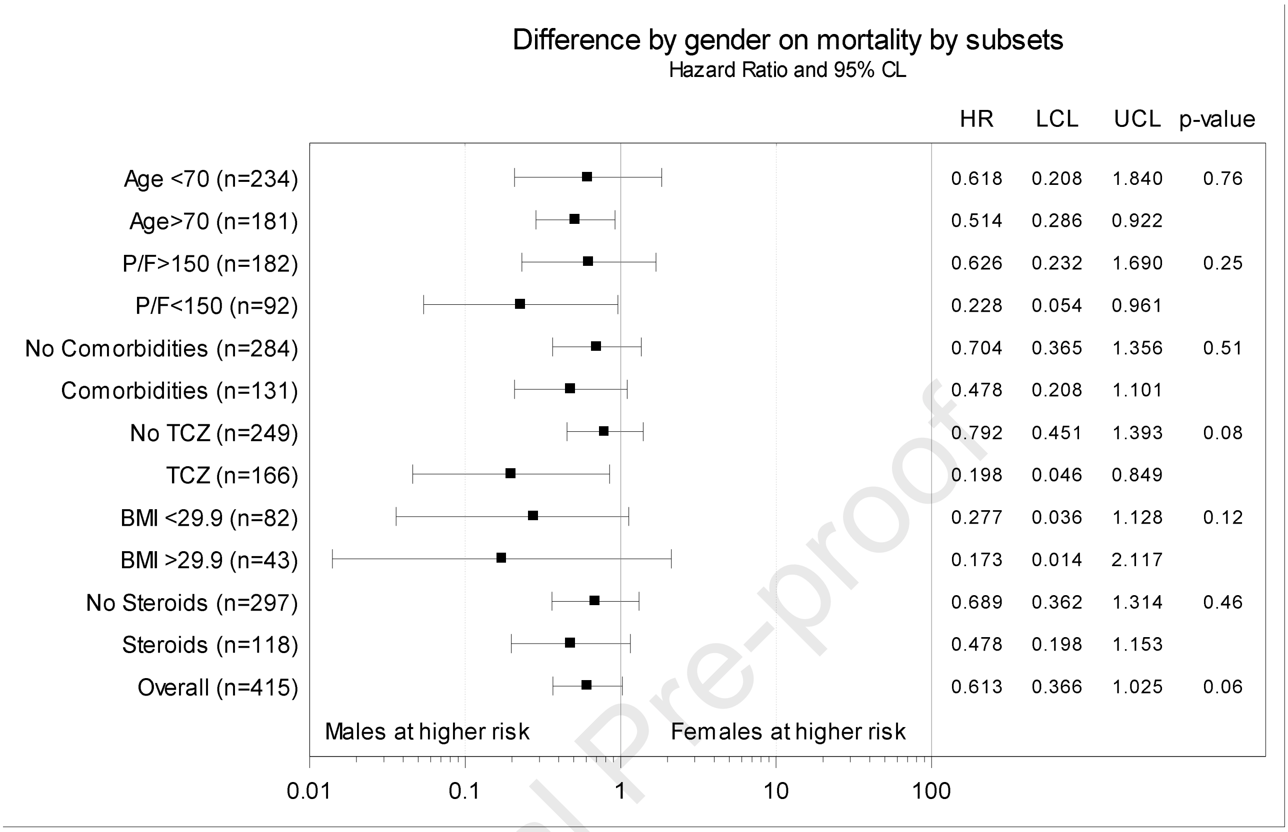
Difference by gender on risk of mechanical ventilaton/death by subsets

Hazard Ratio and 95% CL

	HR	LCL	UCL	p-value
Age <70 (n=234)	0.372	0.167	0.829	0.87
Age>70 (n=181)	0.387	0.222	0.673	
P/F>150 (n=182)	0.417	0.173	1.006	0.18
P/F<150 (n=92)	0.163	0.050	0.529	
No Comorbidities (n=284)	0.455	0.257	0.803	0.53
Comorbidities (n=131)	0.335	0.156	0.716	
No TCZ (n=249)	0.536	0.311	0.924	0.1
TCZ (n=166)	0.255	0.108	0.600	
BMI <29.9 (n=82)	0.097	0.013	0.711	0.04
BMI >29.9 (n=43)	0.396	0.079	1.983	
No Steroids (n=297)	0.542	0.289	1.016	0.25
Steroids (n=118)	0.355	0.181	0.696	
Overall (n=415)	0.403	0.256	0.634	0.0001

*Defintion: Indication for mechanical ventilation 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).

Figure 2B - HR of death associated with Sex overall and in subsets from fitting a standard unadjusted Cox regression model



	HR	LCL	UCL	p-value
Age <70 (n=234)	0.618	0.208	1.840	0.76
Age>70 (n=181)	0.514	0.286	0.922	
P/F>150 (n=182)	0.626	0.232	1.690	0.25
P/F<150 (n=92)	0.228	0.054	0.961	
No Comorbidities (n=284)	0.704	0.365	1.356	0.51
Comorbidities (n=131)	0.478	0.208	1.101	
No TCZ (n=249)	0.792	0.451	1.393	0.08
TCZ (n=166)	0.198	0.046	0.849	
BMI <29.9 (n=82)	0.277	0.036	1.128	0.12
BMI >29.9 (n=43)	0.173	0.014	2.117	
No Steroids (n=297)	0.689	0.362	1.314	0.46
Steroids (n=118)	0.478	0.198	1.153	
Overall (n=415)	0.613	0.366	1.025	0.06

Table 4 - Mediation analysis

A)

	Risk of invasive ventilation/death Female vs. Male				
	Direct Effect		Indirect Effect		% of Tot 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%

B)

	Risk of death Female vs. Male				
	Direct Effect		Indirect Effect		% of Tot 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%

SUPPEMENTARY MATERIALS

Supplementary Table 1. Mean log10 CRP by Sex from fitting a linear mixed model

	Estimates from the mixed model (Mean values 95% CI) - log10 scale					
	Baseline CRP	**Difference in baseline**	**CRP change/year**	**Difference in change/year**	**Adjusted* CRP change/year**	**Adjusted* differencein change/year**
Sex						
Female	0.62 (0.51, 0.73)		-0.06 (-0.08, -0.05)		-0.06 (-0.08, -0.05)	
Male	0.94 (0.86, 1.02)	0.32 (0.18, 0.45)	-0.07 (-0.08, -0.06)	-0.00 (-0.02, 0.01)	-0.07 (-0.08, -0.06)	-0.00 (-0.02, 0.01)
		<.001		0.798		0.648

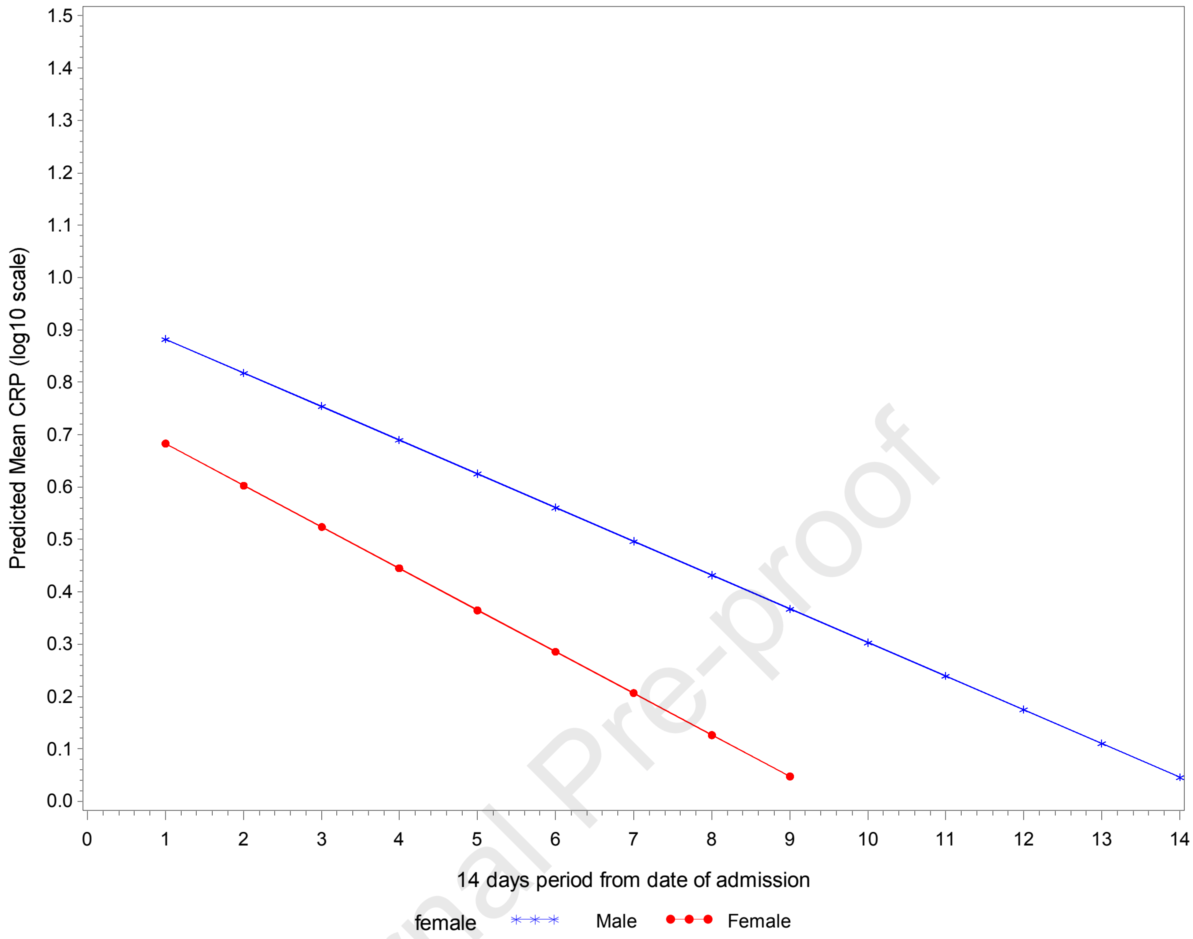
*Adjusted for age and total SOFA Score

Supplementary Table S2
Relative Hazards from fitting a Cox regression model

	Unadjusted and adjusted relative hazards of mechanical ventilation/death			
	N	**No. events**	**RH (95% CI)**	**p-value**
RH of female vs. male				
Unadjusted	415	123	0.40 (0.26, 0.63)	<.001
Adjusted[1]	331	82	0.40 (0.22, 0.71)	0.002
Adjusted[2]	299	69	0.42 (0.22, 0.79)	0.007
Adjusted[3]	203	52	0.33 (0.15, 0.70)	0.004
Adjusted[4]	295	69	0.40 (0.21, 0.75)	0.004
Adjusted[5]	161	35	0.25 (0.09, 0.67)	0.006

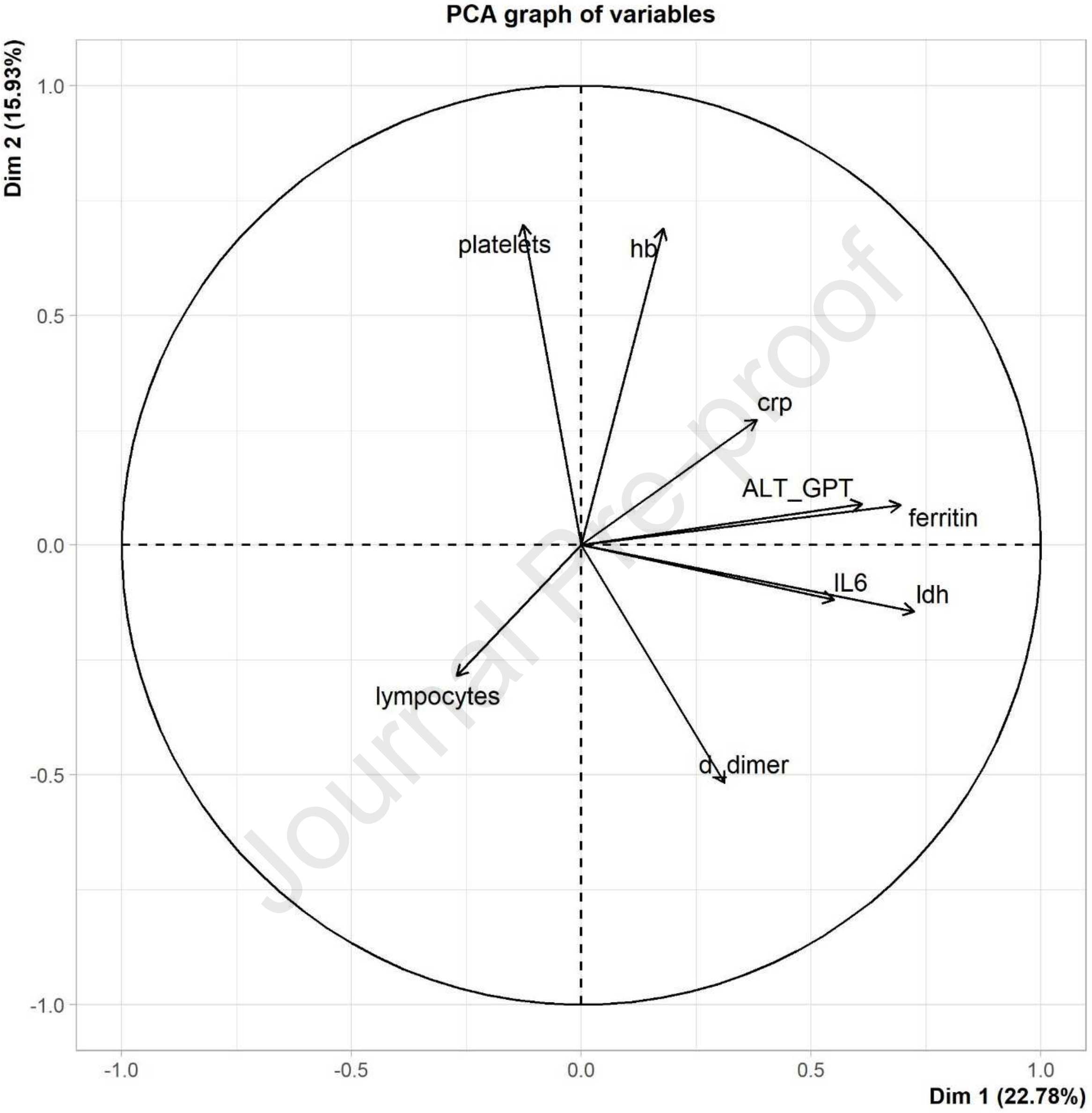
[1] adjusted for age, Charlson Index, Sofa Score and duration of symptoms
[2] adjusted for factors included in (1) plus baseline CRP
[3] adjusted for factors included in (1) plus baseline IL-6
[4] adjusted for factors included in (1) plus baseline d-dimer
[5] adjusted for factors included in (1) plus baseline ferritin

Supplementary Figure 1 Mean log10 CRP by Sex from fitting a linear mixed model

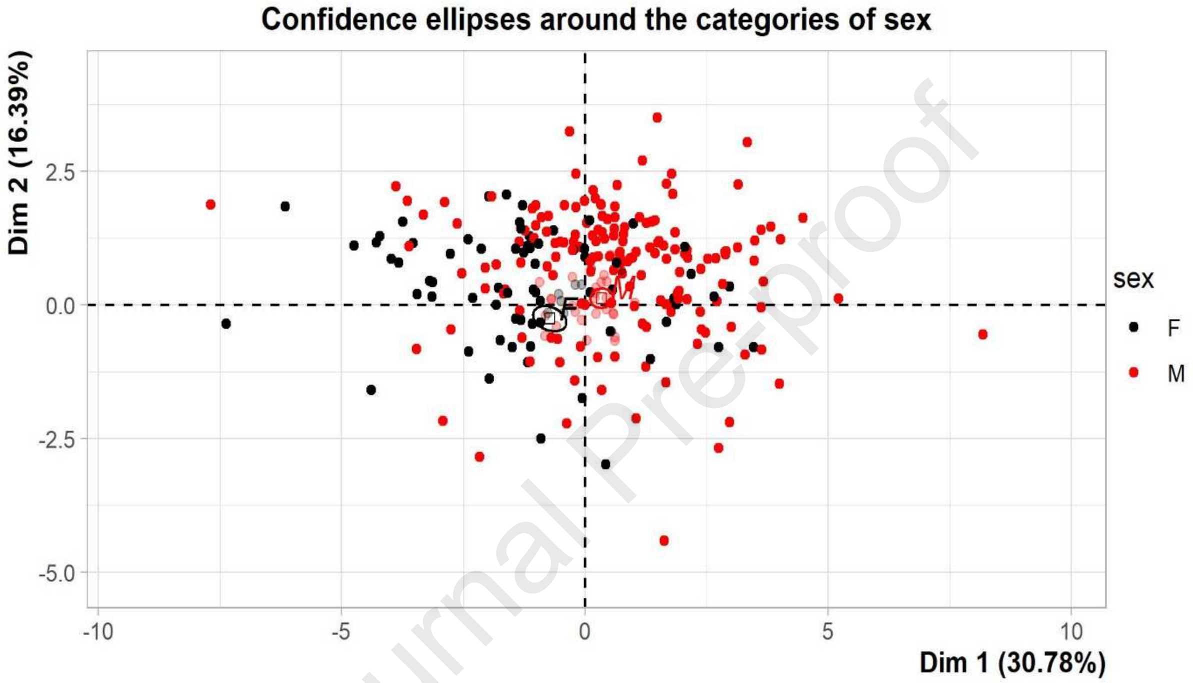


Supplementary Figure 2

A) **Post-baseline biomarkers AUC values clustering in the first two dimensions of PCA analysis**



B) Individuals clustering in the same first two dimensions of PCA analysis, labelled by sex



Supplementary Figure S3
Weighted Kaplan-Meier Curve comparing the cumulative risk of mechanical ventilation or death by sex