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► To cite this version:

Marc Vasse, Dmitry Sukhachev, Marie-Christine Ballester, Frédérique Delcominette, François Mellot, et al.. Prognostic value of cellular population data in patients with COVID-19. *Informatics in Medicine Unlocked*, 2023, 38, pp.101207. 10.1016/j.imu.2023.101207 . hal-04488567

HAL Id: hal-04488567

<https://hal.science/hal-04488567v1>

Submitted on 4 Mar 2024

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Prognostic value of cellular population data in patients with COVID-19.

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Keywords:

COVID-19, cellular population data, prognosis

Abstract

Background and aims: Beckman Coulter hematology analysers identify leukocytes by their volume (V), conductivity (C) and scatter (S) of a laser beam at different angles. Each leukocyte sub-population [neutrophils (NE), lymphocytes (LY), monocytes (MO)] is characterized by the mean (MN) and the standard deviation (SD) of 7 measurements called “cellular population data” (@CPD), corresponding to morphological analysis of the leukocytes. As severe forms of infections to SARS-CoV-2 are characterized by a functional activation of mononuclear cells, leading to a cytokine storm, we evaluated whether CPD variations are correlated to the inflammation state, oxygen requirement and lung damage and whether CPD analysis could be useful for a triage of patients with COVID-19 in the Emergency Department (ED) and could help to identify patients with a high risk of worsening.

Materials and method: The CPD of 825 consecutive patients with proven COVID-19 presenting to the ED were recorded and compared to classical biochemical parameters, the need for hospitalization in the ward or ICU, the need for oxygen, or lung injury on CT-scan.

Results: 40 of the 42 CPD were significantly modified in COVID-19 patients in comparison to 245 controls. @MN-V-MO and @SD-V-MO were highly correlated with C-reactive protein, procalcitonin, ferritin and D-dimers. $SD-UMALS-LY > 21.45$ and > 23.92 identified, respectively, patients with critical lung injuries ($> 75\%$) and requiring tracheal intubation. $@SD-V-MO > 25.03$ and $@SD-V-NE > 19.4$ identified patients required immediate ICU admission, whereas a $@MN-V-MO < 183$ suggested that the patient could be immediately discharged. Using logistic regression, the combination of 8 CPD with platelet and basophil counts and the existence of diabetes or obesity could identify patients requiring ICU after a first stay in conventional wards (area under the curve = 0.843).

Conclusion: CPD analysis constitutes an easy and inexpensive tool for triage and prognosis of COVID-19 patients in the ED.

Introduction:

Infection by the SARS-CoV-2 virus (COVID-19) has been characterized by an evolution in several waves, corresponding to different variants, leading to rapid and massive contamination of patients who arrive in large numbers at the emergency department (ED). Severe forms of the disease are associated with an overproduction of proinflammatory cytokines and lung infiltration by immune cells [1]. Severity of the disease is usually evaluated by different biological parameters such as lymphopenia, increased neutrophil count, blood oxygen saturation, C-reactive protein (CRP) levels and D-dimers [2] and chest computed tomography (CT) scans [3]. Due to a huge influx of patients at the same time in the ED, laboratory and radiology departments can be overwhelmed because of this massive activity and possibly the absence of medical workers, affected themselves by the disease, leading to a delay in the triage of the patients. Therefore, a test that could easily and quickly determine the clinical severity would be welcome.

The white blood cell (WBC) differential analysis performed on Beckman Coulter hematology analyzers is based on "VCS technology." Briefly, WBC are characterized and identified by their volume "V" (measured by direct impedance), their conductivity "C" (analyzed by conductivity in radio frequency current), and their scatter "S" of a laser beam. For each leukocyte sub-population, mean (MN) and standard deviation (SD) of the mean is calculated for these seven parameters. Therefore, an increase in SD is a marker for the heterogeneity of the analyzed leukocyte subpopulation, which may be due to a functional activation of the leukocyte subpopulation [4] or to a monoclonal component [5]. Several reports have indicated that VCS parameters, called "cellular population data" (CPD), are modified in case of infectious diseases [4 – 6, 7], including SARS-CoV-2 infection [8 - 10]. One of the CPD, called monocyte distribution width (MDW), is derived from the measurement of the SD of the volume of monocytes (@SD-V-MO) [11]. It has been regulatory cleared as an aid for sepsis detection in the ED and CE marked as an aid in identifying the patients with risk of having SARS-CoV-2 viral infection when presenting to the ED. Some studies have indicated that MDW increases with the severity of the disease, [8, 9 – 12, 13] especially for patients in intensive care units [7]. We have previously described that CPD, (not only @SD-V-MO) are dramatically modified in patients with COVID-19 and could be useful for the diagnosis of SARS-CoV-2 infection [10]. Therefore, the aim of this study was to analyze whether

variations in CPD could be correlated with the inflammatory state of patients, the size of lung lesions, and therefore could be useful for a triage of patients in the ED (hospitalization in “conventional” wards immediate discharge, or intensive care unit admission). For a large series of 825 consecutive COVID-19 patients from ED, we observed that CPD, particularly from monocyte lineage, were highly correlated to the classical markers of inflammation (C-reactive protein, ferritin, procalcitonin), but also to D-dimers and oxygen requirements as well as the extent of lung damage, quantified on the CT-scan. Forty patients, initially hospitalized in conventional wards, then required admission to intensive care a few days later. Using logistic regression, we observed that the consideration of 8 CPD, with platelet and basophil counts and the existence of diabetes or obesity, could identify COVID-19 patients with a high risk of worsening. Thus, we suggest that analysis of CPD could be used for the rapid and inexpensive estimation of the severity of SARS-CoV-2 infection, instead of using a battery of expensive and time-consuming tests.

Methods

Study population

The study was performed between March 7, 2020, and January 17, 2021. On January 17, 2021, the variant B.1.1.7 was detected in approximately 1–2 % of the patients, therefore most of the patients were infected with the original SARS-CoV-2, at a time when vaccination against SARS-CoV-2 was not available in France. Figure 1 presents a diagram of the patient flow in this study: 974 consecutive patients from the ED with both a positive SARS-CoV-2 RT-qPCR and a complete blood count with differential WBC analysis (CBC-Diff) were included. In 15 cases, patients were excluded because the women were pregnant, in 54 cases because patients were under immunosuppressive agents for solid organ transplantation, in 46 cases because patients were treated by corticosteroids due to chronic inflammatory or autoimmune diseases, in 25 cases because patients had been treated by antineoplastic therapy within the last month before COVID-19 diagnosis and in 9 cases because the patients had a hematological disease (lymphoproliferative disease or myelodysplastic syndrome). Therefore, 825 patients were eligible for the study. A group of 245 subjects with a normal CBC and differential referred to our institution from September 29, 2019, to February 9,

2020, when the prevalence of SARS-CoV-2 was very low in France, without evidence of infectious diseases, was used as controls to define the normal values of CPD.

WBC count and differential

Blood was collected in 3 mL S-Monovettes (Sarstedt, Marnay, France) containing 4.8 mg EDTA-K3. CBC-Diff were performed within 6 hours after collection on the DxH 800 analyzer (Beckman Coulter, Inc., Villepinte, France). WBC differential analysis performed by the Beckman Coulter DxH 800 is based on the measurement of seven distinct parameters for each cellular event. In addition to the volume (V) and conductivity (C), there are different measurements of the scatter: median angle light scatter (MALS), lower median angle light scatter (LMALS), low angle light scatter (LALS) and upper median angle light scatter (UMALS)]. These give information about the granularity of the cytoplasm, nucleus lobularity and membrane surface and axial light loss (AL2) measurement, which analyses cellular transparency [14]. Therefore, for each leukocyte sub-population, mean (MN) and standard deviation (SD) of the mean is calculated for these seven parameters, providing an additional 14 cellular morphometric parameters for each WBC subpopulation [neutrophils (NE), lymphocytes (LY), monocytes (MO) and eosinophils (EO)]. Currently, these morphometric parameters are for research use only (indicated by @ since their clinical utility has not been established).

SARS-CoV-2 RT-qPCR

Diagnoses of SARS-CoV-2 infection was made by RT-qPCR on nasopharyngeal swab, using either the Alinity M SARS-CoV-2 RT-PCR assay (Abbott Molecular, Des Plaines, IL, USA) or the Xpert® Xpress SARS-CoV-2 assay (Cepheid Europe SAS, Maurens-Scopont, France)

Measurement of other biochemistry and coagulation markers

C-reactive protein (CRP), ferritin and creatinine were measured on the Alinity IC analyzer (Abbott Diagnostics, Rungis, France). Procalcitonin (PCT) from Thermo Fisher was quantified

on the Kryptor™ analyzer. D-dimers (D-dimers HS 500 IL) were quantified on the ACL TOP analyzer (Werfen, le Pré-Saint-Gervais, France). These parameters were considered if they were quantified within 24 hours before or after PCR. CRP, ferritin, creatinine, PCT and D-dimers were available for 761, 415, 819, 253 and 480 patients, respectively.

Radiologic evaluation

COVID-19 disease severity was assessed as the percentage of specific lung abnormalities or damage as observed by a radiologist on a chest CT scan: (1) peripheral, bilateral ground glass opacities (GGO) with or without consolidation or visible intra-lobular lines (crazy paving), or (2) multifocal GGO of rounded shape with or without consolidation or visible intra-lobular lines. Patients were then classified as “absence,” “mild,” “moderate,” “extensive,” “severe,” or “critical” based on 0% present, but <10%, 10-25%, 25-50%, 50-75%, or >75% lung damage respectively.

Statistical analyses

As most of the parameters tested were non-normally distributed, results were expressed as the median and the 25th and 75th percentiles. MedCalc software, version 19.2 (MedCalc Software Ltd., Ostend, Belgium) was used. Mann-Whitney tests were used for the comparison of two groups of patients, and the Kruskal-Wallis test and Dunn post hoc tests were employed for multiple group comparisons. For correlation, we used the rank correlation test. To evaluate the performance of individual parameters to discriminate different subgroups of COVID-19 patients, we used receiver operating characteristic (ROC) analysis to obtain the area under the curve (AUC), which demonstrates the discriminative ability of each individual parameter. We considered that a parameter had a discriminating interest when the AUC was above 0.7. Categorical variables were compared using the chi-squared test. To develop the algorithm to differentiate those patients who would need to transfer to the intensive care unit (ICU) after hospitalization in the conventional ward, we considered the patients hospitalized in the conventional ward without the need for the ICU at a later stage (“HOS” group, n=317) as a negative group and patients hospitalized first in conventional wards than in the ICU (“HOS-ICU” group) as a positive group (n=40). We first

included all DxH 800 analyzer parameters into the model and excluded parameters based on its p-value (this is the significance value of an individual parameter into the logistic regression model) with the highest value first. We excluded one parameter on each step, as p-values of remained parameters are changed in the updated model on each step. The termination criteria of the process were not low p-value for all parameters, as recommended by MedCalc, but the balance between false-negative/false-positive results, when we could not further decrease those numbers. The number of false-negative/false-positive were calculated on each step of the backward procedure. Finally, the cutoff of the model was adjusted to achieve a clinically meaningful balance between false-positive and false-negative results.

Ethics committee approval

The study was performed in agreement with French ethical laws (where patients and their families are informed that their biological samples and data used for routine care may be used anonymously unless they expressed their opposition) and conducted under a blanket protocol for COVID-related research at Hospital Foch—established by the local ethics committee of the Foch Hospital (IRB00012437) (“Information réutilisation des données personnelles et des échantillons biologiques à des fins de recherche V2 - 9 avril 2020”).

Results

1 – Characteristics of the population

The population studied included 825 patients (344 females; 481 males), the median age was 67 [54 - 80] years, with no significant differences between women and men (68 and 67 years, respectively). Eighty-five patients were asymptomatic, with a similar proportion in women (11.6 %) and men (9.4 %, $p = 0.347$). The mean time between the onset of symptoms and the diagnosis was significantly higher ($p = 0.001$) in men than women (table 1). The biological inflammatory state was more pronounced in men than in women as it could be seen on CRP, PCT, ferritin and D-Dimer levels. Creatinine clearance was also significantly lower ($p = 0.005$) in men than in women. Concerning CBC, men had higher levels of

hemoglobin and WBC than women ($p < 0.001$), because of a higher level of PMN ($p < 0.001$). In contrast, absolute lymphocyte count was lower in men than in women ($p < 0.001$), as well as platelet levels ($p < 0.001$). The frequency of diabetic patients was higher in males than in females—males required oxygen more frequently, and tracheal intubation and/or ECMO than females. Mortality was significantly higher in males than in females.

2- Variations of CPD in COVID-19 patients, correlations with markers of inflammation.

We evaluated the modifications of CPD in the 825 patients with proven COVID-19 in comparison to the group of controls. As previously described [10], most of the CPD were significantly different except for the mean conductivity of neutrophils (@MN-C-NE) and the mean of the light scatter at low angle of lymphocytes (@MN-LALS-LY) (See supplementary table 1). There were no differences between men and women, except for the standard deviation of the light scatter at low angle of neutrophils (@SD-LALS-NE), which was significantly higher in women than in men (31.8 *versus* 30.6, respectively, $p < 0.001$), and for the mean of axial light loss of monocytes (@MN-AL2-MO), which was significantly higher in men than in women (133 *versus* 129, respectively, $p = 0.015$).

We evaluated whether the variations of CPD were related to the inflammatory state of the patients, usually quantified by the determination of CRP, PCT and ferritin in COVID-19 patients [2]. We also analyzed whether there was any correlation between CPD and D-dimers because it has been shown that this marker of activation of coagulation is related to the severity and prognosis of COVID-19 [2], activation of coagulation being induced notably by the expression of tissue factor by monocytes [15]. CRP was correlated with 31 CPD, mainly CPD concerning lymphocytes (12/14) and monocytes (13/14), PCT was correlated with 18 CPD (5/14 CPD of lymphocytes and 9/14 CPD of monocytes), ferritin was correlated with 22 CPD (7/14 CPD of lymphocytes, 10/14 CPD of monocytes), and D-dimers were correlated with 28 CPD (11/14 CPD of neutrophils and 11 /14 CPD of lymphocytes) (figure 2). The mean volume of monocytes (@MN-V-MO) and the standard deviation of monocytes (@SD-V-MO) were significantly positively correlated with these four parameters, with a high Spearman's coefficient of rank correlations (ρ) and the mean of light scatter at median angle of monocytes (@MN-MALS-MO) demonstrated strong positive correlation with CRP.

Therefore, the variations of CPD are related to the inflammatory state of COVID-19 patients (See supplementary table 2).

3 – Variations of CPD according to lung injury and oxygen requirement, comparison with classical markers of severity of COVID-19.

In a second step, we wondered if the variations of the CPD could be related to the pulmonary lesions of the patients, measured with the CT-scan, or with their need in oxygen. A CT-scan at admission was available for 493 patients. The Kruskal-Wallis test was < 0.05 for 16 CPD (See supplementary table 3). Among them, the standard deviation of the light scatter at median angle of lymphocytes (@SD-MALS-LY), the standard deviation of light scatter at median of lymphocytes (@SD-UMAL-LY), @MN-V-MO, @SD-V-MO and @SD-AL-2-MO increased significantly with the severity of lung injuries (Fig 3 A), as did CRP, PCT, D-dimers and ferritin. Using ROC curve analysis, we calculated whether CPD or other classical biochemical markers could identify patients with a low level of lung injury ($< 10\%$) on the CT-scan. @SD-AL2-MO, @SD-V-MO, @MN-V-MO had an AUC near the cut-off of 0.7 that we considered as useful to discriminate patients (0.694, 0.689 and 0.678 respectively). Only CRP and ferritin had an AUC above 0.7 (table 2). We then evaluated whether CPD could identify patients with critical lung injury ($> 75\%$). @SD-MALS-LY and @SD-UMALS-LY had an AUC of 0.699 and 0.712, respectively. With a cut-off of 21.45, the sensitivity and specificity of @SD-UMALS-LY were 85 and 57.6 %, respectively. CRP, PCT and ferritin were also discriminant, with a cut-off of 83.1 mg/L, 0.51 $\mu\text{g/L}$ and 1330 $\mu\text{g/L}$, respectively (table 2). WBC count and neutrophil, lymphocyte and monocyte counts were also significantly correlated with the importance of the lung injury. A WBC count above $7.6 \times 10^9/\text{L}$ and neutrophil count above $6.1 \times 10^9/\text{L}$ discriminated patients with more than 75 % lung injury (AUC were 0.748 and 0.737, respectively, sensitivity was 85 and 70 %, and specificity 66.5 and 69.7 %, respectively).

We also evaluated the variations of CPD according to the need for oxygen of patients. Patients were divided into five groups according to their oxygen needs: 390 (204 discharged and 186 hospitalized) patients did not require oxygen supplementation, 234 required fewer than 6 liters of oxygen/min, 106 required between 6 and 15 L/min, 38 required high flow

nasal oxygen (HFNO) and 54 needed intubation and/or extracorporeal membrane oxygenation (ECMO). The Kruskal-Wallis test was < 0.05 for 26 CPD (See supplementary table 4). Using ROC curve analysis, we then calculated whether CPD and other classical biochemical markers could identify patients who would not require oxygen supplementation. As observed for patients with a lung damage $< 10\%$, @SD-V-MO and @MN-V-MO had the highest AUC (0.687 and 0.689 respectively), but only CRP, PCT and ferritin had an A.U.C above 0.7 (table 2). We then evaluated whether CPD could identify patients requiring intubation and/or ECMO. @SD-MALS-LY and @SD-UMALS-LY had significant AUCs of 0.727 and 0.726, respectively (fig 3B). With a cut-off of 23.92, the sensitivity and specificity of @SD-UMALS-LY was 68.5.4 and 69.1 %, respectively, whereas with a cut-off of 18.37, the sensitivity and specificity of @SD-MALS-LY was 57.4 and 82 %, respectively. For the other CPD, AUC was below 0.7. CRP, PCT, D-dimer and ferritin were also discriminant, with the highest specificity for D-dimer, like the specificity of @SD-UMALS-LY (table 2). Thus, the levels of @MN-V-MO, @SD-V-MO and SD-V-NE can be an indication of the extent of lung damage and therefore of the patient's oxygen requirements.

4 – Variations of CPD according to the severity of COVID-19

We also evaluated the variations of CPD according to the clinical evolution of the patients. As most of the patients over 80 years, after collegial decision, were not admitted to the ICU for this analysis, for the statistical analysis, they were excluded from the patients hospitalized in conventional wards ($n = 194$). Patients were divided into five groups: 193 patients were discharged from the hospital within 24 hours (DIS), 317 were hospitalized in the conventional ward (HOS) or in the ICU ($n = 71$). Two other groups were considered: 10 patients who were discharged but were hospitalized in conventional wards some days later (DIS-HOS), and 40 patients who were first hospitalized in conventional wards but worsened and needed admission to the ICU (HOS-ICU). The Kruskal-Wallis test was < 0.05 for 24 CPD (See supplementary table 5). Increased levels of @SD-V-MO and @SD-V-NE were able to identify patients requiring immediate ICU admission (fig 4). For @SD-V-MO, the cut-off for immediate ICU admission was 25.03, specificity and sensitivity were 66.2 % for both parameters, (AUC = 0.713), and for SD-V-NE, the cut-off was 19.4, with a sensitivity of 81 % and a specificity of 53.5 % (AUC = 0.702). For the other CPD, AUC was below 0.7.

Then we analyzed whether CPD could be useful to identify patients who could be immediately discharged. Two CPDs were discriminant: @MN-V-MO and @SD-V-MO (fig 4). The cut-off for @MN-V-MO was 183, the sensitivity and the specificity were 66.1 % and 66.8 %, respectively, and the AUC was 0.73. For @SD-V-MO, the cut-off was 24.34, with sensitivity and specificity of 59.3% and 73.6%, with an AUC of 0.73. CRP, PCT, D-dimers and ferritin were also discriminant for immediate ICU admission and for immediate discharge (Table 2). Consequently, @MN-V-MO, @SD-V-MO and SD-V-NE can constitute markers to be considered to identify patients requiring ICU admission or who can be immediately discharged.

5 – Interest of CPD to identify patients with a high risk of worsening.

Approximately 20% of patients with COVID-19 hospitalized in conventional wards require admission in ICU some days later [16, 17]. Therefore, we analyzed whether CPD alone or in association with clinical data and other biological markers could identify patients who would require ICU admission after hospitalization in conventional wards. Forty patients fulfilled this criterion (median time between hospitalization and conventional wards: 4 days, range 1 -16). Six CPD [the mean conductivity of monocytes (@MN-C-MO), the mean of the lower median angle of the light scatter (@MN-LMAL-MO), the standard deviation of the conductivity of lymphocytes (@SD-C-LY), the standard deviation of the axial light loss of lymphocytes (@SD-@AL-2-LY), @SD-MALS-LY and @SD-UMALS-LY] were significantly higher in patients who worsened, but their AUC was below 0.7 using ROC curve analysis (table 3). Lymphocyte and platelet counts were significantly lower in patients who worsened, but the AUC for platelet count was marginally significant (0.698). In contrast, CRP, PCT, D-dimers and ferritin were not significantly different between the two groups of patients (not shown). Using logistic regression, we obtained an “ICU factor,” which allowed us to identify patients who would require transfer to the ICU. The “ICU factor”, based on 8 CPDs (@MN-LALS-NE, @MN-LALS-LY), @MN-C-MO, @MN-UMALS-MO, @SD-MALS-NE, @SD-UMALS-LY, @SD-V-MO and @SD-C-MO), the platelet and basophil counts, presence of obesity (BMI > 30) and diabetes (See supplementary table 6) demonstrated an AUC of 0.843 (fig 5A), a sensitivity of 40% and a specificity of 97.5% at cut-off 0.39 (fig 5B). This approach would allow us to correctly identify 16 out of 40 (40%) patients who required ICU hospitalization, with only 8 (2.5%) incorrectly

classified patients from the “hospitalization only” group (fig 5B). Adding CRP did not improve the performance of the model.

DISCUSSION

Cellular population data are parameters used by Beckman Coulter analyzers for differential determination. They correspond to a morphological analysis of leukocyte subtypes [18] and several studies have shown that their analysis could constitute a useful aid for the diagnosis of viral, parasitic, or bacterial pathologies [19 - 21]. Among the different CPD, @SD-V-MO and its derivative, MDW, was shown to be useful for the detection of early sepsis associated to bacterial infections [4, 6, 7], and was regulatory cleared. Very quickly, it appeared that @SD-V-MO or MDW was increased in patients with COVID-19, and this increase was correlated with the severity of the disease [8, 9, 12, 13]. However, none of these studies, often because the number of patients included was small or because the design did not have this purpose, defined CPD thresholds for triaging ED patients according to their need for care: hospitalization in conventional wards or ICU, or in contrast to be discharged. Additionally, as in a previous study, we showed that variations of CPD in COVID-19 patients were not restricted to monocyte CPD [10].

We observed significant correlations between CPD of different lineages and the classical severity markers of COVID-19 (CRP, PCT, ferritin and D-dimers). CRP was already shown to be significantly correlated with MDW [9, 13], but we also observed that Spearman's rank correlation coefficient was higher for the mean monocyte volume (@MN-V-MO) and mean scatter at median angle (@MN-MALS-MO) than for the @MN-V-MO ($\rho = 0.52$ for @MN-V-MO and @MN-MALS-MO *versus* 0.47 for SD-V-MO). In the same way, Spearman's rank correlation coefficient was higher for PCT and @MN-V-MO ($\rho = 0.368$) than for PCT and @SD-V-MO ($\rho = 0.187$). Most of the CPD of lymphocytes were also correlated with these four "classical" biomarkers of the severity of COVID-19, while neutrophil CPD were less often correlated, except for SD-V-NE, and in a less extend, MN-V-NE (not correlated to D-dimers). This is in fact logical, since it has been clearly established that circulating monocytes participate in all stages of SARS-CoV-2 infection [22]. Lymphocytes, in combination with monocytes, are known to be involved in the defense against viral diseases [23], and their absolute count is decreased in COVID-19 and serum cytokine levels of IL-6, TNF- α and IL-10 were inversely correlated with CD4⁺ and CD8⁺ T-cell counts [24]. In contrast, neutrophils are in the normal range in mild forms of the disease, but an increase of their count during the

disease course is associated with the mortality, reflecting either a stronger stressed reaction or a secondary bacterial infection [25].

In addition, we also observed that CPD of the different lineages (particularly for lymphocytes and monocytes) varied according to the need for hospitalization, and the level of care required by the patients (hospitalization in conventional wards or ICU). However, a cut-off with sufficient significance to identify patients requiring hospitalization in the ICU was only observed for @SD-V-MO and @SD-V-NE. The @SD-V-MO cut-off was 25.03, which is similar to that considered in the study of Polilli *et al.* [26] or Alsuwaidi *et al.* [13]. The predictive value for ICU admission of @SD-V-NE was not surprising since we had seen earlier that in the most severe forms, the neutrophil count increased [25] and had different immunophenotypes [27]. Consequently, these neutrophil subsets contributed to the neutrophil anisocytosis observed.

Additionally, we observed, for the first time to our knowledge, that CPD variations were correlated to the severity of lung lesions on the CT-scan. The AUC of @SD-V-MO (0.689) was close to 0.7, and a cut-off of 22.65 was obtained to identify patients with low injury (< 10%) on the CT-scan. This cut-off was similar to the cut-off obtained in the study of Polilli *et al.* [26] to identify patients who did not require hospitalization. In contrast to this study [26], we observed a higher threshold (≤ 24.34) to identify patients who did not require hospitalization. This could be due to different management of COVID-19 patients between our institution and the General Hospital of Pescara. Indeed, the first wave of COVID-19 cases reached Italy before France, and there may have been more hospitalizations at the beginning of the pandemic as a precaution, given the uncertainty of patient outcomes.

Furthermore, our study revealed that SD-UMALS-LY could discriminate patients with critical lung injury and severe respiratory failure: a @SD-UMALS-LY > 21.45 was associated with critical lesions on the CT-scan, and > 23.92 was observed in patients requiring tracheal intubation and/or ECMO, whereas @SD-V-MO was not discriminant. This variations of lymphocyte CPD could be related to the acquisitions of unusual markers on the lymphocyte membrane, as it has been shown, for example in CD48 [28].

Lastly, we showed that the consideration of 8 CPD of the different lineages in association with some elements of the CBC and two clinical data (obesity and the presence of diabetes)

that could identify hospitalized patients with a high risk of worsening. The main advantage of this approach can be cost effective, since it requires only a CBC-Diff, whereas most of the previously published reports required several additional marker measurements such as lactate dehydrogenase, CRP, creatinine, D-dimers, albumin, PCT, glucose, total bilirubin, calcium, vitamin D, zinc, or lactate levels [12, 29 – 32]. In addition, despite its simplicity (only a CBC) the performances of our predictive model for ICU admission in patients who will worsen (AUC = 0.843) are similar to multiple more sophisticated prognosis models previously described [33].

MDW has been regulatory cleared as a marker of sepsis on the DxH 900 analyzer. The calculation of MDW is a bit different from SD-V-MO [11]. However, the similarity of the thresholds of our study with those of Alsuwaidi *et al.* [13], performed on a DxH 900, suggested that consideration of @SD-V-MO on the DxH 800 analyzer is highly efficient for the stratification of patients with COVID-19.

Of course, our study has several limitations. The first one is that CPD are specific to Beckman-Coulter hematology analyzers. In addition, as most of the studies on COVID-19, it is a retrospective study carried out in a single hospital during the initial phase of the COVID-19 outbreak. Vaccination has changed the severity of the disease. As underlined before, a better knowledge of the disease has changed practices, particularly in terms of admission to intensive care. In a recent analysis (unpublished data), we observed in a series of 349 patients with the Omicron variant (from December 2021 to March 2022) that CPD variations were less pronounced than in this initial cohort, correlated with a much lower frequency of hospitalization in the ICU. However, the validation of the model that we describe is needed in a prospective way and in a multicentric approach. The influence of a previous SARS-CoV-2 infection, the variant of SARS-CoV-2 and/or vaccination remains to be established.

CONCLUSIONS

Many studies have been published concerning the interest of different biological markers for the prognosis of COVID-19 patients. Most of them used the same makers (CRP, D-Dimers, procalcitonin, absolute lymphocyte count...) but only a few of them investigated the morphological variations of blood cells induced by the SARS-CoV-2 and the few studies on

CPD included a limited numbers of patients and/or were limited to the analysis of the MDW. Our study suggests that CPD consideration of the different lineage are useful parameters to identify patients at risk for severe forms of COVID-19. Because of a sufficient number of patients included, we were able to determine thresholds suggesting severe forms of COVID-19. If @SD-V-MO and @MN-V-MO are useful markers for determining the severity of the disease, @SD-UMAL-LY is associated with the severity of lung damage and the need for mechanical ventilation. In addition, CPD consideration of the three lineages with some blood count parameters and morbidities (diabetes and obesity) can help identify patients with a high risk of worsening and ICU admission. This also makes it possible to anticipate the management of intensive care beds—a limiting factor when patients arrive in large numbers at the hospital with a new aggressive variant. These findings should be continually re-evaluated in the coming months as more data becomes available in prospective cohorts, and with longer follow-up.

Credit authorship contribution statement:

Marc Vasse: Writing - review & editing, Conceptualization; Dmitry Sukhachev: Formal analysis, review & editing; Marie-Christine Ballester: Resources and review; Frédérique Delcominette: Resources and review; François Mellot: Resources and review; Florence Habarou: Resources and review; Aurélie Védrenne: Resources and review; Emilie Jolly: Resources and review, Elena Sukhacheva Review & Editing, Eric Farfour: Resources, review & editing; Tiffany Pascreau: review & editing.

Acknowledgments

The authors would like to thank Romane Donato for help to develop the data base and Polly Gobin for her editorial assistance.

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Table 1: Main characteristics of the patients with proven COVID-19 included in the study.

	Total population	Females	Males
n	825	344	481
Age (years)	67 [54 – 80]	68 [53 – 82]	67 [54 – 79]
Asymptomatic n (%)	85 (10.3)	40 (11.6)	45 (9.4)
Time since the onset of symptoms (days)	6 [3 – 10]	5 [2 – 9]	7 ** [3 – 10]
C-reactive protein (mg/L)	62 [16 – 131]	32.7 [8 – 105]	79 *** [26 - 146]
Procalcitonin (ng/mL)	0.13 [0.07 – 0.3]	0.10 [0.06 – 0.24]	0.16 *** [0.09 – 0.37]
Ferritin (µg/L)	668 [264 - 1595]	333 [117 - 731]	936 *** [430 – 2220]
D-dimers (mg/L)	0.751 [0.429 – 1.407]	0.700 [0.360 – 1.188]	0.771 [0.470 – 1.500]
Creatinine clearance (mL/min)	80.7 [56.5 – 96.9]	84.6 [60.7 – 99.9]	79.6 ** [54.1 – 94.3]
White blood cells (x10 ⁹ /L)	6.3 [4.7 – 8.5]	5.8 [4.4 – 8.2]	6.7 *** [5.0 – 9.0]
<i>Neutrophils</i> (x10 ⁹ /L)	4.5 [3.8 – 6.8]	3.9 [3.6 – 4.3]	5.0 *** [3.4 – 7.0]
<i>Lymphocytes</i> (x10 ⁹ /L)	0.9 [0.7 – 1.4]	1.1 [0.8 – 1.5]	0.9 *** [0.6 – 1.2]
<i>Monocytes</i> (x10 ⁹ /L)	0.5 [0.4 – 0.7]	0.5 [0.4 – 0.7]	0.5 [0.4 – 0.8]
Hemoglobin (g/dL)	13.3 [12 – 14.4]	12.8 [11.5 – 13.7]	13.8 *** [12.4 – 14.8]
Platelets (x10 ⁹ /L)	208 [161 – 270]	222 [175 – 286]	194 *** [153 – 255]
Comorbidities n (%)			
<i>Arterial Hypertension</i>	304 (36.8)	121 (35.2)	183 (38)
<i>Diabetes</i>	209 (25.3)	68 (19.8)	141 (29.3)**
<i>Obesity[#] (BMI > 30)</i>	99 (29.6)	42 (35.6)	57 (26.3)
Oxygen Requirement n (%)			
<i>Absence</i>	391 (47.4)	196 (57)	195 (40.5)***

<i>Between 1 and 6 L/min</i>	234 (28.4)	84 (24.4)	150 (31.2)*
<i>Between 7 and 15 L/min</i>	106 (12.8)	44 (12.8)	62 (12.9)
<i>High flow oxygen</i>	41 (5)	13 (3.8)	28 (5.8)
<i>Tracheal intubation and/or ECMO</i>	53 (6.4)	7 (2)	46 (9.6)***
Lung injury on CT scan ^{##} n (%)			
< 10 %	198 (40.2)	87 (45.1)	111 (37)
<i>Between 10 and 25 %</i>	122 (24.7)	53 (27.5)	69 (23)
<i>Between 25 and 50 %</i>	109 (22.1)	38 (19.6)	71 (23.7)
<i>Between 50 and 75 %</i>	44 (8.9)	11 (5.7)	33 (11)
> 75 %	20 (4.1)	4 (2.1)	16 (5.3)
Death n (%)	98 (11.9)	15 (4.4)	83 (17.3)***

Results are expressed as median and [quartiles]. Mann-Whitney test was used to compare females and males. ** p < 0.01; *** p < 0.001.

data available for 334 patients; ## data available for 493 patients

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CT-scan: computed tomography

Table 2: Cut-offs, sensitivity, specificity and area under the curve (AUC) of C-reactive protein (CRP), procalcitonin (PCT), D-dimers or ferritin to identify COVID-19 patients in the emergency department with low (< 10 %) or severe (> 75%) lung injury, requiring no oxygenotherapy or intubation and/or extracorporeal membrane oxygenation, or requiring either immediate admission in intensive care unit (ICU) or who could be immediately discharged.

	Cut-off	Sensitivity	Specificity	AUC
Low lung injury on CT scan				
CRP (mg/L)	< 79.1	78.0	67.5	0.788
Ferritin (µg/L)	< 616	76.8	63.7	0.721
More than 75 % lung injury on CT scan				
CRP (mg/L)	> 83.1	95.0	56.1	0.797
PCT (ng/mL)	> 0.51	80.0	80.4	0.804
Ferritin (µg/L)	> 1329	68.7	74.9	0.740
No oxygen				
CRP (mg/L)	< 40	63.3	78.3	0.764
PCT (ng/mL)	< 0.11	68.0	71.0	0.710
Ferritin (µg/L)	< 64.2	60.2	74.1	0.724
Intubation and/or extracorporeal membrane oxygenation				
CRP (mg/L)	> 83.1	82.4	62.1	0.770
PCT (ng/mL)	> 0.14	90.0	56.6	0.717
D-dimers (mg/L)	> 1634	57.1	82.4	0.708
Ferritin (µg/L)	> 1062	73.0	69.0	0.721
Immediate ICU Admission				
CRP (mg/L)	> 107.7	75.0	85.1	0.855
PCT (ng/mL)	> 0.37	81.0	66.7	0.800
D-dimers (mg/L)	> 1428	84.6	50.0	0.728
Ferritin (µg/L)	> 652	55.4	85.5	0.751
Immediate discharge				
CRP (mg/L)	< 22.4	82.6	73.5	0.840
PCT (ng/mL)	< 0.09	73.2	76.2	0.789
D-dimers (mg/L)	< 600	65.8	73.1	0.750
Ferritin (µg/L)	< 642	60.2	77.6	0.733

Table 3: Values of 6 cellular population data, platelet and lymphocyte counts which are significantly different among 317 COVID-19 patients (< 80 years) requiring hospitalization in conventional wards (HOS) and 40 patients requiring ICU admission after conventional hospitalization (HOS-ICU).

	HOS (n = 317)	HOS-ICU (n = 40)	<i>p</i>	AUC
@SD-C-LY	8.6	9.5	0.016	0.617
@SD-MALS-LY	17.4	18.3	0.022	0.611
@SD-UMALS-LY	21.3	23.1	0.0006	0.633
@SD-AL2-LY	13.7	14.3	0.035	0.603
@MN-C-MO	123	125	0.013	0.620
@MN-LMALS-MO	75	77	0.049	0.596
Platelets (x 10 ⁹ /L)	214	150	< 0.001	0.698
Lymphocytes (x 10 ⁹ /L)	1	0.8	0.019	0.614

AL2: axial light loss; AUC: area under the curve of ROC curve analysis; C: conductivity; LMALS: lower median angle light scatter; LY: lymphocytes; MALS: median angle light scatter; MN: mean; MO: monocytes; SD: standard deviation; UMALS: upper median angle light scatter.

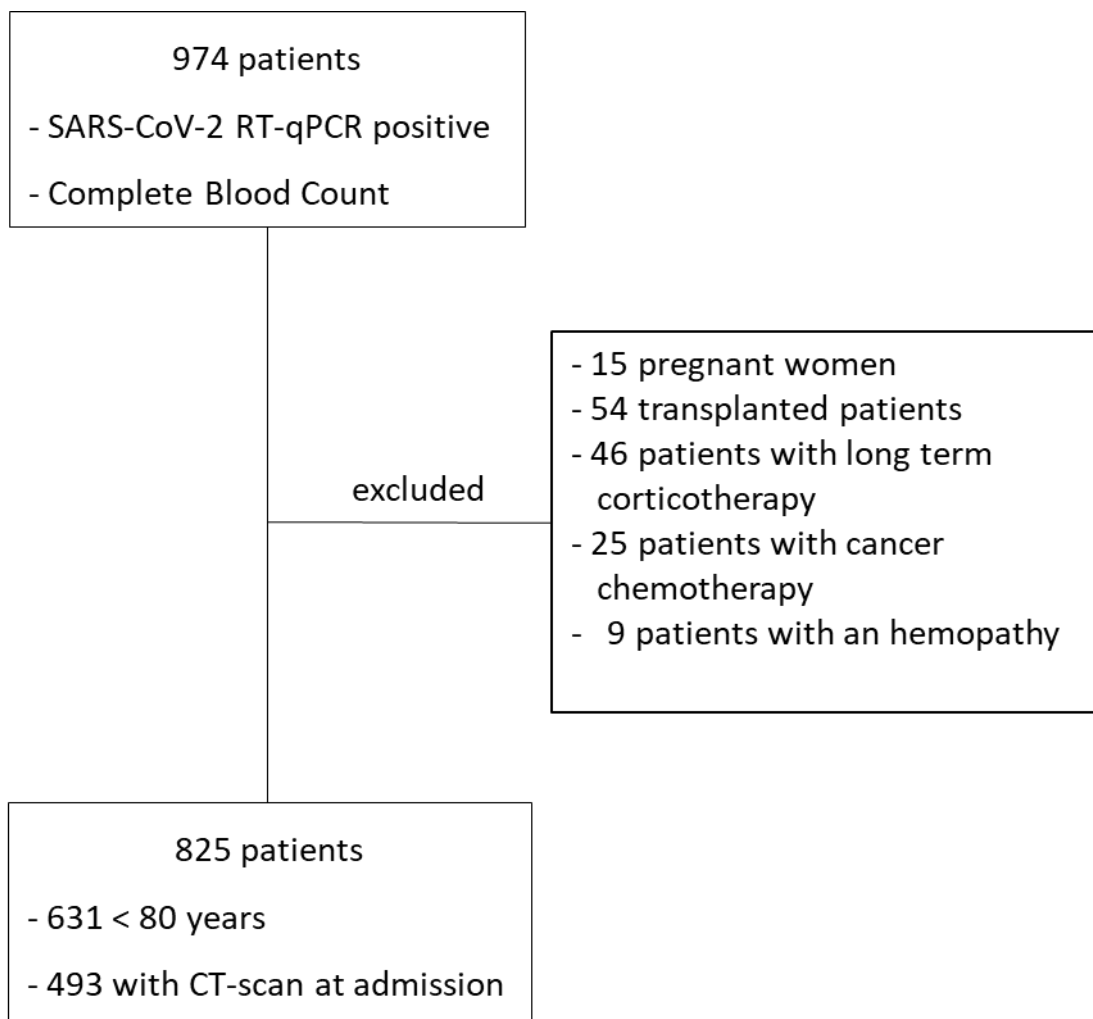


Fig 1: Selection of study patients.

Patients were recruited from the emergency department. To be included, they had to have both a positive SARS-CoV-2 RT-qPCR and a complete blood count. Exclusion criteria were pregnancy, because a possible influence of pregnancy on cellular population data (CPD) can be hypothesized, immunosuppressive and antineoplastic treatments, which modify leukocyte counts and hemopathies, because abnormal blood cells interfere with the determination of the CPD of the normal lineages.

CT-scan: computed tomography-scan

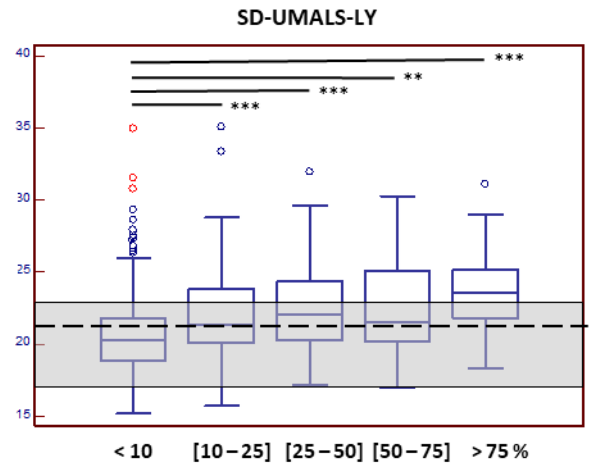
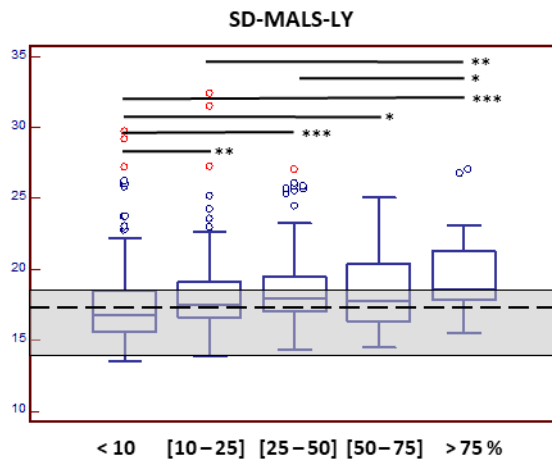
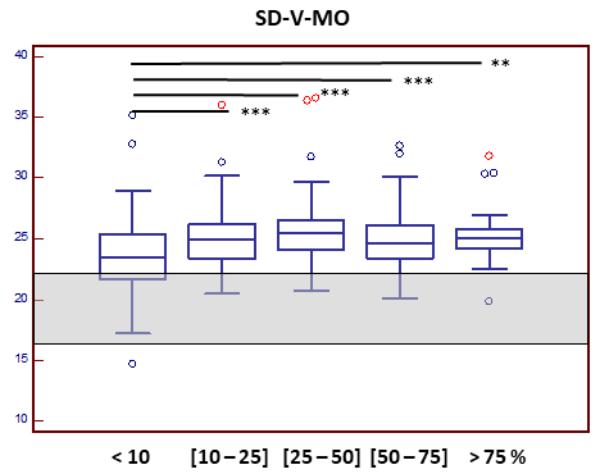
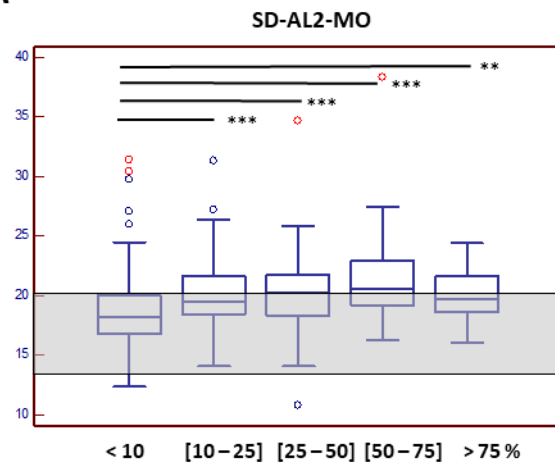
		CRP	PCT	Ferritin	D-dimers
Neutrophils	@MV-V				
	@SD-V				
	@MN-C				
	@SD-C				
	@MN-MALS				
	@SD-MALS				
	@MN-UMALS				
	@SD-UMALS				
	@MN-LMALS				
	@SD-LMALS				
	@MN-LALS				
	@SD-LALS				
	@MN-AL2				
	@SD-AL2				
Lymphocytes	@MV-V				
	@SD-V				
	@MN-C				
	@SD-C				
	@MN-MALS				
	@SD-MALS				
	@MN-UMALS				
	@SD-UMALS				
	@MN-LMALS				
	@SD-LMALS				
	@MN-LALS				
	@SD-LALS				
	@MN-AL2				
	@SD-AL2				
Monocytes	@MV-V				
	@SD-V				
	@MN-C				
	@SD-C				
	@MN-MALS				
	@SD-MALS				
	@MN-UMALS				
	@SD-UMALS				
	@MN-LMALS				
	@SD-LMALS				
	@MN-LALS				
	@SD-LALS				
	@MN-AL2				
	@SD-AL2				

 p < 0.05;
  p < 0.01;
  p < 0.001

Fig 2: Correlations between cellular population data (CPD) of 825 patients with proven COVID-19 and C-reactive protein (CRP), procalcitonin (PCT), ferritin and D-dimers

For each CPD, correlations with the 4 classical markers of severity of COVID-19 were indicated by a colored rectangle which indicated the p value, calculated by the rank correlation test. The exact value of “p” as well as the Pearson’s correlation coefficient are indicated in the appendix (see Supplementary table 2). CRP, PCT, ferritin, and D-dimer levels were available for 761, 253, 415 and 480 patients, respectively.

A



B

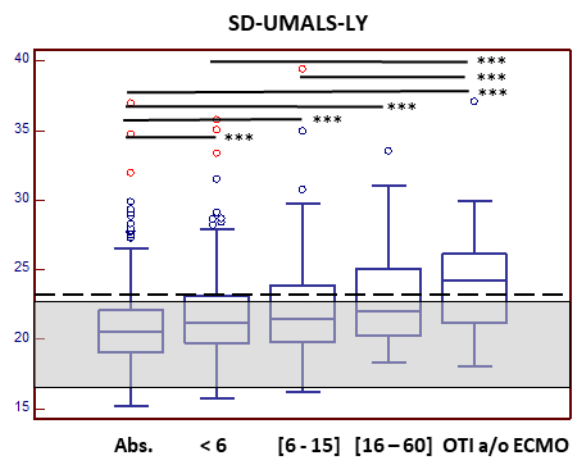
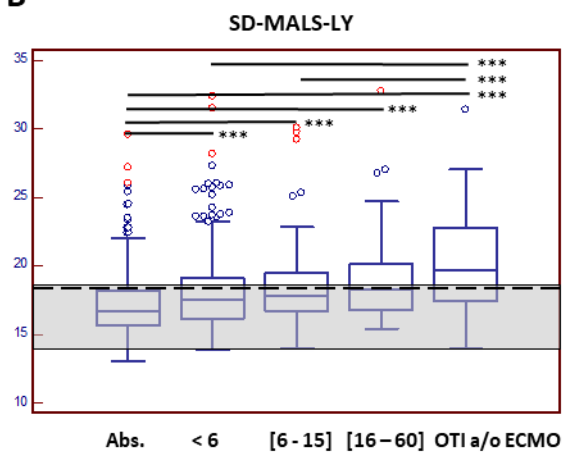


Fig 3: Variations of different cellular population data according (A) to lung injury quantification on computed tomography-scan of 493 patients with COVID-19 or (B) needs in oxygen.

Abscissa indicates the percentages of lung injury on CT-Scan (A) or the volume of oxygen in L/min distributed (B).

Abs: absence, OTI a/o ECMO: orotracheal intubation and/or extracorporeal membrane oxygenation

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Mann-Whitney test)

The gray zone corresponds to the normal range (5th – 95th percentile). The dotted lines correspond to the cutoffs determined by ROC curve analysis to identify patients with more than 75 % injury or requiring orotracheal intubation and/or ECMO. Only significant statistical differences between the groups with a low (< 10%) or critical (> 75%) lung injuries were indicated, or without the need for oxygen or orotracheal intubation and/or ECMO.

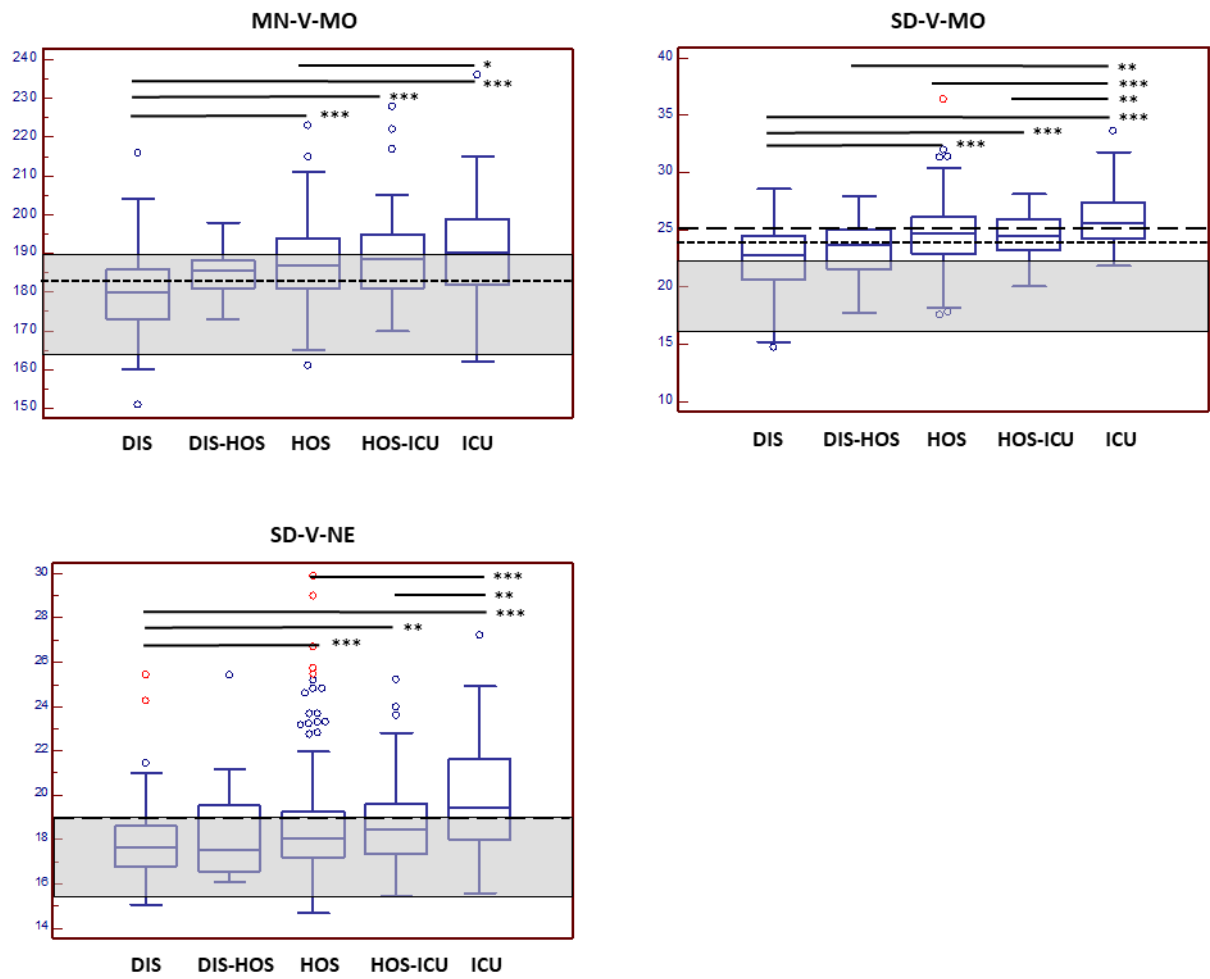


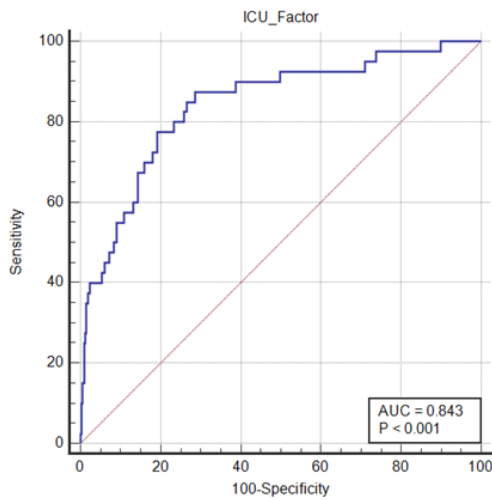
Fig 4: Variations of @MN-V-MO, SD-V-MO and @SD-V-NE according to the need for hospitalization in 362 patients with COVID-19, and the type of hospitalization.

DIS: discharged, HOS: Hospitalization in conventional wards, ICU: Intensive care unit

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Mann-Whitney test)

The gray zone corresponds to the normal range (5th – 95th percentile). The dotted lines correspond to the cutoffs determined by ROC curve analysis to identify patients that can be discharged (small dots) or require immediate ICU admission (large dots). Only statistical differences between the groups of discharged and ICU patients are indicated.

A



B

Cut-off	True negative	False positive n – (%)	False negative	True positive	Sensitivity	Specificity	Positive predictive value	Negative predictive value
0.43	311	6 (1.9)	25	15	35	98.11	70	92.3
0.39	309	8 (2.5)	24	16	40	97.48	66.7	92.8
0.31	296	21 (6.6)	22	18	45	93.69	47.4	93.1

Figure 5: (A) ROC curve analysis of the ICU factor obtained by logistic regression to identify patients hospitalized in conventional wards who will worsen and require transfer to the Intensive Care Unit. The clinical performance of different cut-offs of the “ICU factor” are indicated in the table (B).

The ICU factor is composed of the following parameters: mean light scatter at the low angle of neutrophils (@MN-LALS-NE) and of lymphocytes (@MN-LALS-LY), mean of the monocyte conductivity (@MN-C-MO), mean of the light scatter at upper median angle of monocytes (@MN-UMALS-MO) and standard deviation at the median angle of the light scatter of neutrophils (@SD-MALS-NE), the standard deviation of light scatter at upper median angle of lymphocytes (@SD-UMALS-LY) and the standard deviation of the volume and conductivity of monocytes (@SD-V-MO and @SD-C-MO), the absolute count of platelets and basophils, and the existence of a diabetes and obesity.