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CORRESPONDENCE

Vaccination does not affect leukocyte morphologic abnormalities of severe COVID-19

To the Editor:

In April 2020, we described the morphological abnormalities in circulating leukocytes of the first set of SARS-CoV-2 infected patients admitted to our institution at the outbreak of the pandemic.[1] The most typical anomalies concerned neutrophil granulocyte nuclei, with hyposegmentation and dark chromatin, and cytoplasm, with increased or decreased granularity, and Döhle bodies. We also described the presence in peripheral blood (PB) of a neutrophil left shift, with immature granulocytes and apoptotic cells, as well as occasional decrease of neutrophil myeloperoxidase. Such changes disappeared during or after recovery, when reactive lymphocytes became predominant. The existence of unusual, dysplasia-like morphological features in neutrophil granulocytes in COVID-19 has been confirmed, integrated and corroborated by many studies, verting on morphology,[2–5] electron microscopy,[5] immunophenotype,[6] and genomics-transcriptomics-genetic analysis.[7]

Two years after our report, with almost 14.6 millions of COVID-19 cases diagnosed in Italy and more than 150 000 deaths (https://www.epicentro.iss.it/en/coronavirus/sars-cov-2-dashboard accessed on April 3, 2022), we have retrospectively re-evaluated PB film leukocyte morphology in light of the massive vaccination campaign implemented in our country (89.85% of the Italian population above 12 years of age has completed the vaccination cycle, see https://www.governo.it/it/cscovid19/report-vaccini/ accessed on April 3, 2022). Two expert observers prospectively recorded the qualitative alterations found in leukocytes at the microscope examination of anonymized PB films of 77 consecutive hospitalized patients with severe COVID-19 and positive PCR test for SARS-CoV-2 from January 22 to February 15, 2022. Results of blood cell counts in K2-EDTA, analyzed with a Siemens ADVIA 2120 hematologic analyzer (Siemens Healthcare, Milan, Italy) within 6 h from blood collection were also recorded. We also investigated a possible relationship with the vaccine status of the patients. All clinical data were anonymized prior to analysis.

Study patients (49 males/28 females) had a mean age of 69.2 years (range 20–96); 37 (48%) of them were unvaccinated, while 40 (52%) had received anti-SARS-COv-2-189 vaccines (14 fully vaccinated with a booster dose, 24 with two doses, and 2 with one dose). Co-morbidities were present in most patients, while the absence of co-morbidities was observed in seven unvaccinated cases. The respiratory situation was similar in the two groups, except for tracheostomy (two patients in the unvaccinated group). There were 11 deaths in unvaccinated patients and seven deaths in the vaccinated group.

Hemoglobin concentration was similar among the unvaccinated and the vaccinated patents (mean HB 11.4 g/dL [range: 7.4–14.1] vs. 10.6 [range: 8.0–12.9]). Mean platelet count was slightly higher in the vaccinated group (270×10^9/L [range: 32–649] vs. 212 [range: 16–722]). Unvaccinated patients displayed slightly higher white blood cell count (WBC) (mean 11.4×10^9/L [range: 7.1–15.0] vs. 6.8 [range 1.7–21.9]) and similar lymphocyte counts (1.26×10^9/L [0.30–1.95]) in unvaccinated cases vs. 1.33 [0.15–12.0]). Such differences were not statistically significant, except for PLT ($p < .05$).* The mean peroxidase neutrophil index (MPXI, laboratory reference range −10 to +5) was −0.25 in the vaccinated group (range: −18.1 to +6.8) and −5.1 in the unvaccinated COVID-19 patients (range −31.9 to +17.3) ($p > .1$). Partial neutrophil myeloperoxidase deficiency (MPXI <−10) was observed in 15/77 COVID-19 patients (18.2%, almost equally divided between seven vaccinated and eight unvaccinated); the MPXI was >10% in two vaccinated COVID-19 patients (+17.3 and +17.2, respectively).

Examinations of the PB films confirmed the presence of neutrophil morphological atypia in about one-third of COVID-19 patients. Table 1 shows the frequency of the main anomalous findings. A hyposegmented, pseudo-Pelger-like neutrophil nucleus, with hypercondensed chromatin, was the most frequent anomaly (not to be confounded with band cell nucleus): it was observed in both vaccinated and unvaccinated patients with a similar frequency of about 18% of PB films. Next in frequency, increased, toxic-like cytoplasm hypergranularity was more common in vaccinated patients (20%), while pale-blue, Döhle-body-like cytoplasmic areas were more frequent in unvaccinated patients (21.6%). The presence in the PB films of pyknotic and smudged cells was relatively common and difficult to quantify due to the variability of film preparation and possible artifacts. Lymphopenia with activated lymphocytes was also occasionally seen in patients with different vaccination statuses.

In conclusion, we have confirmed that neutrophil morphological abnormalities are observed in patients with severe SARS-CoV-2 infection who require hospital admission, independently of their vaccination status. Their frequency and severity appear to have decreased compared with our previous observation.[1,2] Nuclear hyperdense chromatin with decreased segmentation still is the most common morphological feature, together with increased density and number of

TABLE 1 Neutrophil morphological and cytochemical abnormalities observed in hospitalized patients with COVID-19, considering the vaccination status

	Total cases ($n = 77$)	Vaccinated ($n = 40$)	Unvaccinated ($n = 37$)
Hyposegmented neutrophils	14 (18.2%)	7 (17.5%)	7 (18.9%)
Hypersegmented neutrophils	4 (5.2%)	2 (5.0%)	2 (5.4%)
Hypogranular neutrophils	7 (9.1%)	2 (5.0%)	5 (13.5%)
Hypergranular neutrophils	10 (13.0%)	8 (20%)	2 (5.4%)
Pale-blue cytoplasmic areas	8 (10.4%)	3 (7.5%)	5 (13.5%)
Nucleated red blood cells (any)	9 (11.7%)	5 (12.5%)	4 (10.8%)
Immature granulocytes (>1%)	7 (9.1%)	2 (5.0%)	5 (13.5%)
Advia MPXI <10	14 (18.2%)	7 (17.5%)	8 (21.6%)

cytoplasmic granules. In addition, using the ADVIA 2120 hematologic analyzer with automated cytochemistry, we have found an increased frequency of cases of partial neutrophil myeloperoxidase deficiency, confirming our preliminary observation.[1] The morphological and the cytochemical abnormalities can be related to the general inflammatory state and cytokine storm that characterize the clinical evolution of severe COVID-19 cases[7–10] and seem to be generally not affected by prior vaccination.

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CONFLICT OF INTEREST

None to declare.

DATA AVAILABILITY STATEMENT

Anonymized data available upon request.

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ENDNOTE

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