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# Two different patterns of lymphocyte alterations in critically ill COVID-19 patients

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Dear Editor,

Lymphocytes play a decisive role in maintaining immune homeostasis and guiding the inflammatory response. However, investigating properly the immunological features of COVID-19 patients is particularly challenging since reported alterations may intertwine disease triggers and consequences. Among all analyzed immunological parameters, lymphopenia appears as a signature of COVID-19, reported in over 80% of patients and involving all lymphocyte subsets [1]. The occurrence of lymphopenia is a hallmark of COVID-19 disease severity [1–3], but may also be promoted by host factors including age or preexisting comorbidities such as immunosuppression.

The several factors leading to lymphopenia suggest a time-dependent operating mechanism. Indeed, SARS-CoV-2-related lymphocyte apoptosis through the ACE2 receptor [2] might prevail in patients admitted to the intensive care unit (ICU) immediately following infection. In patients later admitted to the ICU, at the time of hypercytokinemia, lymphocyte migration through the endothelium and lymphocyte deficiency induced by TNF- $\alpha$  and IL-6 might be additional causes of lymphopenia [2, 4]. Last, lymphopenia being a reliable indicator of disease severity [1–3], the extent of organ dysfunction and associated lactate imbalance suppress lymphocytes proliferation [5]. Therefore, it is likely that the nature and the depth of lymphocyte dysfunction vary over

time, particularly between the time of viral symptoms onset and the time of ICU admission.

Studies investigating COVID-19-associated lymphopenia have primarily assessed its association with mortality and treatment success [3]. To our knowledge, the effect of the abovementioned timeline on baseline quantitative and qualitative peripheral lymphocyte alterations has never been investigated.

In this study of critically ill COVID-19 patients, we investigated the relationship between baseline levels of peripheral lymphocyte subsets and mortality, independently from age and disease severity. We hypothesized that the association between lymphocyte subsets and mortality might differ according to the time between symptoms onset and ICU admission.

One hundred consecutive critically ill patients with laboratory-confirmed SARS-CoV-2 infection admitted to the Saint-Louis Hospital, Paris, were prospectively included between March 7th and April 28th 2020. All patients had a positive real-time reverse transcriptase–polymerase chain reaction assay of nasopharyngeal or rectal swabs. Data from lymphocyte phenotyping, gamma globulins levels and monocyte HLA-DR (mHLA-DR) expression upon ICU admission were analyzed. A Cox proportional hazard model was used to assess the relation between lymphocyte subsets count and mortality, before and after adjustment for age and severity. The effect of time of symptoms onset to ICU admission on mortality adjusted for lymphocytes subsets counts, gammaglobulines and mHLA DR was also assessed.

Most patients were male (70%) and the median age was 59 (IQR [53–67]) years. Main comorbidities included hypertension (55%), diabetes (30%), obesity (25%) and malignancies (16%). The median time between symptoms onset and ICU admission was 8 [IQR 6–12] days. The reason for ICU admission was an acute respiratory failure in 94% of patients. Overall 55% underwent mechanical ventilation and the 28-day mortality was 30%.

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A lymphopenia ( $< 1500$  cells/mm<sup>3</sup>) was reported in 80% of patients with a median value of 749 [545–1091] cells/mm<sup>3</sup> (laboratory reference values: 1425–2030), affecting T lymphocytes (540 [344–769] cells/mm<sup>3</sup>) (laboratory reference values: 770–2000), CD4 + T cells (332 [184–464] cells/mm<sup>3</sup>) (laboratory reference values: 480–1320), CD8 + T cells (190 [118–269] cells/mm<sup>3</sup>) (laboratory reference values: 192–720), and B lymphocytes (106 [56–191] cells/mm<sup>3</sup>) (laboratory reference values: 67–270).

The association between unadjusted and adjusted mortality and levels of lymphocyte subsets, gamma globulins, and mHLA-DR is shown in Fig. 1. In the overall population, mortality was associated with CD4 + T cells ( $P=0.02$ ) and B cells ( $P=0.01$ ) depletions. After adjustment for age and SOFA score, B lymphopenia  $< 200$ /mm<sup>3</sup> was the only independent predictor of mortality [HR 5.6 (1.3–25);  $P=0.0003$ ].

Strikingly, the relationship between mortality and all investigated variables (except mHLA DR) displayed different patterns when comparing the data from patients admitted to the ICU within the first week after symptoms onset and patients admitted  $\geq 8$  days after symptoms onset.

In patients admitted within the first week, the mortality was not associated with any lymphocytes subset. In contrast, the mortality for patients admitted  $\geq 8$  days was associated with total lymphopenia ( $P=0.03$ ) and T lymphocytes depletion ( $P=0.04$ ) involving CD4 + T cells ( $P=0.03$ ) (with preserved CD8 + T cells). A similar trend was observed between mortality and B lymphocytes depletion ( $P=0.06$ ) in patients admitted  $\geq 8$  days. Similar results were obtained in a sensitivity analysis excluding patients with lymphoma.

The significant contribution of the dysfunctional immune response to the progression of COVID-19 disease has been demonstrated and multiple targeted anti-inflammatory and immunomodulatory therapies are currently evaluated in ongoing randomized control trials [2].

The COVID-19 associated inflammation phase encompasses many overlapping signaling pathways [2] and probably masks different immunological profiles that remain to be defined. In this purpose, a recent study identified 2 distinct patterns promoting hypercytokinemia, namely the hemophagocytic lymphohistiocytosis (driven by IL-1 $\beta$ ) and the immune dysregulation (driven by IL-6) [2].

Our study provides new insights into the immunological heterogeneity in severe COVID-19 patients [3]. These findings suggest that the association between mortality and lymphopenia may involve different lymphocyte subpopulations alterations according to the disease time course.

In patients admitted  $\geq 8$  days, the mortality was correlated with the baseline depletion of T lymphocytes, CD4 + T cells and B lymphocytes. Of note, none of the

patients included in this study received corticosteroids before admission in the intensive care unit and lymphocyte phenotyping. Consistently with the previous studies [3], these immunological features argue for a time-dependent dysfunctional immunity and suggest that trials investigating antivirals or anti-inflammatory therapies should stratify clinical outcomes according to time since viral symptoms onset.

In conclusion, in critically ill COVID-19 patients, the dysfunctional immune response embraces heterogeneous immunological patterns. The comparison of baseline lymphocyte subsets according to the delay from symptoms onset to ICU admission discloses different patterns that should contribute, together with other immunological biomarkers, to the distinction of different immunophenotypes. This immunological heterogeneity should be considered as a predominant feature driving the therapeutic strategy. Last, trials investigating antivirals or anti-inflammatory therapies should stratify clinical outcomes according to time since viral symptoms onset.

The blue lines indicate the adjusted predicted mortality (on age and SOFA score after Cox multivariate analysis) according to the lymphocytes counts, and the shaded areas 95% confidence intervals.

In the overall population (left panel), mortality was associated with CD4 + T cells ( $P=0.02$ ) and B cells ( $P=0.01$ ) depletions. After adjustment for age and SOFA score, B lymphopenia  $< 200$ /mm<sup>3</sup> was the only independent predictor of mortality [HR 5.6 (1.3–25);  $P=0.0003$ ].

The relationship between mortality and all investigated variables (except mHLA DR) displayed different patterns when comparing the data from patients admitted to the ICU within the first week after symptoms onset (central panel) and patients admitted  $\geq 8$  days after symptoms onset (right panel).

In patients admitted within the first week, the mortality was not associated with any lymphocytes subset. In contrast, the mortality for patients admitted  $\geq 8$  days was associated with total lymphopenia ( $P=0.03$ ) and T lymphocytes depletion ( $P=0.04$ ) involving CD4 + T cells ( $P=0.03$ ) (with preserved CD8 + T cells). A similar trend was observed between mortality and B lymphocytes depletion ( $P=0.06$ ) in patients admitted  $\geq 8$  days.

$P = P$ -value for the relation between mortality and lymphocytes subsets counts, gammaglobulines and mHLA DR.

$P_a = P$  value for the adjusted relation between mortality and lymphocytes subsets counts, gammaglobulines and mHLA DR.

$P_t = P$  value for the relation between time from symptoms onset to ICU admission and mortality adjusted for lymphocytes subsets counts, gammaglobulines and mHLA DR.

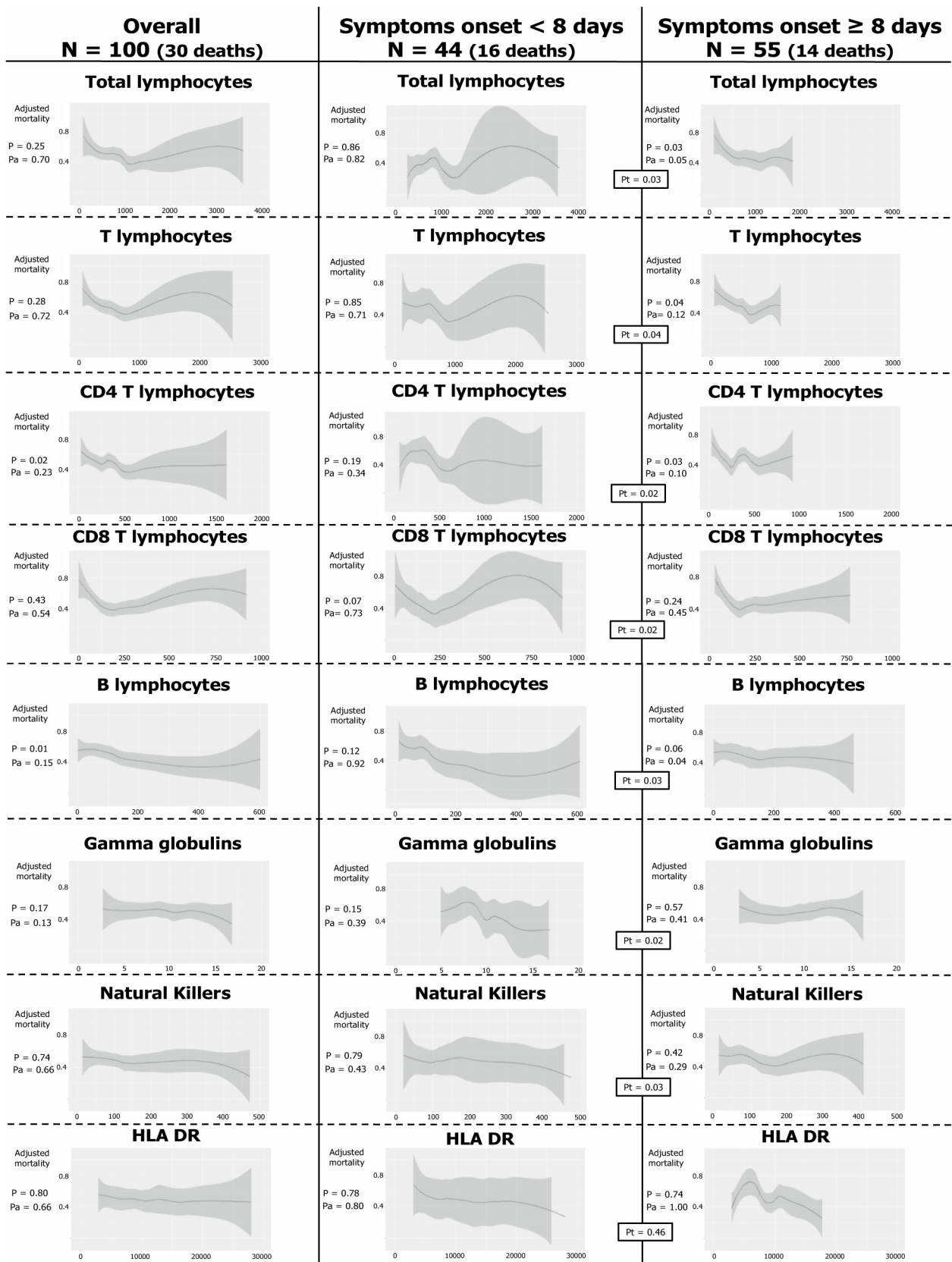


Fig. 1 Association between lymphocyte subsets, gamma globulins, monocyte HLA-DR (mHLA-DR) expression and adjusted mortality in COVID-19 patients according to the time between symptoms onset and ICU admission



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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

**Ethical approval** This study was approved by the Ethics Committee of the French Intensive Care Society (FICS; CE SRLF n°20-32).

**Statement of human and animal rights** This study was conducted in accordance with the ethical standards of the responsible committee on human experimentation.

**Informed consent** All patients included provided informed consent.

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