The Age Again in The Eye of The Covid-19 Storm: Evidence-Based Decision Making.

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Research

Keywords: severe acute respiratory syndrome coronavirus 2, COVID-19, immunosenescence, lockdown, immunity, renin-angiotensin-aldosterone system inhibitors, cut-off points, lymphocytes, area under the curve.

Posted Date: March 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-228480/v1

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ABSTRACT

Background: One hundred million of contagions, more than 2 million deaths and less than one year of COVID-19 have changed our lives and our health management systems forever. Ageing is known to be one of the significant determinants for COVID-19 severity. Two main reasons underlie this: immunosenescence and age correlation with main COVID-19 comorbidities such as hypertension or dyslipidaemia. This study has two aims. The first is to obtain cut-off points for laboratory parameters that can help us in clinical decision-making. The second one is to analyse the effect of pandemic lockdown on epidemiological, clinical, and laboratory parameters concerning the severity of the COVID-19. For these purposes, 257 of SARSCoV2 inpatients during pandemic confinement were included in this study. Moreover, 584 case records from a previously analysed series, were compared with the present study data.

Results: Concerning the characteristics of lockdown series, mild cases accounted for 14.4%, 54.1% were moderate and 31.5%, severe. There were 32.5% of home contagions, 26.3% community transmissions, 22.5% nursing home contagions, and 8.8% corresponding to frontline worker contagions regarding epidemiological features. Age >60 and male sex are hereby confirmed as severity determinants. Equally, higher severity was significantly associated with higher IL6, CRP, ferritin, LDH, and leukocyte counts, and a lower percentage of lymphocyte, CD4 and CD8 count. Comparing this cohort with a previous 584-cases series, mild cases were less than those analysed in the first moment of the pandemic and dyslipidaemia became more frequent than before. Age, lymphocyte count and LDH had similar distributions at both moments. IL-6, CRP and LDH values above 69 pg/mL, 97 mg/L and 328 U/L respectively, as well as a CD4 T-cell count below 535 cells/µL, were the best cut-offs predicting severity since these parameters offered reliable areas under the curve.

Conclusion: Age, sex and dyslipidaemia together with selected laboratory parameters on admission can help us predict COVID-19 severity and, therefore, make clinical and resource management decisions. Demographic features associated with lockdown could affect the homogeneity of the data and the robustness of the results.

Keywords: severe acute respiratory syndrome coronavirus 2, COVID-19, immunosenescence, lockdown, immunity, renin-angiotensin-aldosterone system inhibitors, cut-off points, lymphocytes, area under the curve.

BACKGROUND

SARS-CoV-2 infection became widespread [1], being possibly the worst trouble worldwide, as its effects have altered virtually any feature in our lives. Health, economy and individual freedom are seriously threatened all around the world. Almost a year after the first diagnosed case, several waves and strains have hit global health. The virus has infected 102.390.915 people and killed 2.214.302 with an overall case-fatality rate of 2.16% [2]. Identifying risk or severity factors for COVID-19 will help clinicians and clinical managers to make decisions about the best therapy [3], and the kind and amount of resources necessary to face new waves [4]. Severity factors might be related to health-based restrictions and should be considered before making public health decisions [5, 6].

Since the early days of the pandemic, enormous efforts have been made to identify epidemiological and clinical factors that can predict the severity of the disease [7-11, 12-15]. These efforts have firmly established age as one of the crucial elements along with comorbidities closely associated with age, most notably hypertension, diabetes, and obesity [16-23]. Independently, the male sex has also been described to be related to a severe evolution [16, 18-20, 22, 23]. Likewise, the analytical parameters related to an exacerbated inflammatory state and an exhausted adaptive immune system have been described in association with the most severe forms [16, 24-26]. Despite the similarity in the overall description of the parameters associated with the severity of COVID-19 disease, there are notable differences in the risk factors and the analytical parameter values, among the articles published [27]. The causes for these differences can rely on the geographical origin of the study populations, the different study designs and the severity criteria adopted. Additionally, some elements, which are not usually reflected in the scientific literature, might influence the pandemic landscape. One of these elements would be the effect of the pandemic lockdown restrictions, with the meaning of “stay-at-home orders” in the patient baseline characteristics. These differences may be especially relevant in defining cut-off points for analytical parameters that could be extrapolated to different populations and different pandemic moments. The objective of this study is twofold. First, to obtain cut-off points for laboratory parameters that can help us

in clinical decision-making. Secondly, to evaluate the effect that confinement may have on the patient demographic and clinical characteristics. This study aims to analyse the effect of pandemic lockdown on epidemiological, clinical, and laboratory parameters concerning the severity of the COVID-19; for this purpose, we have compared the baseline demographic and clinical characteristics as related to the severity of COVID-19 inpatients infected before complete lockdown, with those of patients admitted to hospital during close lockdown period.

RESULTS

A total of 257 inpatients from 13 Spanish Hospitals with SARS-CoV-2 infection were included. Mild cases accounted for 14.4%, 54.1% moderate and 31.5% severe (Table 1), with 32.5% of home contagions, 26.3% community transmissions, 22.5% nursing home contagions and 8.8% corresponding to frontline workers.

Descriptive baseline characteristics of the population (valid n, frequencies, percentages, mean, median, standard deviation and interquartile range) are shown in Table 1. Categorical variables stratified by severity are shown in Table 2.

Males accounted for 58% of cases. Ages in our cohort ranged from 18 to 97 years, with a median of 68 years (IQR 54-90). Concerning comorbidities, 16.9% had obesity, 15.8% were smoker or ex-smokers, 49.2% had hypertension, 32.1% of them were treated with ACEIs (angiotensin converting enzyme inhibitors) and 33% with ARBs (angiotensin receptor blockers). 41.6% had dyslipidaemia and 27.6% suffered diabetes mellitus. The presence of immunodeficiency was most often secondary to other processes, such as a transplantation or chemotherapy treatment; it accounted for 19% cases (n=45) as seen in Table 1.

Age above 60 ($p=0.046$), male gender ($p=0.049$) and institution or community transmission ($p<0.001$) arose as severity determinants in our series (Table 2).

Neither hypertension nor the use of renin-angiotensin system blockers (RAABs) was significantly associated with severity. Mild cases accounted for 10.1% of patients with age ranged between 60 and 75 years, and 17.1% of patients over 75, whereas only 5.6% of home nursing cases were mild.

Most comorbidities were age-related, such as hypertension, dyslipidaemia, diabetes and primary immunodeficiency. Smoking status was both age and sex-related (Table 3) (Figure 1).

On admission, the median of laboratory parameters, IL6, CRP, ferritin, D-dimer and lactate dehydrogenase (LDH) were above reference ranges; but both, percentage and median lymphocyte counts were under reference ranges (Table 1). Higher severity was significantly associated with higher IL6, CRP, ferritin, LDH, and leukocyte counts, and lower percentage and lymphocyte counts (Table 4). Results from 76 cases with data of lymphocyte subpopulations on admission showed that higher severity was significantly associated with lower CD4 and CD8 counts (Table 4).

Comparison of the two series: patients recruited on the very first days of pandemic vs. close confinement

Our group has previously published data on the risk factors and laboratory parameters of a multicentre series of patients admitted by COVID-19 during the first weeks of the pandemic [16]. A comparison of data corresponding to the close confinement (phase 2 from now on) with the previous series (phase 1 from now on) was performed.

Even with the same inclusion criteria, along with current data and the previous compilation, there were significantly less mild inpatients in phase 1 in comparison with phase 2 ($p<0.001$). Age was higher ($p=0.027$) within the second period. More cases were reported to have dyslipidaemia ($p<0.001$), a history of secondary immunodeficiency ($p<0.001$) and fewer patients were on treatment with angiotensin II receptor blockers ($p=0.002$) (Supplementary tables 1 y 2).

Laboratory parameters such as IL-6, CRP and ferritin, although increased, were significantly lower during confinement than in the early months of the pandemic ($p=0.028$, <0.001 and <0.001 respectively). In contrast, lymphopenia and declining CD8+ cell counts were more evident in the second phase but did not reach significance (Supplementary table 2).

Severity distribution within male inpatients was almost identical within the two series, but mild female cases decreased as moderate ones grew ($p<0.001$, Figure 2). Severity was significantly higher for all age groups during confinement, especially within the 60 to 75 years old group. Cases above 75 years were predominantly severe both at the beginning of the pandemic and during confinement. Mild inpatients were older in May ($p=0.01$) than in March and so were ($p=0.028$) severe ones (Figure 3).

Normolipidemic cases were less frequently mild to become moderate in May ($p<0.001$) as compared to march and the same effect was observed within non-SID cases ($p<0.001$). SID COVID-19 inpatients almost doubled as a percentage ($p<0.001$) the proportion of march (Figure 4).

Regarding raw data stratified by severity, some laboratory parameters such as IL-6, CRP, ferritin and LDH, although high, showed values significantly lower than those in phase one. IL6, CRP and ferritin upon arrival were lower in May moderate cohort and CRP as well in mild inpatients. LDH was lower in May mild group as compared to that of March. In the same line, the lowering of CD8 cell count was more evident in phase two but did not reach the significance. All the other parameters compared yielded similar results in the first and second cohorts (data not shown).

Diagnostic accuracy of laboratory parameters:

To look for cut-off points in laboratory parameters at admission that would allow us to predict the severity of COVID-19 disease, data from the two series of patients were pooled. Kurtosis and asymmetry were calculated for both cohorts and both fell apart >10% for every parameter but age, lymphocyte count and LDH. In terms of diagnostic accuracy, only IL-6, CRP, LDH levels and lymphocyte CD4+ count offered a reliable area under the curve. The optimal cut-off and the diagnostic statistics for each parameter are shown in Table 5. Moreover, two extreme thresholds were determined in the merged cohort. A threshold with a likelihood ratio positive (LR+) appropriate for predicting severe COVID-19 and a point with a likelihood ratio negative (LR-) suitable for discarding severe COVID-19 was calculated. Extreme cut-offs for each parameter are shown in Table 5. When assessing diagnostic accuracy, CD19 count had an unacceptable AUC of 0.59 (95% CI: 0.47-0.71).

DISCUSSION

This study raises the question of whether the decision values for both clinical and laboratory parameters associated with severe COVID-19 might change, depending on external or environmental factors such as lockdown. Even working with the same population, selection criteria, and observers, data heterogeneity arises and searching for prognostic risk factors or laboratory cut-off values becomes troublesome. Noise factors should be therefore discarded to simplify and improve triage algorithms.

Regarding severity of SARS-CoV-2 infection, different risk factors or comorbidities and laboratory parameters have been reported to date, but none of them is consistent across published studies [27]. Our group has previously described the relationship among demographic, clinical and laboratory parameters with COVID19 severity in a retrospective study including 584 patients infected for SARS-CoV-2, just before the alarm state declaration and the close confinement in our country in March 2020 [16]. In the present work, with the same case selection and severity criteria, we describe the relationship between clinical risk factors and laboratory parameters at admission in a group of 257 patients admitted to Spanish hospitals during confinement in May 2020.

Concerning clinical risk factors found at the first moment (age, male sex, hypertension, diabetes, hyperlipidaemia, ARBs intake) only age and male sex remain relevant in this second moment, together with dyslipidaemia. The epidemiological background, which was not recorded in the first phase, was a risk factor for severest COVID-19. Laboratory parameters such as leukocytes, neutrophils, IL-6, CRP, ferritin, D-dimer, and LDH increased with the severity conversely to the decrease in lymphocyte percentage.

However, as comparing both cohorts, severity, and the presence of comorbidities such as dyslipidaemia and secondary immunodeficiency (SID) was significantly higher in patients hospitalized during confinement while the use of ARBs was significantly lower.

Strikingly, some laboratory parameters whose elevations are associated with greater severity in both phases were lower in this second phase. Lymphopenia, however, seemed more evident in this phase.

In the second phase, the epidemiological background was recorded, and it is worth noticing that 25% of the cases were institutionalized in nursing homes. One of the major differences that could be a decisive factor is that cases included in the first series were infected just before strict containment measures were decreed in Europe. Meanwhile, cases included in the second series were admitted during close confinement. We may guess that during strict confinement, the epidemiological background would have been oscillating from intrafamily to institutional contagions. A higher number of institutionalized cases would mainly impact data because the older age and a higher rate of pre-existing morbidities of second stage patients. Additionally, medical prescribing habits for such as the use of RAASBs might have been

influenced either by institutionalization or by the highly changing huge number of variable-quality scientific reports at the beginning of pandemics.

A remarkable effort has been made to identify clinical and laboratory factors that can help us predict the evolution of COVID-19 disease. This effort crystallized in a vast amount of original articles and meta-analyses. As in our previous study, in most meta-analyses, age, as well as the presence of hypertension, diabetes and cardiovascular disease, are identified as COVID-19 risk factors (17, 18). Regarding laboratory data upon admission, in line with our findings, several studies reveal a significant increase in IL-6 [17, 27-29], CRP [17, 27-29], D-dimer [17, 18, 27, 28], ferritin [17, 29], LDH [17, 18], leukocytes [17, 28, 29], neutrophils [27-29], and a decrease of lymphocytes [17, 18, 27-30], a decrease of T lymphocytes CD4+ [27, 29, 30] and CD8+ [18, 27, 29, 30], related to the severity. However, in some of these papers [17, 28] the authors warn about heterogeneity across different studies selected for meta-analysis, pointing out as possible causes the origin of the data, the sample size and the month of publication.

In our current work, some previously identified factors associated with severity along the first period lost their significant relationships while age and gender were consolidated as severity factors. In addition to remark, age remains a determinant of the main comorbidities initially identified as risk factors. Furthermore, significant quantitative differences within laboratory values have been detected as comparing both periods, pointing out a temporal bias.

Particular attention has been paid as well to clarify the role of RAASBs in SARS-CoV-2 infection and the severity of COVID-19 disease. Several meta-analyses have addressed this central issue, but no consensus is met to date. An overall protective effect of RAASBs use is described, this would be mainly attributable to the use of ACEIs (OR:0.652; 95% CI: 0.478-0.891), but not similar effect is observed with concomitant ACEIs plus ARBs (OR:0.867; 95% IC:0.638-1.179) or ARBs alone intakes (OR:0.810; 95% IC:0.629-1.044) [31]. In another meta-analysis where the relationship of the use of RAASBs with the probability of COVID-19 is stated, geographical differences are evidenced, detecting that the use of RAASBs is generally associated with a better prognosis only in studies carried out in Asian countries (OR.0.37; 95% CI: 0.16-0.89) whereas, in those carried out in North America, it is commonly associated with an even more significant increase in ICU admissions (OR: 1.75; 95% CI: 1.37-2.23) and in those carried out in Europe it is related with a higher death probability (OR: 1.68; 95% CI: 1.05-2.70). The authors note that ACEIs would

be mainly protective and conversely, ARBs would be associated with an increased risk of death [32]. In a different sense are the findings of the meta-analyses of Megaly et al. [33] and Chan et al. [34]. In the first one, the use of RAASBs is associated with a lower risk of death (OR: 0.57; 95% CI: 0.32-0.98) [33]. In the latter, the use of RAASBs is not globally associated with an increased risk of infection (ACEIs OR: 0.95; 95% CI: 0.86-1.05), (ARBs OR: 1.05; 95% CI: 0.97-1.14) [34]. However, ARBs increase the risk of infection in young subjects, while ACEIs do not increase the susceptibility to infection, not the severity or mortality from COVID-19 [34]. In the phase 1 of our study, a protective effect promoted by ACE inhibitors' intake was described, while the use of ARBs was associated with increased severity [16]. However, our current study does not find any effect of the use of RAAS inhibitors, neither protection nor higher risk. Due to the widespread use of this drug and the apparent beneficial effects concerning COVID-19 disease of ACEIs compared with the deleterious effect of ARBs, more controlled studies are necessary to delve into this major concern.

In order to search for cut-off points of laboratory parameters at the time of admission, which would help us predict the evolution of the patients, data from both series were merged. In terms of diagnostic accuracy, only IL-6, LDH, CRP and CD4+lymphocyte counts offered a good area under the curve (Table 5). Accordingly, the extreme threshold values that would allow us to confirm or rule out a serious evolution, for these parameters or the NK count were only informative. On the other hand, leukocyte, neutrophil and lymphocyte counts, although predictable, yielded cut-off points with adequate LR+, that could help foresee a severe evolution.

Several studies analyse the ability of cut-off points in laboratory parameters to predict the evolution of COVID-19. For LDH, several cut-off points have been described. LDH (250 - 500 U/L) (HR 2.5, 95% CI 1.2 - 5.2) and LDH > 500 U/L (HR 9.8, 95%CI 2.8 - 33.8) (20); LDH >277 U/L (sensitivity 58.7%, specificity 82% for severe disease) and 359.5 U/L (sensitivity 93.8%, specificity 88.2% for death) [35]; LDH >450 U/L (AUC 0.76, sensitivity 75%, specificity 70%) for respiratory failure) [36]; LDH >325 U/L (AUC 0.762 for severe disease)[23]. In our study the optimal cut-off for LDH was similar to those previously described, 328.5 U/L (AUC 0.71, 95% CI:0.67-0.75) (Sensitivity: 66.6%; Specificity: 75.2%) for severe disease (Table 5). Additionally, two extreme thresholds were calculated; the value 574 IU/L had an LR+ of 5.3 for diagnosing severe disease, and a value of 177.5 IU/L had an LR- of 0.30 for discarding severe COVID-19.

Several cut-off points have been described for the CRP. A value of 11 µg/dL showed an AUC of 0.78 (sensitivity 72%, specificity 71%) for respiratory failure [36]; CRP>25.95 mg/L has an AUC of 0.84 (95% CI 0.780-0.905) for severity [37]; CRP > 46 mg/L has an AUC of 0.777 for severity [23]; CRP> 38.2 mg/L has and AUC of 0.875 (95% CI 0.867-0.883) (sensitivity 84.6%, Specificity 92.3%) for severity [28]. In our study the optimal CRP cut-off was higher than those previously published, 97.3 mg/L (AUC 0.69, 95% CI 0.65-0.73) (Sensitivity: 62.2%; Specificity: 66.1%) (Table 5). Concerning the extreme thresholds, the value 291.85 mg/L had an LR+ of 5.16 for diagnosing severe disease and a value of 7 mg/L had an LR- of 0.21 for discarding severe COVID-19.

Like other acute phase reactants, ferritin is elevated in the moderate and severe forms of COVID-19. Tahtasakal et al. have proposed a ferritin value > 303 µg/L (303 ng/mL) (AUC 0.698) as a predictor of severity [23]. Ferritin > 200 ng/mL is also part of a model to predict patients who will need high-flow O2 input (HR 7.5) [4]. In the present study, ferritin had an AUC of 0.67 (95% CI:0.62-0.72), and the best cut-off was much higher than those reported in the previous publications, 632.5 ng/mL (Sensitivity: 72.7%; Specificity: 56.8%) (Table 5). Concerning the extreme thresholds, the value 2688.5 ng/mL had an LR+ of 5.51 for diagnosing severe disease and a value of 162 ng/mL had an LR- of 0.17 for discarding severe COVID-19.

Zhou et al. have proposed a D-dimer value >1 mg/L (1000 ng/mL) (OR 18.42, 95% CI 2.64-128.55) for COVID-19 associated mortality [19]. The optimal cut-off proposed by Tahtasakal et al. is 574 µg/L (574 ng/mL) for severe COVID-19 (AUC 0.694) [23]. Elshazli et al. in a meta-analysis have found 0.48 µg/L (480 ng/mL) as the optimal value for predicting severity (AUC 0.876, 95% CI 0.868-0.884) (sensitivity 88.9%, specificity 77.8%) [28]. A value of 0.65 mg/L (650 ng/mL) has been proposed by Zhang et al. as a predictor for severity in older adults [38]. In our study, D-dimer had an AUC of 0.62 (95% CI:0.57-0.66) and the optimal cut-off was 1068 ng/mL (Sensitivity: 45.3%; Specificity: 76.1%) (Table 5). The extreme high threshold showed no utility because of their obviousness, 34744 ng/mL with an LR+ of 4.6 for diagnosing severe disease. No D-dimer value had a reliable LR- less than 0.5 for discarding severe COVID-19.

IL-6 levels are used in the context of COVID-19 disease for patient follow-up and clinical decision-making. Several levels of IL-6 have been proposed in different studies to predict severity progression. In the meta-analysis by Elshazli et al. a cut-off point of 22.9 pg/mL obtained an AUC of 0.63 (95% CI 0.616-0.648)

(Sensitivity 71.4%, Specificity 71.4%) [28]. A similar cut-off point, 34.9 pg/mL, showed an AUC of 0.760 and an OR of 12.750 (95% CI 2.2-75.3) to predict ICU admission [3]. A 64 pg/mL cut-off point for IL-6 is also part of a model to predict patients who will need high-flow O2 input (hazard ratio 18) [4]. A higher cut-off, 163.4 pg/mL, has been proposed for predicting death with a 91.7% sensitivity and 57.6% specificity [39]. In our study a value of 69.08 pg/mL has been found as a predictor of severity (AUC 0.70, 95% CI:0.64-0.76) pg/mL (Sensitivity: 51.2%; Specificity: 79.8%) (Table 5). Concerning the extreme thresholds, the value 175 pg/mL had an LR+ of 5.2 for diagnosing severe disease and a value of 5.56 pg/mL had an LR- of 0.19 for discarding severe COVID-19.

An elevated neutrophil count, lymphopenia, and an elevated neutrophil/Lymphocyte ratio are characteristic of severe COVID-19 [23, 28, 40]. Lymphopenia is also part of the infection pathogenesis and is both a cause and a consequence of the severity [41]. Ji et al. have proposed a cut-off point of $1*10^3$ lymphocytes/µL for the diagnosis of severe disease (HR 3.7, 95% CI 1.8-7.8) [20]. Tahtasakal et al. have proposed a cut-off point of $1.04*10^3$ cells/µL for the diagnosis of severe disease (AUC 0.678) [23]. In a meta-analysis with 6320 patients, a cut-off point of $0.98*10^3$ cells/µL (AUC 0.867, 95% CI 0.861-0.873) is proposed (sensitivity 81.2%, specificity 87.5%), as a marker of severe COVID-19 [28]. In our study, the AUC of this parameter was low 0.61 (95% CI:0.57-0.65) and the best cut-off was 0.725 cells$*10^3$/µL (Sensitivity: 39.9%; Specificity: 77.7%) (Table 5). Regarding extreme thresholds, the value 0.365 cells$*10^3$/µL had an LR+ of 5.34 for diagnosing severe disease. No lymphocyte count value had a reliable LR- under 0.5 for discarding severe COVID-19. In summary, both the low AUC and the absence of extreme values with adequate LR- mean that no reliable lymphocyte count values were found to help predict evolution in our study.

Total lymphocyte count, lymphocyte populations, especially the T ones, are affected in the severest cases of COVID19. Different cut-offs for CD8+ lymphocytes have been reported. Du et al. [42] propose a cut-off point of 75 cells/µL to predict a fatal outcome. In our study, the CD3+CD8+ count had a low AUC of 0.63 (95% CI:0.52-0.75) and the best cut-off was 163 cells/µL (Sensitivity: 51.6%; Specificity: 75%) (Table 4). Concerning the extreme thresholds, no CD3+CD8+ count had an acceptable LR+ for diagnosing severe disease nor LR- less than 0.5 for discarding severe COVID-19. For CD4+ T-lymphocytes, there are few studies with predictive cut-off points, Zhang et al. have proposed a cut-off point of 268 cells/µL (AUC

0.804, 95% CI 0.695-0.912) for predicting severe disease in older adults with COVID-19 [38]. However, in our study. CD3+CD4+ count had an AUC of 0.70 (95% CI:0.61-0.80) and the optimal cut-off was 535 cells/µL (Sensitivity: 90.3%; Specificity: 50.5%) (Table 4). The value 95.74 cells/µL had an LR+ of 4.79 for diagnosing severe disease, and a value of 660.6 cells/µL had an LR- of 0.16 for discarding severe COVID-19.

Finally, age has been ratified as a crucial factor in COVID-19 severity in our series. Age correlates with endothelial damage and coagulation dysfunction, immunosenescence, inflammaging, including the effects of chronic cytomegalovirus infection, increased prevalence of COVID-19-associated comorbidities, and low levels of vitamin D [43]. Immunosenescence refers to age-related changes in the immune system [44]. Older individuals are more susceptible to infections due to immunological changes associated with the ageing process [45]. These progressive changes affect both innate and adaptive immunity. They include a decrease in naive lymphocytes, the contraction of lymphocyte repertoire, increased memory lymphocytes, fibrotic changes in lymph node architecture, and dysregulation in cytokine production [46]. The low-grade chronic inflammatory state that accompanies ageing, called inflammaging, may predispose older adults to severe COVID-19 by impairing the immune response to SARS-CoV-2. Inflammaging is characterized by high levels of acute-phase proteins and pro-inflammatory cytokines [47]. It has been suggested that individuals with more severe SARS-CoV-2 infection may have a cytokine storm syndrome characterized by increased levels of cytokines and chemokines [45]. Cytokine storm in elderly with severe SARS-CoV-19 is associated with age-related pathophysiological processes, including senescent cell inflammatory phenotype, excess oxygen radical production, immunosenescence, and lack of vitamin D [48]. Endothelial damage is a critical point that allows us to identify patients prone to develop severe COVID19. Endothelial barrier mechanisms are independently compromised by diabetes, obesity, age [49] and hypertension, that are known to determine bad COVID-19 prognosis. The hypothesis that attributes the severity of COVID-19 evolution to age-related changes is partly speculative, but supported by different experimental studies. Thus, Rydyznski et al., studying the specific humoral and cellular response to SARS-Cov-2, point out that age correlates with a more severe specific antigen immune response. Older individuals present an uncoordinated humoral and cellular response to SARS-CoV-2. This coordination is notably affected in those over 65 years of age. T lymphocytes' shortage is associated with age and worse COVID19 prognosis [50].

Baas et al. performing genomic analysis of the response to SARS-CoV-1 in a murine model, point out that older individuals present an exacerbated immune response and that the expression of the genes of TNF-α, IL-6, CCL2, CCL3, CXCL10 and INF-γ exhibit a biphasic pattern that correlates with the peak of viral replication and with the flow of lymphocytes to the areas of more severe histopathological damage in the lungs [51]. Sims et al. characterize the cytokine storm that accompanies severe COVID-19 and find a panel of markers, such as IL-6, PTX3, IL-1RA, CTSL1, IL-18 and RAGE that would reflect vascular endothelial disruption [52]. An unbalanced production of pro-inflammatory cytokines has been described in immunosenescence in healthy individuals. Thus, Shurin et al. demonstrate that INF-γ-inducible chemokines (MIG and IP-10) increase with age [53]. Concerning the above, Tincati et al. analysing the phenotype of cytokines and chemokines that characterizes the worsening of COVID-19 in the second week of the disease, point out that this critical point in the evolution of the disease is associated to higher levels of CXCL8/IL-8, CXCL-9/MIG and CXCL10/IP-10, and that the presence of circulating neutrophils is associated to these levels [54]. Likewise, Xiong et al., employing the transcriptomic analysis of the characteristics of bronchoalveolar lavage of individuals with COVID-19 pneumonia, point out the association between the pathogenesis of the disease and the excessive release of cytokines such as CCL2/MCP-1, CXCL10/IP-10, CCL3/MIP-1A and CCL4/MIP-1B [55]. Moreover, IP-10 (CXCL10) [56, 57] and MCP-1 [56] have been proposed as biomarkers related to the risk of death in COVID-19 [56) and severity [57]. In the autopsies of the patients who died due to COVID-19, besides the mononuclear inflammatory infiltration, the diffuse alveolar damage and the formation of hyaline membranes, the particular presence of vascular affectation with epithelial damage points to a probable direct cytopathic role of the virus stands out [58]. A hypothesis could be built, where the changes associated with ageing, such as epithelial dysfunction and changes in basal levels of cytokines and chemokines (standing out CXCL-10/IP-10 and CCL2/MCP-1) would enhance an exaggerated response triggered by the direct cytopathic action of the virus on endothelium. The specific response against SARS-CoV-2, when uncoordinated due to ageing, would contribute to a worse evolution.

CONCLUSIONS

The heterogeneity within data from two different moments, might reduce the possibility of valid diagnostic calculations regarding areas under the curve. Nevertheless, some reliable and informative parameter cut-offs could help us coping with COVID-19.

The relationship between the use of RAABs and the COVID-19 severity was not conclusively established. The conflicting results of our study and others guarantee future controlled studies.

Concerning risk factors, age is confirmed to play a pivotal role in COVID-19 severity in our series. A hypothesis can be drawn where the changes associated with ageing, such as epithelial dysfunction and basal levels of cytokines and chemokines would play a key role enhancing an exacerbated response to the direct cytopathic action of SARS-CoV2 on vascular endothelium. A specific immune response against SARS-CoV-2, when uncoordinated to different extents depending on age, would determine a bad COVID19 prognosis.

METHODS

Study design and participants

A retrospective multicentre analysis was performed on a consecutive set of SARS-CoV-2 infected inpatients, microbiologically confirmed by positive polymerase chain reaction (CRP) test, admitted to the 13 hospitals, during May 2020. Cases were tracked for a five-week follow-up period from admission to discharge. A minimum sample size of 20 patients was considered for every hospital. A total of 260 individuals over 18 years old, from 13 Spanish hospitals were recruited. After data quality assessment, 257 patients were included in the analyses. Cases were stratified into three severity groups before any statistical analyses according to the following criteria:

- Mild: whenever clinical symptoms were mild with no abnormal radiological findings.
- Moderate: cases with confirmed pneumonia that was not considered severe
- Severe: when at least one of the following criteria was met: acute respiratory distress, shock, admission to the intensive care unit (ICU), the process was so considered by the physician in charge. Any death was as well classified as severe.

This retrospective observational study was conducted according to national regulations, institutional

policies and in the tenets of the Helsinki Declaration. It was approved by the local institutional Ethics Committee of any involved hospitals.

Data from 584 case records from the previously analysed series [16], were compared with data from the 257 cases in the present study and both were pooled to explore cut-off values.

Data collection

Any data analysed were extracted from electronic medical records. The collection form included demographic, epidemiological and clinical data: age, sex, history of diabetes mellitus (DM), dyslipidaemia, hypertension (HTA), renin-angiotensin-aldosterone system blockers (RAASBs) and statins intake, the smoking status, obesity, time from onset to diagnosis, laboratory data on admission, and COVID-19 severity using the criteria previously defined.

Additionally, data from a former study conducted by our group the pre-confinement phase (16) were compared to those in the current study and merged for cut-off analyses. Inclusion and severity criteria were the same for both cohorts.

Laboratory data

Major laboratory markers were extracted from medical records on admission. Routine blood examinations included leukocyte, neutrophil and lymphocyte counts (cells/µL) and percentages. Serum biochemical tests recorded were ferritin (µg/L), lactate dehydrogenase (LDH, U/L), C- reactive protein (CRP) and D-dimer (µg/L). Immunological tests recorded were interleukin-6 (IL6, pg/mL), Lymphocyte population count (cells/µL) and percentage, complement factors C3 and C4 and immunoglobulins IgG, IgA and IgM (mg/dL).

Statistical analysis

Demographic and clinical characteristics of patients were expressed as their mean and standard deviation (SD); when not adjusting to a normal distribution, the median was used to represent non-parametric data for continuous variables and frequency distributions represented categorical variables.

Kolmogorov-Smirnov test was performed on each continuous variable to contrast normality. To analyse the overall differences between the three groups: mild, moderate, and severe type, the ANOVA was tested on variables with normal distribution and n>30 (% and CD4 lymphocyte count, % of CD8 lymphocytes, % of NK and C3 concentration). The Kruskal-Wallis test was used to analyse the relationship

with severity for non-parametric variables. To contrast the Ho of independence within categorical variables, Chi-square and Fisher's exact test were used.

A comparison of data collected during the lockdown period with our published data collected outside the lockdown (16) was performed as independent samples' Wilcoxon test for medians.

Pooling data from the two series searching for predictive cut-off values: We have previously published the association of laboratory parameters with severity from a cohort of COVID-19 hospitalized patients before complete lockdown. We merged the data from this cohort with those obtained during our country home confinement. To determine the diagnostic validity of each laboratory parameter, sensitivity, specificity, the receiver operating curve (ROC), the area under the curve (AUC) and the optimal cut-off (Youden index) were calculated. Moreover, to maximize the specificity (to rule in severe COVID-19) and the sensitivity (to rule out severe COVID-19), likelihood ratio positive and negative were calculated.

Abbreviations.

Angiotensin-converting enzyme inhibitors (ACEIs); angiotensin II receptor blockers (ARBs); renin-angiotensin-aldosterone system blockers (RAASB); lactate dehydrogenase (LDH); C- reactive protein (CRP); interleukin-6 (IL-6); Natural Killers (NK) immunoglobulin G (IgG); immunoglobulin A (IgA); immunoglobulin M (IgM); polymerase chain reaction (RCP); standard deviation (SD); interquartile range (IQR); receiver operating curve (ROC); area under the curve (AUC); likelihood ratio (LR).

DECLARATIONS

Ethics approval

This study was conducted according to national regulations, institutional policies, and in the tenets of the Helsinki Declaration. This study was approved, with the Valladolid Health Area Drug Research Ethics Committee acting as the main committee, in a meeting held on March 31, 2020, and with the reference number "PI 20-172-NO-HCU". Moreover, it was approved by each of the local institutional Ethics Committee of the 19 hospitals involved.

Availability of data and materials

All collected data, including fully anonymized participant data, are available to others. Available information includes fully anonymized participant data and data dictionary. Related documents are available from the date of publications henceforth: study protocol, statistical analysis, and approval of the ethical board. These documents are available from the date of publications henceforth at email address cmartinalo@saludcastillayleon.es or aurora.jurado.sspa@juntadeandalucia.es

Data will be shared after approval of proposals by the Valladolid Este Ethical Committee.

Competing interests

The authors stated no conflicts of interest.

Funding

This work has been carried out without funding.

Authors' contributions

MCM and AJ conceived the idea for this study, designed the protocol, analysed the data and drafted the manuscript. The remaining authors collected the data, and assessed for data quality. All authors provided critical revisions and approved the final version of the manuscript.

Acknowledgements

The authors thank all caregivers who indirectly contributed to this multicentre study. They also thank the Spanish Society for Immunology (Sociedad Española de Inmunología, SEI) for its support and endorsement

REFERENCES

1. Du Toit A. Outbreak of a novel coronavirus. Nat Rev Microbiol. 2020;18(3):123.

2. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). https://coronavirus.jhu.edu/map.html. Accessed 30 January 2021.

3. Guirao JJ, Cabrera CM, Jiménez N, Rincón L, Urra JM. High serum IL-6 values increase the risk of mortality and the severity of pneumonia in patients diagnosed with COVID-19. Mol Immunol. 2020;128:64-68.

4. Riveiro-Barciela M, Labrador-Horrillo M, Camps-Relats L, González-Sans D, Ventura-Cots M, Terrones-Peinador M et al. Simple predictive models identify patients with COVID-19 pneumonia and poor prognosis. PLoS One. 2020;15(12):e0244627.

5. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med. 2020;8(4): e21.

6. Hanff TC, Harhay MO, Brown TS, Cohen JB, Mohareb AM. Is There an Association Between COVID-19 Mortality and the Renin-Angiotensin System—a Call for Epidemiologic Investigations. Clin Infect Dis. 2020;ciaa329. doi: 10.1093/cid/ciaa329.

7. Zheng F, Tang W, Li H, Huang YX, Xie YL, Zhou ZG. Clinical characteristics of 161 cases of coronavirus disease 2019 [COVID-19] in Changsha. Eur Rev Med Pharmacol Sci. 2020;24(6):3404-3410.

8. Liang WH, Guan WJ, Li CC, Li YM, Liang HR, Zhao Y et al. Clinical characteristics and outcomes of hospitalized patients with COVID-19 treated in Hubei [epicenter] and outside Hubei [non-epicenter]: A Nationwide Analysis of China. Eur Respir J. 2020;2000562. doi: 10.1183/13993003.00562-2020

9. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z. et al. Characteristics of COVID-19 infection in Beijing. J Infect. 2020;80[4]:401-406.

10. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J. COVID-19 with Different Severity: A Multi-centre Study of Clinical Features. Am J Respir Crit Care Med. 2020; doi: 10.1164/rccm.202002-0445OC.

11. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu 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;28;395(10229):1054-1062.

12. Li LQ, Huang T, Wang YQ, Wang ZP, Liang Y, Huang TB. COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. J Med Virol. 2020;doi: 10.1002/jmv.25757.

13. 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;13;doi: 10.1001/jamainternmed.2020.0994

14. Cao J, Tu WJ, Cheng W, Yu L, Liu YK, Hu X. Clinical features and short-term outcomes of 102 patients with coronavirus disease 2019 in Wuhan, China. Clin Infect Dis. 2020; doi.org/10.1093/cid/ciaa243

15. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA. 2020; doi: 10.1001/jama.2020.5394.

16. Jurado A, Martín MC, Abad-Molina C, Orduña A, Martínez A, Ocaña E et al. COVID-19: age, Interleukin-6, C-reactive protein, and lymphocytes as key clues from a multicentre retrospective study. Immun Ageing. 2020;17:22.

17. Mudatsir M, Fajar JK, Wulandari L, Soegiarto G, Ilmawan M, Purnamasari Y et al. Predictors of COVID-19 severity: a systematic review and meta-analysis. F1000Res. 2020;9:1107.

18. Setiati S, Harimurti K, Safitri ED, Ranakusuma RW, Saldi SRF, Azwar MK et al. Risk factors and laboratory test results associated with severe illness and mortality in COVID-19 patients: A systematic review. Acta Med Indones. 2020;52(3):227-245.

19. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu 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;395(10229):1054-1062. Erratum in: Lancet. 2020 Mar 28;395(10229):1038.

20. Ji D, Zhang D, Xu J, Chen Z, Yang T, Zhao P et al. Prediction for Progression Risk in Patients With COVID-19 Pneumonia: The CALL Score. Clin Infect Dis. 2020;71(6):1393-1399.

21. Wu G, Yang P, Xie Y, Woodruff HC, Rao X, Guiot J et al. Development of a clinical decision support system for severity risk prediction and triage of COVID-19 patients at hospital admission: an international multicentre study. Eur Respir J. 2020;56(2):2001104.

22. Weng Z, Chen Q, Li S, Li H, Zhang Q, Lu S et al. ANDC: an early warning score to predict mortality risk for patients with Coronavirus Disease 2019. J Transl Med. 2020;18(1):328.

23. Tahtasakal CA, Oncul A, Sevgi DY, Celik E, Ocal M, Turkkan HM et al. Could we predict the prognosis of the COVID-19 disease? J Med Virol. 2020;doi: 10.1002/jmv.26751.

24. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L et al. Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19). Front Immunol. 2020; Doi.org/10.3389/fimmu-2020.00827

25. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020;doi.org/10.1093/cid/ciaa248

26. Koff WC, Williams MA. COVID-19 and Immunity in Aging Populations. A new research agenda. N Engl J Med. 2020;doi: 10.1056/NEJMp2006761

27. Moutchia J, Pokharel P, Kerri A, McGaw K, Uchai S, Nji M et al. Clinical laboratory parameters associated with severe or critical novel coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. PLoS One. 2020;15(10):e0239802.

28. Elshazli RM, Toraih EA, Elgaml A, El-Mowafy M, El-Mesery M, Amin MN et al. Diagnostic and prognostic value of hematological and immunological markers in COVID-19 infection: A meta-analysis of 6320 patients. PLoS One. 2020;15(8):e0238160.

29. Akbari H, Tabrizi R, Lankarani KB, Aria H, Vakili S, Asadian F et al. The role of cytokine profile and lymphocyte subsets in the severity of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. Life Sci. 2020;258:118167.

538 30. Huang W, Berube J, McNamara M, Saksena S, Hartman M, Arshad T et al. Lymphocyte Subset
539 Counts in COVID-19 Patients: A Meta-Analysis. Cytometry A. 2020;97(8):772-776.

540 31. Pirola CJ, Sookoian S. Estimation of Renin-Angiotensin-Aldosterone-System (RAAS)-Inhibitor
541 effect on COVID-19 outcome: A Meta-analysis. J Infect. 2020;81(2):276-281.

542 32. Patoulias D, Katsimardou A, Stavropoulos K, Imprialos K, Kalogirou MS, Doumas M. Renin-
543 Angiotensin System Inhibitors and COVID-19: a Systematic Review and Meta-Analysis. Evidence for
544 Significant Geographical Disparities. Curr Hypertens Rep. 2020;22(11):90.

545 33. Megaly M, Glogoza M. Renin-angiotensin system antagonists are associated with lower mortality
546 in hypertensive patients with COVID-19. Scott Med J. 2020;65(4):123-126.

547 34. Chan CK, Huang YS, Liao HW, Tsai IJ, Sun CY, Pan HC et al. National Taiwan University Hospital
548 Study Group of ARF, the Taiwan Primary Aldosteronism Investigators and the Taiwan Consortium for
549 Acute Kidney Injury and Renal Diseases. Renin-Angiotensin-Aldosterone System Inhibitors and Risks of
550 Severe Acute Respiratory Syndrome Coronavirus 2 Infection: A Systematic Review and Meta-Analysis.
551 Hypertension. 2020;76(5):1563-1571.

552 35. Li C, Ye J, Chen Q, Hu W, Wang L, Fan Y et al. Elevated Lactate Dehydrogenase (LDH) level as an
553 independent risk factor for the severity and mortality of COVID-19. Aging (Albany NY). 2020;12(15):15670-
554 15681.

555 36. Poggiali E, Zaino D, Immovilli P, Rovero L, Losi G, Dacrema A et al. Lactate dehydrogenase and C-
556 reactive protein as predictors of respiratory failure in COVID-19 patients. Clin Chim Acta. 2020;509:135-
557 138.

558 37. Cheng B, Hu J, Zuo X, Chen J, Li X, Chen Y et al. Predictors of progression from moderate to severe
559 coronavirus disease 2019: a retrospective cohort. Clin Microbiol Infect. 2020;26(10):1400-1405.

560 38. Zhang XY, Zhang L, Zhao Y, Chen L. Risk Assessment and Prediction of Severe or Critical COVID-
561 19 Illness in Older Adults. Clin Interv Aging. 2020;15:2145-2153.

562 39. Mandel M, Harari G, Gurevich M, Achiron A. Cytokine prediction of mortality in COVID19
563 patients. Cytokine. 2020;134:155190.

564 40. Wang C, Deng R, Gou L, Fu Z, Zhang X, Shao F et al. Preliminary study to identify severe from
565 moderate cases of COVID-19 using combined hematology parameters. Ann Transl Med. 2020;8(9):593.

566 41. Jin M, Shi N, Wang M, Shi C, Lu S, Chang Q et al. CD45: a critical regulator in immune cells to
567 predict severe and non-severe COVID-19 patients. Aging (Albany NY). 2020 16;12(20):19867-19879.

568 42. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M et al. Predictors of mortality for patients with
569 COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J. 2020
570 7;55(5):2000524.

571 43. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed
572 mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child.
573 2020:archdischild-2020-320338.

574 44. Pera A, Campos C, López N, Hassouneh F, Alonso C, Tarazona R, et al. Immunosenescence:
575 implications for response to infection and vaccination in older people. Maturitas. 2015;82(1):50-5.

576 45. Pietrobon AJ, Teixeira FME, Sato MN. Immunosenescence and Inflammaging: Risk Factors of
577 Severe COVID-19 in Older People. Front Immunol. 2020;11:579220.

578 46. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine
579 immune responses. Immun Ageing. 2019;16:25.

580 47. Hazeldine J, Lord JM. Immunesenescence: A Predisposing Risk Factor for the Development of
581 COVID-19? Front Immunol. 2020;11:573662.

582 48. Meftahi GH, Jangravi Z, Sahraei H, Bahari Z. The possible pathophysiology mechanism of cytokine
583 storm in elderly adults with COVID-19 infection: the contribution of "inflame-aging". Inflamm Res.
584 2020;69(9):825-839.

585 49. Hanchard J, Capó-Vélez CM, Deusch K, Lidington D, Bolz SS. Stabilizing Cellular Barriers: Raising
586 the Shields Against COVID-19. Front Endocrinol (Lausanne). 2020;11:583006.

587 50. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D et al. Antigen-
588 Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease
589 Severity. Cell. 2020;183(4):996-1012.e19.

590 51. Baas T, Roberts A, Teal TH, Vogel L, Chen J, Tumpey ™ et al. Genomic analysis reveals age-
591 dependent innate immune responses to severe acute respiratory syndrome coronavirus. J Virol.
592 2008;82(19):9465-76.

593 52. Sims JT, Krishnan V, Chang CY, Engle SM, Casalini G, Rodgers GH et al. Characterization of the
594 cytokine storm reflects hyperinflammatory endothelial dysfunction in COVID-19. J Allergy Clin Immunol.
595 2021;147(1):107-111.

596 53. Shurin GV, Yurkovetsky ZR, Chatta GS, Tourkova IL, Shurin MR, Lokshin AE. Dynamic alteration of
597 soluble serum biomarkers in healthy aging. Cytokine. 2007;39(2):123-9.

598 54. Tincati C, Cannizzo ES, Giacomelli M, Badolato R, d'Arminio Monforte A et al. Heightened
599 Circulating Interferon-Inducible Chemokines, and Activated Pro-Cytolytic Th1-Cell Phenotype Features
600 COVID-19 Aggravation in the Second Week of Illness. Front Immunol. 2020;11:580987.

601 55. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A et al. Transcriptomic characteristics of
602 bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg
603 Microbes Infect. 2020;9(1):761-770.

604 56. Chen Y, Wang J, Liu C, Su L, Zhang D, Fan J et al. IP-10 and MCP-1 as biomarkers associated with
605 disease severity of COVID-19. Mol Med. 2020;26(1):97.

606 57. Lev S, Gottesman T, Sahaf Levin G, Lederfein D, Berkov E, Diker DO, et al. Observational cohort study
607 of IP-10's potential as a biomarker to aid in inflammation regulation within a clinical decision support
608 protocol for patients with severe COVID-19. PLoS One. 2021;16(1):e0245296.

58. Calabrese F, Pezzuto F, Fortarezza F, Hofman P, Kern I, Panizo A et al. Pulmonary pathology and COVID-19: lessons from autopsy. The experience of European Pulmonary Pathologists. Virchows Arch. 2020;477(3):359-372.

Table 1. Baseline characteristics of the study population

Clinical and demographic characteristics	All patients n=257; (%)
Severity	
Mild	37 (14.4)
Moderate	139 (54.1)
Severe	81 (31.5)
Sex	
Male	148 (57.6)
Female	109 (42.4)
Hypertension	121 (49.2)
ACEI[a] intake	
No	74 (67.9)
Yes	35 (32.1)
ARB[a] intake	
No	74 (67)
Yes	36 (33)
Dyslipidaemia	102 (41.6)
Statins intake	
No	57 (52.8)
Yes	51 (47.2)
Diabetes	68 (27.6)
Obesity	34 (16.9)
Primary Immunodeficiency	2 (0.9)
Secondary Immunodeficiency	41 (18.1)
Epidemiological background	
Nursing home resident	36 (22.5)
Live-in relative	52 (35.5)
Frontline worker	14 (8.8)
Community transmission	42 (26.3)

	Ref.v[b]	n	Mean	Median	SD[c]	IQR[d]
Age		257	66.0	68.0	17.0	54-90
laboratory data on admission						
IL6[e] (pg/mL)	<4.4	139	62.2	29.9	94.4	10-296.6
CRP[f] (mg/L)	<10	242	105	47	246	10.8-324.6
ferritin (ng/mL)	20-250	197	882	499	111	211-2718
D-dimer (ng/mL)	<500	237	2532	741	10683	410-6258
LDH[g] (U/L)	120-246	234	336	289	168	212-681
days from onset to admission		240	8	7	5	4-16
Leucocyte count (cells*10^3/µL)	4-12.4	252	8.31	6.60	7.99	4.90-17.60
Neutrophil count (cells*10^3/µL)	1.9-8	252	6.20	5.06	4.14	3.26-15.07
Lymphocyte count (cells*10^3/µL)	0.9-5	252	2.18	1.00	10.75	0,7-2,59
Lymphocyte %	19-48	232	17.30	14.50	12.00	9.5-40.3
CD3+CD4+ %	25-65	76	44.20	45.80	13.20	36.6-67.9
CD3+CD4+ count (cells*10^6/µL)	500-1400	75	541	460	365	257-1263
CD3+CD8+ %	12-40	76	21.70	21.10	10.50	14.5-40
CD4+CD8+ count (cells*10^6/µL)	250-1000	75	268	182	234	117-685
CD19+ %	5-20	68	14.20	13.30	8.30	8.1-32.8
CD19+ count (cells*10^6/µL)	100-500	67	164	127	151	66-409

Natural Killer %	5-20	68	16.30	14.40	9.00	9-36.8
Natural Killer count (cells*10^6/µL)	50-500	64	171	148	107	103-335
Immunoglobulin G (mg/dL)	650-1600	60	961.0	924.0	431.0	728-1629
Immunoglobulin A (mg/dL)	40-350	60	264.0	228.0	152.0	162-587
Immunoglobulin M (mg/dL)	50-300	60	105.0	89.0	83.0	70-256
C3 (mg/dL)		71	133.0	127.0	46.0	108-225
C4 (mg/dL)		70	30.0	28.0	13.0	23-56
Total days in hospital		245	16	12	11	7-38

Abbreviations: RASB[a], renin-angiotensin system blockers; Ref.v[b], reference values; SD[c], standard deviation; IQR[d], interquartile range; IL6[e], interleukin 6; CRP[f], C-reactive protein; LDH[g], lactate dehydrogenase.

Table 2. Risk Factors by severity n (%)

	Mild	Moderate	Severe
Age (p=0.046)	n (%)	n (%)	n (%)
<30	0(0)	7(87.5)	1(12.5)
30-45	3(13)	16(69.6)	4(17.4)
45-60	12(19.7)	36(59)	13(21.3)
60-75	9(10.1)	45(50.6)	35(39.3)
>75	13(17.1)	35(46.1)	28(36.8)
Sex (p=0.050)			
Male	21(14.2)	73(49.3)	54(36.5)
Female	16(14.7)	66(60.6)	27(24.8)
Hypertension			
No	15(12)	74(59.2)	36(28.8)
Yes	19(15.7)	59(48.8)	43(35.5)
ACEI[a] intake			
no	6(8)	42(56.8)	26(35.1)
yes	7(20)	15(42.9)	13(37.1)
ARB[b] intake			
no	8(11)	37(50.7)	28(38.4)
yes	4(11.1)	19(52.8)	13(36.1)
Dyslipidaemia			
No	127 (32.2)	159 (40.4)	108 (27.4)
Yes	30 (18.9)	75 (47.2)	54 (34.0)
Statins intake			
No	19(12.8)	88(59.1)	42(28.2)
Yes	9(11.3)	39(48.8)	32(40)
Diabetes			
No	23(12.9)	102(57.3)	53(29.8)
Yes	9(13.2)	33(48.5)	26(38.2)
Obesity			
No	20(12)	95(56.9)	52(31.1)
Yes	3(8.8)	17(50)	14(41.2)
Primary Immunodeficiency			
No	27(11.6)	130(55.8)	76(32.6)
Yes	0(0)	2(100)	0(0)
Secondary immunodeficiency			
No	23(12.4)	103(55.7)	59(31.9)
Yes	3(7.3)	24(58.5)	14(34.1)
Epidemiological background (p=0.001)			
Nursing home resident	2(5.6)	19(52.8)	15(41.7)
Live-in relative	10(19.2)	37(71.2)	5(9.6)
Frontline worker	0(0)	11(78.6)	3(21.4)
Community transmission	6(14.3)	16(38.1)	20(47.6)

Abbreviations: ACEI[a], angiotensin-converting enzyme inhibitors; ARB[b], angiotensin II receptor blockers

643

644

645

Table 3. Influence of age and gender on comorbidities

		Age					Gender	
		<30	**30-45**	**45-60**	**60-75**	**>75**	**Male**	**Female**
		n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Hypertension[a]	no	7(5.6)	19(15.2)	41(32.8)	41(32.8)	17(13.6)	67(53.6)	58(46.4)
	yes	0(0)	2(1.7)	17(14)	45(37.2)	57(47.1)	73(60.3)	48(39.7)
Dyslipidaemia[a]	no	7(4.9)	17(11.9)	45(31.5)	47(32.9)	27(18.9)	81(56.6)	62(43.4)
	yes	0(0)	4(3.9)	15(14.7)	38(37.3)	45(44.1)	59(57.8)	43(42.2)
Obesity	no	5(3)	14(8.4)	42(25.1)	56(33.5)	50(29.9)	103(61.7)	64(38.3)
	yes	1(2.9)	2(5.9)	7(20.6)	18(52.9)	6(17.6)	17(50)	17(50)
Diabetes[a]	no	7(3.9)	18(10.1)	55(30.9)	57(32)	41(23)	102(57.3)	76(42.7)
	yes	0(0)	3(4.4)	5(7.4)	31(45.6)	29(42.6)	40(58.8)	28(41.2)
Smoker[a,b]	no	4(2.2)	17(9.1)	50(26.9)	65(34.9)	50(26.9)	101(54.3)	85(45.7)
	yes	3(12)	1(4)	7(28)	6(24)	8(32)	20(80)	5(20)
	ex	0(0)	0(0)	0(0)	8(80)	2(20)	8(80)	2(20)
Primary immunodeficiency[a]	no	7(3)	21(9)	59(25.3)	83(35.6)	63(27)	133(57.1)	100(42.9)
	yes	1(50)	0(0)	0(0)	0(0)	1(50)	1(50)	1(50)
Secundary immunodeficiency	no	5(2.7)	16(8.6)	52(28.1)	60(32.4)	52(28.1)	98(53)	87(47)
	yes	2(4.9)	3(7.3)	6(14.6)	21(51.2)	9(22)	28(68.3)	13(31.7)
Epidemiological background	NHR	1(2.8)	3(8.3)	6(16.7)	8(22.2)	18(50)	18(50)	18(50)
	LIR	1(1.9)	5(9.6)	15(28.8)	16(30.8)	15(28.8)	31(59.6)	21(40.4)
	FW	1(7.1)	1(7.1)	7(50)	4(28.6)	1(7.1)	5(35.7)	9(64.3)
	CT	3(6.7)	7(15.6)	12(26.7)	23(51.1)	13(51.1)	38(65.5)	20(34.5)

all p-values either age[a] or gender[b] were <0.001

Abbreviations: NHR: nursing home resident; LIR: live-in relative; FW: frontline worker; CT: community transmission.

Table 5. Age and Laboratory results by COVID-19 severity.

	severity p-value	n	mean	median	SD[a]	IQR[b]
Age	0.002					
Mild		37	68.22	68	16.64	54-82
Moderate		138	62.87	66	16.84	49.32-76
Severe		80	70.59	70	13.19	61.5-80.5
On admission						
IL6[c] (pg/mL)	<0.001					
Mild		11	25.01	10.6	32.31	5.2-37.1
Moderate		88	44.85	29.46	56.46	8.41-56.5
Severe		40	110.47	43.45	143.75	20.05-143.62
CRP[d] (mg/L)	0.006					
Mild		34	43.87	22.35	54.88	8.16-58.4
Moderate		135	80.74	37.8	200.46	6.8-103.75
Severe		73	177.13	100.6	344.21	43.3-195.3
ferritin (ng/mL)	0.002					
Mild		22	474.00	256.8	540.30	197-535
Moderate		120	731.87	459.75	907.18	181-925
Severe		55	1373.10	929	1488.30	366.4-1805
D-dimer (ng/mL)						
Mild		30	1234.90	684	2069.30	373-1297
Moderate		134	1652.22	715.5	5786.79	431-1150
Severe		73	4680.19	800	17425.42	462-1990
LDH[e] (U/L)	<0.001					
Mild		31	215.39	206	62.63	172-243
Moderate		128	328.92	287	160.07	213.5-408.5
Severe		75	396.96	354	183.43	263-507
Leucocyte count (cells*10^3/µL)	0.011					
Mild		34	424.62	6.39	1813.47	5.53-8.78
Moderate		137	190.92	6.37	1098.34	4.6-8.75
Severe		81	280.85	8.25	1441.86	5.5-12.98
Neutrophil count (cells*10^3/µL)						
Mild		34	286.69	4.85	1244.32	3-6.85
Moderate		137	129.54	4.8	782.33	3.17-7
Severe		81	220.16	7	1147.11	4.19-11.58
Lymphocyte count (cells*10^3/µL)	0.005					
Mild		34	1.44	1.29	0.78	0,84-1,78
Moderate		137	2.1	1.06	9.07	0,7-1,39
Severe		81	1	0.91	0.59	0,68-1,15
Lymphocyte %	0.005					
Mild		34	21.92	18.85	12.74	14-28.2
Moderate		119	18.82	15	12.64	10.9-25
Severe		79	13.10	11	9.27	6.6-16.7
CD3+CD4+ %						
Mild		9	51.17	51	11.79	43.1-56.7
Moderate		48	43.88	45.76	12.71	37.43-51.525
Severe		19	41.78	41	14.46	34.32-52.74
CD3+CD4+ count (cells*10^3/µL)	.007					
Mild		9	729.87	565	445.12	372.015-1035
Moderate		48	586.66	516	365.49	289.252-818.5

Severe		18	325.35	293	210.64	185.934-466.519
CD3+CD8+ %						
Mild		9	20.45	24.4	10.09	14.79-27.1
Moderate		48	21.95	21.84	9.48	14.83-27.05
Severe		19	21.71	18	13.23	12.34-28.9
CD4+CD8+ count (cells*10^3/µL)	0.018					
Mild		9	263.29	177	205.19	143.616-269.955
Moderate		48	283.92	214	195.35	132.5-412.5
Severe		18	226.03	129.22	331.47	82-228
CD19+ %						
Mild		9	12.08	12.76	5.00	9.8-15.8
Moderate		44	13.98	13.47	7.91	7.4-17.18
Severe		15	16.16	14.5	10.81	9-20.68
CD19+ count (cells*10^3/µL)						
Mild		9	166.59	145	108.67	76.23-241.74
Moderate		44	177.19	130	168.64	66.5-217.966
Severe		14	120.52	114.85	108.90	66-132.936
Natural Killer %						
Mild		9	12.91	11	6.85	7.7-15
Moderate		44	16.85	14.63	9.24	9.205-20.75
Severe		15	16.93	17.06	9.66	8.36-23.8
Natural Killer count (cells*10^3/µL)						
Mild		9	159.68	132	89.67	103-169
Moderate		41	188.59	157	116.15	119-223
Severe		14	126.01	127.9	75.10	66-188
IgG (mg/dL)						
Mild		9	853.84	918	212.83	782-980
Moderate		31	981.27	950.3	296.30	772.14-1190
Severe		20	976.96	821	642.17	618.255-1048.5
IgA (mg/dL)						
Mild		9	284.40	239	206.15	175-277
Moderate		31	278.63	251	133.13	184.85-358
Severe		20	232.88	189.97	154.48	152.5-311
IgM (mg/dL)	0.009					
Mild		9	164.57	94	175.42	86.3-133
Moderate		31	103.63	98.7	53.67	71.59-132
Severe		20	81.39	84	43.07	46.45-112.95
C3 (mgdL)						
Mild		8	142.80	139	25.72	125-151.68
Moderate		38	135.03	127	50.55	108-152
Severe		25	126.91	125	44.84	90-153.28
C4 (mgdL)						
Mild		8	31.58	29.5	5.26	28.4-33.85
Moderate		37	30.52	29.3	11.72	23-39
Severe		25	28.30	25.7	15.40	23-29.8

Abbreviations: SD[a], standard deviation; IQR[b], interquartile range; IL6[c], interleukin 6; CRP[d], C-reactive protein; LDH[e], lactate dehydrogenase.

656

Table 5. Diagnostic validity of laboratory parameters

		AUC[a]	AUC 95% IC[b]	LR+[c]	LR-[d]	YI[e]	Sensitivity (%)	Specificity (%)
IL6[f] (pg/mL)								
Best cut-off	69.08	0.70	0.64-0.76			0.31	51.24	79.86
Best cut-off for LR+	175.1			5.2			28.10	94.60
Best cut-off for LR-	5.56				0.19		97.52	12.59
CRP[g] (mg/L)								
Best cut-off	97.3	0.67	0.65-0.73			0.28	62.18	66.05
Best cut-off for LR+	291.85			5.16			14.29	97.23
Best cut-off for LR-	7				0.21		97.90	11.62
ferritin (ng/mL)								
Best cut-off	632.5	0.67	0.62-0.73			0.29	72.66	56.79
Best cut-off for LR+	2688.5			5.51			12.23	87.77
Best cut-off for LR-	162				0.17		97.12	16.62
LDH[h] (U/L)								
Best cut-off	328.5	0.71	0.66-0.75			0.36	65.58	70.62
Best cut-off for LR+	574			5.34			17.21	96.78
Best cut-off for LR-	177.5				0.3		88.37	30.99
D-dimer (ng/mL)								
Best cut-off	1068	0.62	0.57-0.66			0.21	45.28	76.12
Best cut-off for LR+	34744			4.62			1.89	99.59
Best cut-off for LR-	None						na.	na.
Leucocyte count (cells*10^3/µL)								
Best cut-off	7.835	0.62	0.58-0.67			0.22	50.57	71.90
Best cut-off for LR+	15.875			5.14			9.89	98.08
Best cut-off for LR-	None						na.	na.
Neutrophil count (cells*10^3/µL)								
Best cut-off	6.26	0.66	0.62-0.70			0.27	51.33	75.44
Best cut-off for LR+	13.04			5.37			12.17	97.74
Best cut-off for LR-	None						na.	na.
Lymphocyte count (cells*10^3/µL)								
Best cut-off	0.725	0.61	0.57-0.65			0.18	39.92	77.66
Best cut-off for LR+	0.365			5.34			10.27	98.08
Best cut-off for LR-	None						na.	na.
CD3+CD4+ count (cells/µL)								
Best cut-off	535.5	0.70	0.61-0.80			0.41	90.32	50.51
Best cut-off for LR+	95.74			4.79			9.68	97.98
Best cut-off for LR-	660.6				0.16		93.55	39.39
CD4+CD8+ count (cells/µL)								
Best cut-off	162.83	0.63	0.52-0.75			0.22	58.06	63.64
Best cut-off for LR+	None						na.	na.
Best cut-off for LR-	None						na.	na.
CD19+ count (cells/µL)								
Best cut-off	na.[i]	0.59	0.47-0.71			na.	na.	na.
Best cut-off for LR+	na.						na.	na.
Best cut-off for LR-	na.						na.	na.
Natural Killer count (cells/µL)								
Best cut-off	147.5	0.65	0.54-0.77			0.28	75.00	53.26
Best cut-off for LR+	43.29			5.11			16.67	96.74
Best cut-off for LR-	229.38				0.18		95.83	22.83

Abbreviations: AUC[a], Area under the curve; AUC 96% IC[b], 95% Confidence Intervale of the area under the curve; LR[c], Likelyhood ratio positive; LR-[d], Likelyhood ratio negative; YI[e], Youden Index; IL6[f], interleukin 6; CRP[g], C-reactive protein; LDH[h], lactate dehydrogenase; na.[i], non aplicable.

Figure 1. TITLE. Severity factors and comorbidities interactions.

LEGEND. Pearson´s Chi Squared p-values. Abbreviations: Sex(m/f)[a]: Sex (male/female); ACEIs[b]: angiotensin conversor enzyme inhibitors; ARBs[c]: angiotensin II receptor blockers; EB[d]: epidemiological background.

Figure 2. TITLE. Severity distribution by sex of first and second series of COVID-19 inpatients**.**

LEGEND. The pie charts on the top of the figure correspond to the first series and those at the bottom to the second series.

Figure 3. TITLE. Severity distribution by age groups of first and second series of COVID-19 inpatients. **LEGEND.** The upper part of the figure corresponds to the first series and the lower part to the second series.

Figure 4. TITLE. Severity distribution and dyslipidaemia of first and second series of COVID-19 inpatients.

LEGEND. The pies on the top of the figure correspond to the first series and those at the bottom to the second series.

Figures

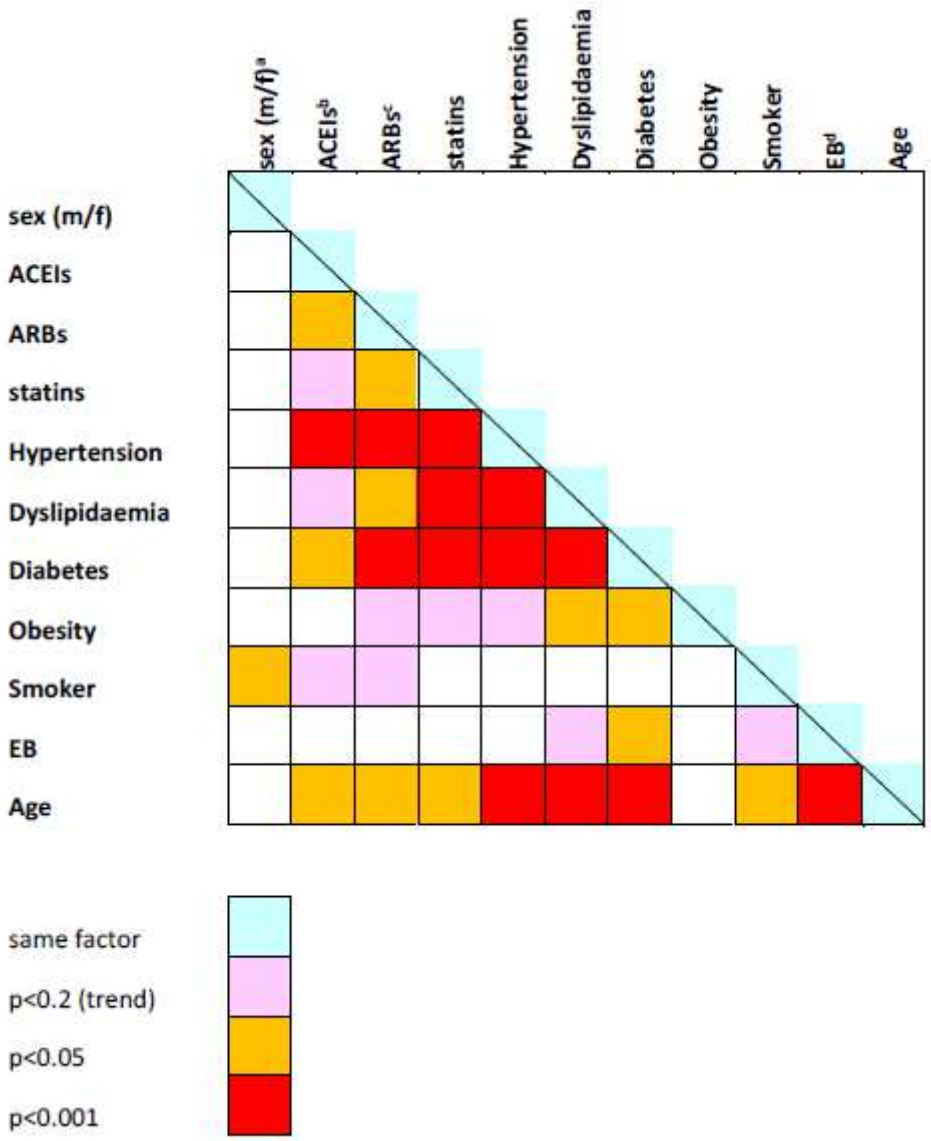

Figure 1

Severity factors and comorbidities interactions. Pearson´s Chi Squared p-values. Abbreviations: Sex(m/f)a: Sex (male/female); ACEIsb: angiotensin conversor enzyme inhibitors; ARBsc: angiotensin II receptor blockers; EBd: epidemiological background.

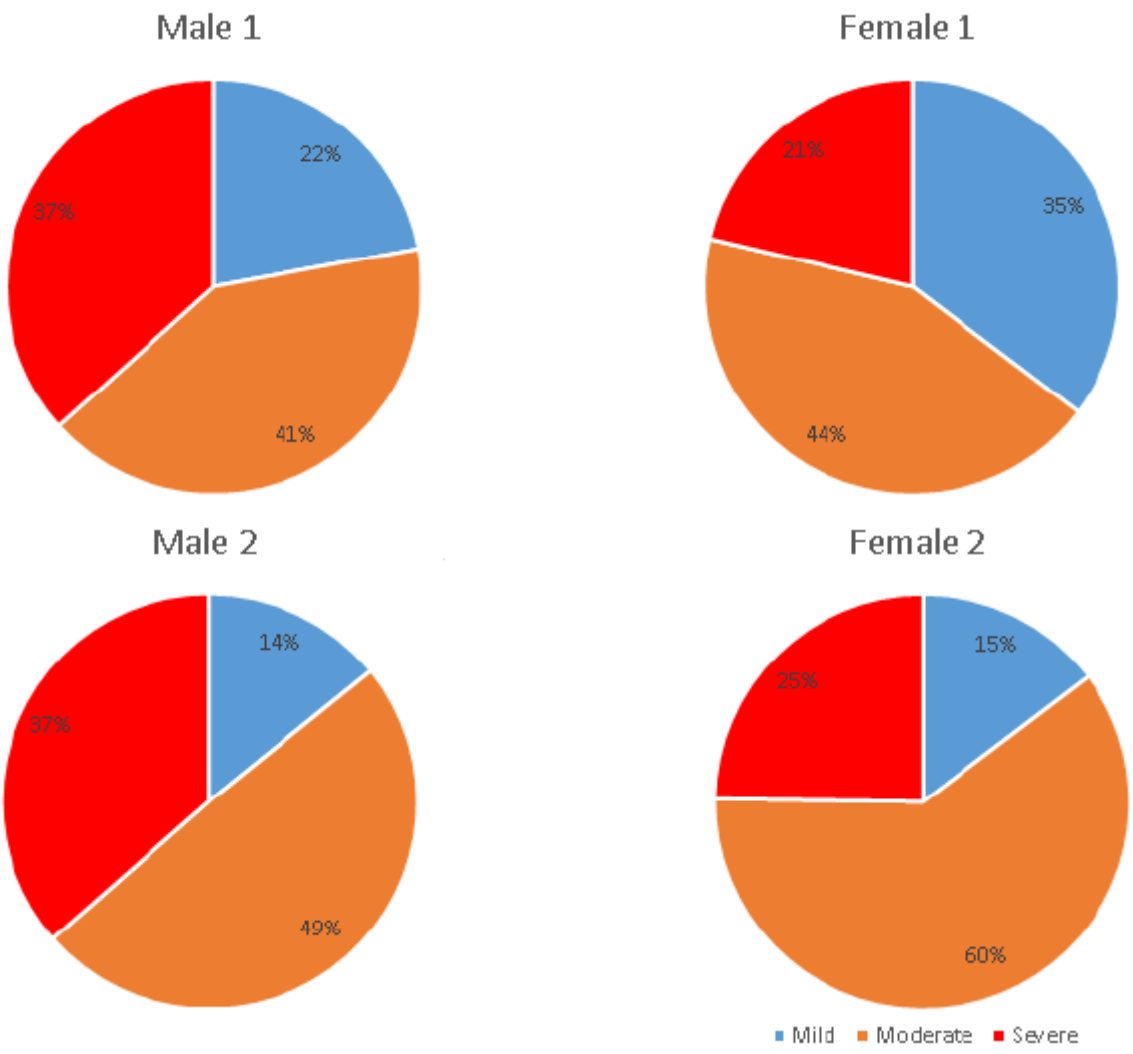

Figure 2

Severity distribution by sex of first and second series of COVID-19 inpatients. The pie charts on the top of the figure correspond to the first series and those at the bottom to the second series.

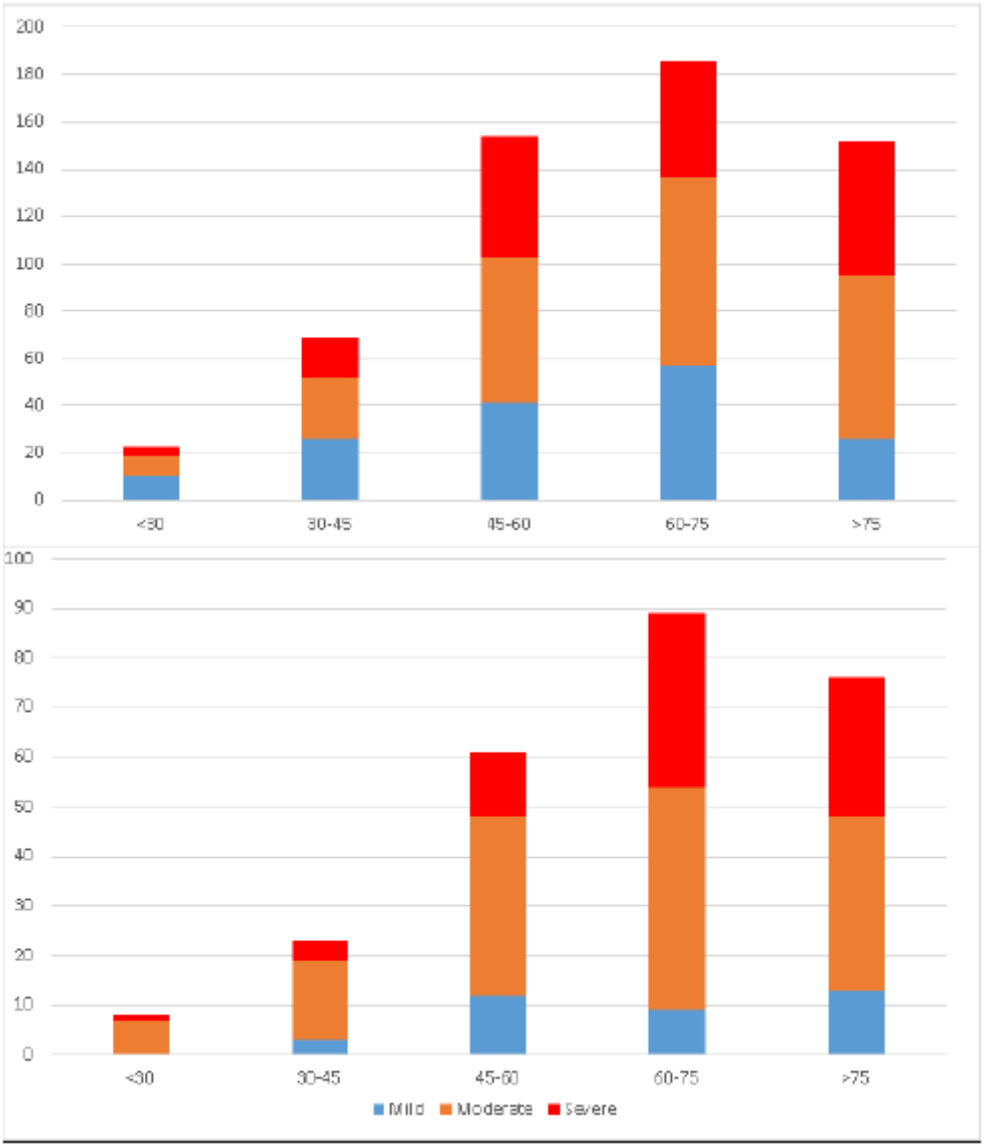

Figure 3

Severity distribution by age groups of first and second series of COVID-19 inpatients. The upper part of the figure corresponds to the first series and the lower part to the second series.

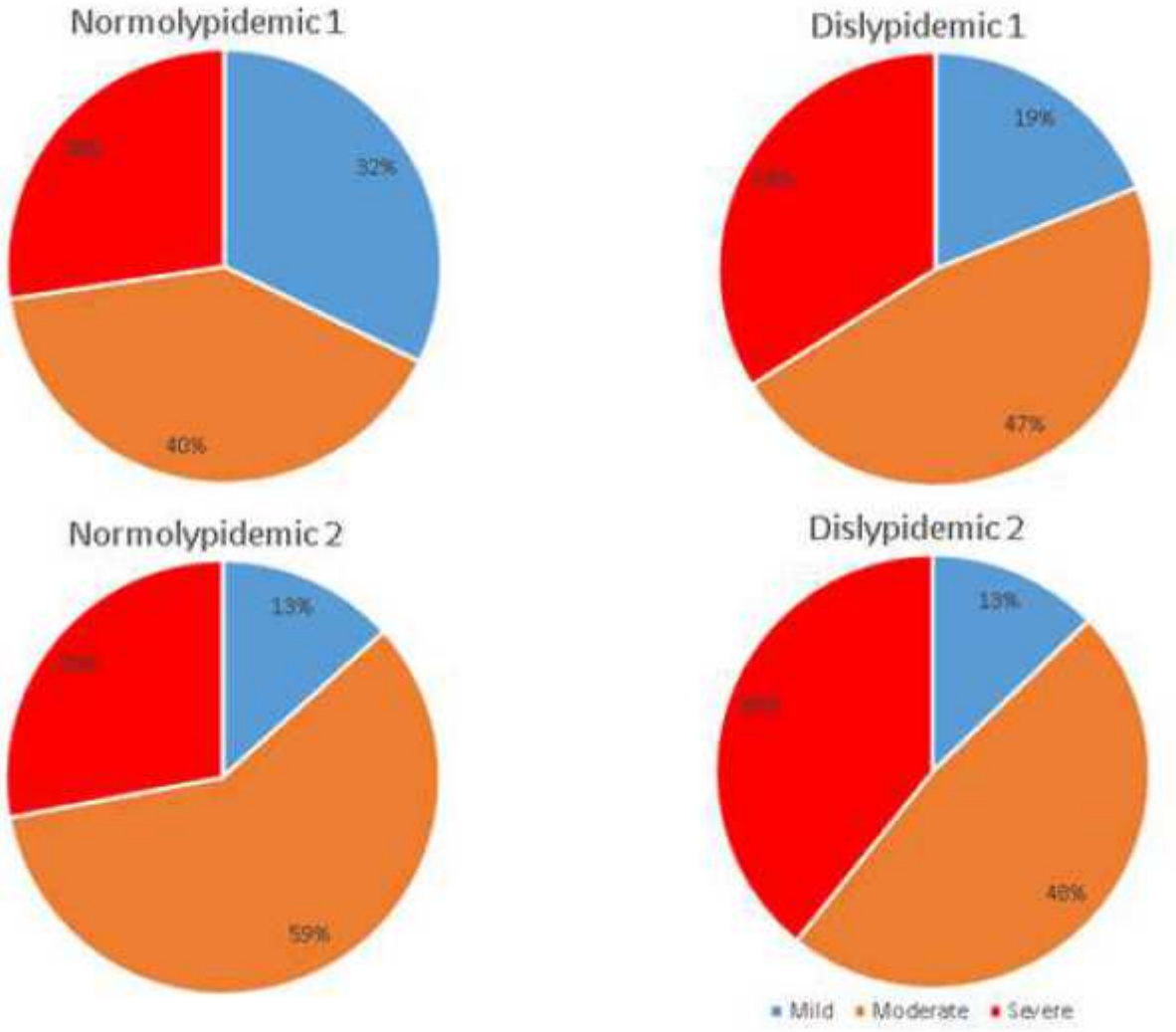

Figure 4

Severity distribution and dyslipidaemia of first and second series of COVID-19 inpatients. The pies on the top of the figure correspond to the first series and those at the bottom to the second series.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx
- SupplementaryTable2.xlsx