To the Editor,

The pathology of COVID-19 results from a dysregulation of the adaptive immune system. Hematological abnormalities have been reported repeatedly in COVID-19 patients since the outbreak of the pandemic [1].

Leukocyte differential present certain features in SARS-CoV2 infected patients neutrophilia, lymphopenia and morphology alterations, which could be useful for screening [2].

Not only differential cell population data (CPD) are research parameters reported as part of leukocyte differentials by some modern analyzers; these are morphometric parameters that characterize leukocytes and classify them according to their volume, granularity and the content in nucleic acids. CPD reflect numerically the changes in morphology and activation status triggered by infections.

With the advent of COVID 19, several studies used the CPD data in the timely diagnosis of the disease. The data were also utilized for monitoring, prognosis and assessment of disease severity [3–7].

We have explored the leukocyte differential and CPD parameters reported by the Mindray BC-6800 Plus analyzer in patients infected with SARS-CoV-2 and those suffering from infections of different etiologies admitted to the Emergency Department. We evaluate the utility of these parameters as laboratory indicators for the detection of COVID-19.

This prospective observational study was conducted after receiving approval from the Comité de Ética de la Investigación de Euzkadi CEIE (Regional Ethics Committee, PL2019090, 17 July 2019).

The training group consisted of consecutive patients with fever and suspected infection admitted to the Emergency Department at Galdakao–Usansolo Hospital between the 1st of December 2020 and the 30th of January 2021. The criterion for inclusion in the validation group was the same as for the training group: the participants were consecutive patients with fever admitted to the Emergency Department (between 1st of February and 30th of April 2021).

The CBC and extended leukocyte parameters were acquired using the Mindray BC-6800 Plus analyzer (Mindray Diagnostics, Shenzhen, China). The counter was calibrated, monitored and maintained following the manufacturer’s recommendations.

Patients with COVID 19 were diagnosed using the current standards and on the basis of positive results of RT-PCR for SARS-CoV-2 in throat swab specimens. Bacterial infections were revealed by positive cultures, while positive serology or molecular testing confirmed other viral infections.
The whole patient sample was divided into two subsets: training and validation set. To evaluate the mean/median differences across both cohorts, the non-parametric Wilcoxon test was used, while the $\chi^2$-test was applied to compare categorical variables in both groups.

Mean and median differences of the laboratory markers by patient’s COVID-19 status were computed in the training set. Likewise, to assess the relationship between the laboratory CPD markers and the COVID-19 status, the non-parametric Wilcoxon test was used. Furthermore, a univariate logistic regression analysis was performed in order to measure the unadjusted odds ratio (OR) with their confidence intervals at 95% level. As second step, a multivariate logistic regression analysis was performed: as dependent variable, patient’s COVID-19 status was considered. The independent factors were those variables whose p-value was <0.20 in the univariate analysis. Using a backward procedure, the final multivariate model was obtained. The model robustness was assessed in terms of discrimination (by means of the area under the ROC curve (AUC)).

Once the multivariate model was found in the training set, its performance was gauged in the validation cohort. Similarly, model discrimination and calibration was calculated.

All the statistical procedures were performed using R 3.5 release. A p-value <0.05 was deemed to be statistically significant.

A sample of 454 patients were recruited in the current study: 237 (52.20%) in training set and 217 (47.80%) patients were derived to the validation set.

The former group included 237 patients; COVID-19 was confirmed in 151 patients. There were 86 patients with other infections; 53 of them suffered from pneumonia, urinary infections, meningitis, bacterial gastroenteritis. Thirty-three patients suffered viral infections (respiratory infections, Epstein-Bar virus, influenza or cytomegalovirus).

The latter group of 217 patients included 115 COVID-19, 102 non-COVID-19 (32 viral, 70 bacterial).

The statistical tests confirmed that both groups were comparable; the exception were the immature neutrophil counts (p 0.03), whereas the values ranged from 0.01–0.05 × 10⁹/L, and showed extensive overlap.

In the training group, summary statistics and their corresponding unadjusted odds ratios of the CPD values according to the patient’s COVID-19 status were computed (Table 1). Lower WBC (OR (95% CI): 0.854 (0.804, 0.908)), lymphocyte count (OR (95% CI): 0.363 (0.254, 0.520)) and monocyte counts (OR (95% CI): 0.014 (0.004, 0.045)) were found to be related to the presence of COVID-19 disease (p < 0.001). As for the CPD features, Neu X and the Lym Z showed statistically significant differences between COVID-19 and non-COVID-19 patients. Low Neu Y values were associated to having COVID-19 disease, but it was not statistically significant (p = 0.11).

Table 1: Summary statistics (median and 25–75 percentile range [P25–P75] of leukocytes [absolute counts, ×10⁹/L] and cell population data [arbitrary optical units] in the training set [n = 237]).

<table>
<thead>
<tr>
<th></th>
<th>No COVID-19 patients (n = 86)</th>
<th>COVID-19 patients (n = 151)</th>
<th>Unadjusted analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (P25–P75)</td>
<td>Median (P25–P75)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>WBC/L</td>
<td>10.79 (6.96–15.79)</td>
<td>7.19 (5.16–9.59)</td>
<td>0.854 (0.804, 0.908)</td>
</tr>
<tr>
<td>Lymph</td>
<td>1.86 (1.11–3.08)</td>
<td>1.00 (0.69–1.34)</td>
<td>0.363 (0.254, 0.520)</td>
</tr>
<tr>
<td>Mono</td>
<td>0.66 (0.46–0.91)</td>
<td>0.26 (0.00–0.45)</td>
<td>0.014 (0.004, 0.045)</td>
</tr>
<tr>
<td>Neut</td>
<td>6.24 (3.71–11.83)</td>
<td>5.69 (3.885–7.74)</td>
<td>0.924 (0.873, 0.978)</td>
</tr>
<tr>
<td>IG</td>
<td>0.02 (0.01–0.10)</td>
<td>0.010 (0.0–0.02)</td>
<td>0.033 (0.003, 0.310)</td>
</tr>
<tr>
<td>Neu X</td>
<td>361 (328–391)</td>
<td>346 (322–372)</td>
<td>0.992 (0.986, 0.998)</td>
</tr>
<tr>
<td>Neu Y</td>
<td>435 (407–461)</td>
<td>428 (412–448)</td>
<td>0.994 (0.986, 1.001)</td>
</tr>
<tr>
<td>Neu Z</td>
<td>1,814 (1,759–1,865)</td>
<td>1,808 (1,749–1,872)</td>
<td>0.999 (0.997, 1.002)</td>
</tr>
<tr>
<td>Lym X</td>
<td>88 (83–95)</td>
<td>88 (85.1–92.4)</td>
<td>0.981 (0.942, 1.022)</td>
</tr>
<tr>
<td>Lym Y</td>
<td>656 (626–699)</td>
<td>650 (627–681)</td>
<td>0.997 (0.992, 1.003)</td>
</tr>
<tr>
<td>Lym Z</td>
<td>956 (932–977)</td>
<td>960 (945–981)</td>
<td>1.010 (1.000, 1.020)</td>
</tr>
<tr>
<td>Mon X</td>
<td>200 (190–211)</td>
<td>200 (193–209)</td>
<td>0.996 (0.979, 1.013)</td>
</tr>
<tr>
<td>Mon Y</td>
<td>936 (884–988)</td>
<td>933 (888–988)</td>
<td>1.000 (0.996, 1.003)</td>
</tr>
<tr>
<td>Mon Z</td>
<td>1,299 (1,275–1,331)</td>
<td>1,303 (1,270–1,332)</td>
<td>0.998 (0.992, 1.001)</td>
</tr>
<tr>
<td>Platelets</td>
<td>241 (141–367)</td>
<td>222 (175–301)</td>
<td>0.999 (0.997, 1.001)</td>
</tr>
<tr>
<td>NLR</td>
<td>3.85 (1.24–9.45)</td>
<td>5.43 (3.82–8.03)</td>
<td>0.999 (0.971, 1.028)</td>
</tr>
</tbody>
</table>

WBC, leukocyte count; Neu, neutrophils; Lymph, lymphocytes; Mono, monocytes; Neu X, neutrophils complexity; Neu Y, neutrophils nucleic acids content; Neu Z, neutrophils size; Lym X, lymphocytes complexity; Lym Y, lymphocytes nucleic acids content; Lym Z, lymphocytes size; Mon X, monocytes complexity; Mon Y, monocytes nucleic acids content; Mon Z, monocytes size; PLT, platelets; IG, immature neutrophils count; NLR, neutrophil/lymphocyte ratio.
As next step, Table 2 displays the odds ratios and the confidence intervals at 95% level of the independent predictor for having the COVID-19 disease, both in the training and validation sets. The lower WBC and Neu Y values as well as higher neutrophil counts were related for predicting the outcome of interest. This model showed good discrimination and calibration properties in both training and validation sets: an AUC value of 0.867, p > 0.05 in the H–L test was obtained.

Despite differences in CPD found in the univariate analysis, only neutrophils derived parameters were found to be useful for the discrimination when multivariate analysis was performed.

Neutrophils constitute an essential component of the leukocyte family and play a critical role in the innate immune response [8, 9]. In COVID-19, the persistent infection and prolonged hypoxia lead to compensatory hyperplasia of the bone marrow and an increase in the number of released granulocytes. These observations explain our data and the contribution of neutrophil counts and Neu Y, a marker for cell activation, in our model, which could help identify COVID-19 patients at admission with high accuracy (AUC 0.867).

Our study has some limitations. First, it is a single-center study; the preliminary results must be validated in a multi-center evaluation including more patients. Second, we aimed to improve the rapid evaluation of an SARS-CoV-2 infection at the Emergency Department; thus, we lack the data on asymptomatic patients and children.

Some prompt initial indicators obtained in the emergency rooms would be beneficial since they could point to the appropriate confirmatory diagnostic test and decide on treatment. Currently, clinicians suspect SARS-CoV-2 infection when a patient presents with a cough, shortness of breath, or fever; however, it should become mandatory to establish and follow better “watchful waiting” systems for such patients.

Abnormalities in routine tests can be used for this purpose, particularly in CBC, the laboratory test most frequently ordered by emergency physicians, can warn of COVID-19 infection [10].

The WBC differential and CPD parameters can be relied on in the evaluation of a patient with a fever of unknown origin. Then, the isolation and safety procedures can be initiated to reduce the risk of transmission of the infection among other patients and health care professionals.

Nevertheless, CPD values largely depend on the analyzer; this fact reveals a weakness of these “upgraded” WBC differentials. CPD from analyzers of different brands are not necessarily comparable, hindering the widespread use of these research parameters.

The transferability and harmonization of such procedures must be ensured before introducing the CPD as a standard in clinical practice.

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Ethical approval: This prospective observational study was conducted after receiving approval from the Comité de Ética de la Investigación de Euzkadi CEIE (Regional Ethics Committee, PI2019090, 17 July 2019).

References


