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Letter to the Editor

Sex-disaggregated data confirm serum ferritin as an independent predictor of disease severity both in male and female COVID-19 patients

Dear Editor,

The current COVID-19 pandemic is revealing profound differences between men and women in disease outcomes. Available sex-disaggregated data for COVID-19 show equal numbers of cases between sexes with fatality rates higher in men than in women. The Italian data confirm this trend with male patients undergoing a worse outcome and a significantly higher lethality at all age groups (13, 9%) compared to females (9, 4%).[1]

We recently reported that increased levels of ferritin were directly related with COVID-19 severity.[2] Particularly, patients who needed admission to the ICU showed 5.8 times higher serum ferritin compared to patients with mild disease. In light of the reported sex differences in COVID-19 severity and lethality, in this letter we present sex disaggregated data of routine serum laboratory testing performed on admission, including serum ferritin, according to disease severity.

141 patients confirmed as COVID-19 were admitted to the isolation ward of Emergency Department at Policlinico Umberto I Hospital in Rome, Italy, between March 2020 and June 2020, were studied. Serum samples were collected from patients upon admission before starting any treatment and tested by Laboratory Department. The patients were 60 females and 81 males, aging 64,48 (16,58) years. The 60 female patients included 44 patients (73.3%) with mild disease and 16 (26.7%) with acute respiratory distress syndrome (ARDS) and systemic inflammation (severe group).The 81 male patients included, 37 patients (45.7%) with mild disease and 44 (54.3%) in the severe group with a significantly higher number of severe cases in males (Chi-squared test $p<0.001$).Disaggregating data by sex, the only parameter that showed a significant difference between male and female patients was ferritin (Table 1, Fig. 1A). Serum ferritin levels were positively correlated with severity of COVID-19 both in male and female patients (Fig. 1B-C). Moreover, ROC curve analysis confirmed the excellent prognostic accuracies of serum ferritin in discriminating patients with severe clinical conditions in both sexes (male patients: AUC 0.961, CI: 0,921 to 1000 $p < 0.001$; female patients: AUC 0.930, CI: 0,865 to 0,996 $p<0,001$) (Fig. 1D-E) with different associated criterion (males: ferritin > 717 µg/L – sensitivity 88.64% specificity 97.30%; females: ferritin > 596 µg/L – sensitivity 81.25%, specificity 93.18%). Based on the severity of pulmonary impairment in CT scan and respiratory failure, the patients were divided in 4 groups according to the WHO guidelines.[3] As shown in Table 2A regarding 60 female patients, 18 did not present CT alterations and did not need mechanical ventilation (Group 0-mild); 17 had changes in CT scan but did not need mechanical ventilation (Group 1-moderate); 15 presented CT scan alterations and needed mechanical ventilation (Group 2-severe); 10 showed CT alterations and needed ICU admission (Group 3-critical). Out of 81 male patients (Table 2B) 11 patients belonged to Group 0-mild; 15 to Group 1-moderate; 23 to Group 2-severe; 32 to Group 3-critical. Sex-disaggregated data obtained by re-analyzing the four groups of patients confirm that male patients have a worse disease severity than women (Chi-squared test for trend $p<0.001$). Table 2A and B also report age and serum levels of C Reactive Protein (CRP), D-Dimer (D-D), Lactate Dehydrogenase (LDH), Neutrophil to Lymphocyte ratio (NLR) and ferritin in the different groups of female and male patients and Fig. 2A-B-C-D shows categorized box and whisker plots of ferritin and age according to COVID-19 severity in female and male patients. Multivariate logistic regression model including age, CRP, D-D, LDH, NLR and ferritin, demonstrated that serum ferritin resulted as an independent predictor of disease severity in male (OR = 1,0058, 95% CI, 1,0013 to 1,0103 $p < 0,001$) and female patients. (OR = 1,0048, 95% CI, 1,0018 to 1,0078 $p < 0,001$).

Table 1
Analysis of variance of serum parameters in male and female patients.

variable	Female patients (60) Mean (SD)	Male patients (81) Mean (SD)	p
Age years	66.31 (16.81)	63.16 (16.39)	n.s.
CRP µg/L	9.21 (10.46)	8.70 (9.26)	n.s.
D-D µg/dL	2160.27 (1696.12)	1783.62 (1525.03)	n.s.
LDH IU/L	335.86 (170.22)	306.98 (154.98)	n.s.
NLR	7.02 (6.27)	9.80 (9.69)	n.s.
Ferritin µg/L	531.13 (563.67)	1027.54 (1013.85)	0.000

The outcome of COVID-19 appears to be influenced by the interaction among several genetic, environmental, gender and sex-dependent factors. COVID-19 can trigger a cytokine response storm (CRS) that is associated with high mortality but for which the underlying pathophysiology and diagnostics are not yet well characterized.[4] Loss of balance between adaptive and innate immune systems may result in a hyper-inflammatory state and CRS.[5] It has been reported that male patients have a greater inflammatory reaction, with higher levels of LDH, ferritin, and CRP but a lower lymphocyte count than females, adjusted by age and comorbidity.[6] It is well known that the innate and adaptive immune responses to viral infections are more intense in females and that sex hormones act as important modulators of the immune response.[7] Taken together our sex-disaggregated data show that serum ferritin levels progressively increase with the severity of the disease but males require intensive care more frequently than women. To note, ferritin is a key mediator of immune dysregulation, especially in conditions of extreme hyperferritinemia, via direct immune-suppressive and pro-inflammatory actions, contributing to the CRS involved in COVID-19 complications.[2,8] Although it should be noted that the normal reference ranges for

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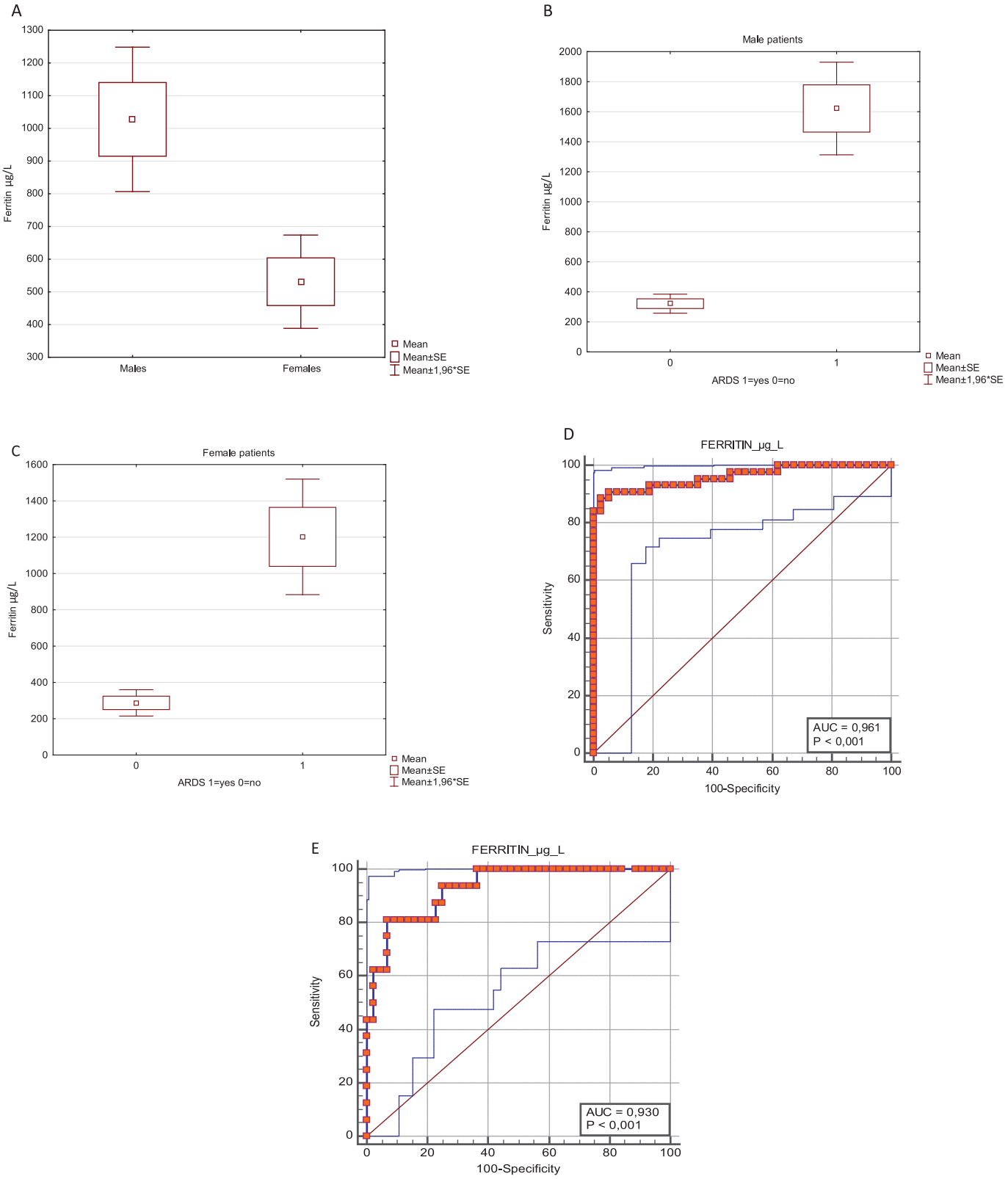

Fig. 1. A. Analysis of Variance of serum ferritin between male (81) and female (60) COVID-19 patients $p<0.001$. B Analysis of Variance of serum ferritin between severe (44) and non severe (37) male COVID-19 patients $p<0.001$. C Analysis of Variance of serum ferritin between severe (16) and non severe (44) female COVID-19 patients $p<0.001$. D ROC curve analysis of serum ferritin levels for the severity of COVID-19 in male patients. E ROC curve analysis of serum ferritin levels for the severity of COVID-19 in female patients.

Table 2A
Analysis of variance of serum parameters in female patients.

	number	Age years	CRP μg/L	D-D μg/dL	LDH IU/L	NLR	Ferritin μg/L
Group*	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
0	18	53.31(18.26)	5.84(11.85)	1624.36(1774.70)	306.57(175.15)	4.88(3.97)	315.85(396.98)
1	17	74.6513.54)	10.31(9.79)	2429.27(1609.08)	356.11(99.15)	6.71(5.65)	279.88(183.85)
2	15	69.73(12.34)	14.87(10.22)	2066.82(1565.46)	265.50(51.44)	6.93(7.43)	592.19(499.38)
3	10	68.00(14.43)	6.49(5.48)	2722.25(1919.16)	590.50(400.93)	11.11(7.26)	1254.19(733.54)
all	60	66.32(16.81)	9.20(10.466)	2160.27(1696.12)	335.68(170.22)	7.02(6.27)	531.13(563.67)
P		0.001	n.s.	n.s.	n.s.	n.s.	0.000

* for group description refer to the text.

Table 2B
Analysis of variance of serum parameters in male patients.

	number	Age years	CRP μg/L	D-D μg/dL	LDH IU/L	NLR	Ferritin μg/L
Group*	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
0	11	51.00(22.91)	2.52(3.31)	930.10(1244.80)	253.00(69.61)	6.37(9.54)	224.00(137.88)
1	15	55.93(16.60)	6.56(7.22)	1468.92(1479.56)	271.40(159.97)	8.13(6.66)	339.93(199.26)
2	23	62.30(13.89)	5.52(5.70)	1583.95(1263.37)	298.20(145.01)	9.90(9.44)	839.56(561.40)
3	32	71.90(10.74)	13.99(10.93)	2548.14(1644.12)	378.46(187.17)	11.59(10.85)	1761.19(1168.41)
all	81	63.16(16.39)	8.70(9.26)	1783.62(1525.03)	306.98(154.98)	9.80(9.69)	1027.54(1013.85)
p		0.000	0.000	0.019	n.s	n.s	0.000

* for group description refer to the text.

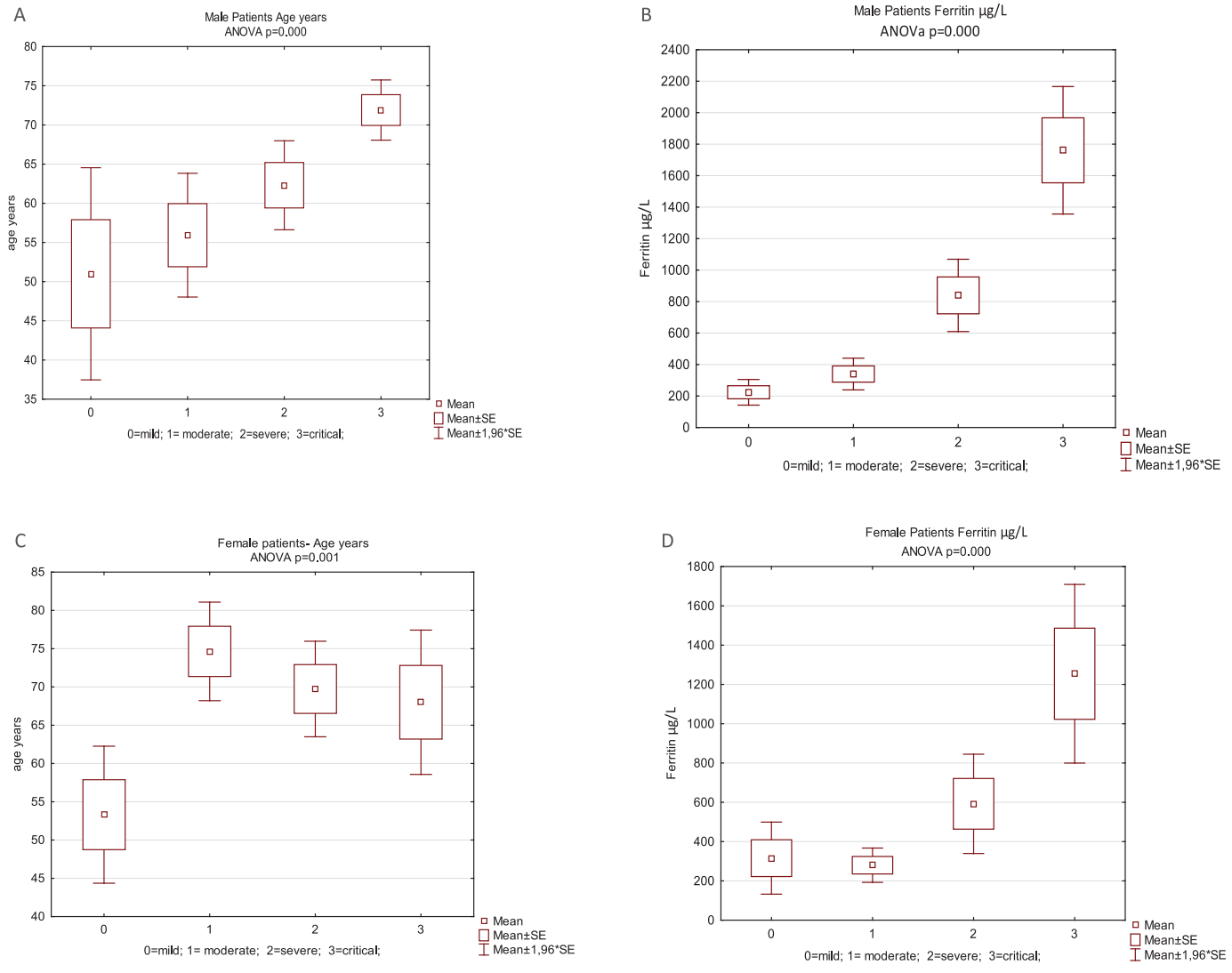

Fig. 2. A-B. Analysis of Variance – Categorized box and whisker plot of age and ferritin according to COVID-19 severity in male patients. C-D Analysis of Variance – Categorized box and whisker plot of age and ferritin according to COVID-19 severity in female patients.

serum ferritin in women show lower values than in men, it could be tempting to speculate that the higher serum ferritin status of males could contribute to the worse outcome of COVID-19 in male patients.

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