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The Association of Low CD4 Expression on Monocytes and Low CD8+ T-Cell Count at Hospital Admission Predicts the Need for Mechanical Ventilation in Patients With COVID-19 Pneumonia: A Prospective Monocentric Cohort Study

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OBJECTIVES: To identify COVID-19-associated immunophenotyping patterns at hospital admission and to determine if some patterns could predict the need for mechanical ventilation (MV).

DESIGN: Prospective observational monocentric cohort study.

SETTING: A university-affiliated hospital in Marseille, France.

PATIENTS: Thirty patients presenting with laboratory-confirmed COVID-19 pneumonia were enrolled within the first 48 hours of hospital admission and compared with 18 healthy controls.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Whole-blood leukocytes were immunophenotyped with a rapid and simplified one-step flow cytometry method. Thirty-eight immune and five laboratory parameters were compared first between COVID-19 patients and controls and then between the COVID-19 patients who received or not MV during their stays. The variables that significantly discriminated MV from non-MV patients in univariate analysis were entered into a multiple stepwise logistic regression analysis. The COVID-19 patients were predominantly male (87%), aged 61 years (50–71 yr), and 93% received early corticosteroid therapy. Sixteen patients (53%) were managed with noninvasive respiratory support, and 14 (47%) required MV. Compared with controls, COVID-19 patients were characterized by an immune signature featuring: 1) decreased HLA-DR expression on monocytes; 2) reduced basophils, eosinophils, T-cells, NK cells, and nonclassical monocyte count; and 3) up regulation of CD169 on monocytes, CD64 on neutrophils, the adhesion/migration markers (CD62L and CD11b), and the checkpoint inhibitor CD274 on myeloid cells. Among the COVID-19 patients, those who received MV had lower level of CD4 and HLA-DR on monocytes, lower CD8+ T-cell count, and higher lactate dehydrogenase at hospital admission. In multivariate analysis, only CD4 on monocytes ($p = 0.032$) and CD8+ T-cell count ($p = 0.026$) were associated with MV requirement. The model combining these two variables provided an area under curve of 0.97 (95% CI, 0.83–0.99).

CONCLUSIONS: The association of low CD4 on monocytes and low CD8+ T-cell count at hospital admission was highly predictive of the need for MV in hospitalized patients with COVID-19 pneumonia.

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KEY POINTS

Question: Does the immunophenotyping pattern of circulating leukocytes at hospital admission predict the need for mechanical ventilation in patients with COVID-19 pneumonia?

Findings: The membrane expression of CD4 on monocytes and the T-CD8+ cell count were independently associated with MV requirement in multivariate logistic regression analysis.

Meaning: The combination of CD4 on monocytes and T-CD8+ cell count, measured at hospital admission using a rapid and simplified one-step flow cytometry method, helps predict the severity of the disease.

KEY WORDS: COVID-19; flow cytometry; immunophenotyping; respiratory distress syndrome; respiratory insufficiency

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can manifest through a broad range of clinical symptoms, but the most severe form of the disease is characterized by hypoxia and may progress to acute respiratory distress syndrome, organ failure, and death (1). Predisposing factors of severe forms include older age, sex (male), obesity, and comorbidities (2–4). Among patients admitted to ICUs, the case fatality rate ranges from 11% to 36% depending on the need for mechanical ventilation (MV) during the stay (5). Predicting early the course of the disease would be, therefore, highly relevant for prognostication and appropriate resource allocation.

Following SARS-CoV-2 viral invasion (6), the host triggers a systemic immune response shown to be dysregulated in patients with the most severe form (7–15). Using flow cytometry, several studies have reported peripheral myeloid and lymphoid dysfunctions, and the extent of these alterations correlated with the severity of the disease (16–21).

Flow cytometry is a powerful technique providing extensive cells characterization and phenotyping (22). Recently, a rapid one-step method incorporating whole blood into a standardized no-wash assay has been developed. This provides robust analyses and paves the way for point-of-care application (23, 24).

Although reduced CD4+ and CD8+ T-cells count at hospital admission is associated with worse outcomes (25, 26), other phenotypic markers of activation, exhaustion, and adhesion/migration on granulocytes have been linked to severity later during the course of the disease (27). Therefore, we sought to investigate at standardized time points the course of immune cell profiles in hospitalized patients with COVID-19 using rapid one-step flow cytometry.

The aim of this study was first to identify immunophenotyping patterns that could discriminate COVID-19 patients from controls, and second to determine if some patterns could predict the need for MV. We hypothesized that a combination of immune markers might be best predictive than common other demographic or laboratory parameters.

MATERIALS AND METHODS

This prospective observational cohort study was conducted at the European Hospital of Marseille from January to June 2021 in accordance with the Helsinki Declaration and French law on research involving humans. The study protocol was approved by an independent national review board on October 29, 2020 (Comité de Protection des Personnes, Ile de France XI, IDRCB 2020-A00756-33) and registered at ClinicalTrials.gov (NCT04816760). All enrolled patients provided written informed consent prior to inclusion.

Patient Selection and Management

Adult patients were eligible for enrollment in the study if they fulfilled the following criteria: 1) hospitalized for less than 48 hours, 2) positive SARS-CoV-2 reverse transcriptase-polymerase chain reaction, 3) patterns of COVID-19 pneumonia on chest computed tomography scan (bilateral ground-glass opacities and/or consolidations), and 4) an acute onset of respiratory symptoms (≤1 wk). The exclusion criteria are available in the **Supplemental Digital Content** (http://links.lww.com/CCX/B98).

Patients with COVID-19 pneumonia were managed according to international guidelines (28), including standard anticoagulation in the absence of thrombosis, corticosteroid therapy (dexamethasone, 6 mg

daily for 10 d) if requiring supplemental oxygen, and interleukin-6 (IL-6) receptor blocker (tocilizumab, 8 mg/kg up to a maximum of 800 mg) in case of severe or critical pneumonia. MV patients received low tidal volume protective ventilation, prone position if PaO_2/FIO_2 was less than or equal to 150 mm Hg, and venovenous extracorporeal membrane oxygenation in accordance with guidelines (29, 30). Patients with clinical features of lung fibrosis received rescue corticosteroid therapy (methylprednisolone, 2 mg/kg, daily).

Blood Samples and Data Collection

Blood samples were collected weekly in the morning from inclusion to day 28 (days 0, 7, 14, and 28). The first sample (day 0) was collected on the day of inclusion, which occurred within the first 48 hours following the hospital admission. Flow-cytometry analysis was performed on blood collected in ethylenediaminetetraacetic acid tubes. Five laboratory indices were also evaluated: C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), high-sensitivity troponin I, and D-dimer.

Details on the clinical data recorded are available in the Supplemental Digital Content (http://links.lww.com/CCX/B98). The Simplified Acute Physiology Score II was obtained at hospital admission (31). The Sequential Organ Failure Assessment score (SOFA) was calculated on days of measurements (32). We used the World Health Organization Clinical Progression Scale (WHO-CPS) (33) to characterize the type of respiratory support at each time point of the study and recorded the maximum value during the stay. The population of COVID-19 patients was separated into MV and non-MV cohorts according to the need for MV during the hospital stay.

Flow Cytometry Analysis

Three premixed antibody panels were used (**eTable 1**, http://links.lww.com/CCX/B98), all obtained from Beckman Coulter (Brea, CA), to assess leucocytes (DURAClone IM Phenotyping Basic dry panel), granulocytes (DURAClone IM Granulocytes dry panel), and myeloid activation markers (IOTest Myeloid Activation Test, liquid cocktail). Leucocyte staining and red blood cell lysis were performed following the one-step method (23). Further details are available in the Supplemental Digital Content (http://links.lww.com/CCX/B98), and the gating strategy is illustrated in **eFigures 1–3** (http://links.lww.com/CCX/B98).

Statistical Analysis

Details on the sample size calculation are available in the Supplemental Digital Content (http://links.lww.com/CCX/B98). Categorical data are presented as numbers and percentages (%) and were compared with the chi-square test. Continuous data are presented as medians and interquartile ranges and were compared with the Mann-Whitney *U* test.

To discriminate COVID-19 patients from controls, we performed univariate analysis of flow cytometry parameters at hospital admission using the response screening platform. This procedure classified the parameters according to their significance level based on the false discovery rate (FDR) *p* value; a method that applied the Benjamini-Hochberg procedure adjustment for multiple comparisons. The parameters with FDR *p* values less than or equal to 0.05 were selected for principal component analysis (PCA) and hierarchical clustering. The response screening procedure was repeated within COVID-19 patients to discriminate those requiring or not MV. The parameters with FDR *p* values less than or equal to 0.05 were introduced into a multivariate stepwise logistic regression analysis. We used receiver operating characteristic analysis to determine the area under curve (AUC) and the Youden index method to determine the criterion value.

Flow cytometry parameters were compared at each time point with the control group using the Steel test. Correlations were established with the Pearson correlation coefficient (*r*). All tests were two-tailed, and the significance level was fixed at 5%. Statistical analyses were performed with JMP Version 16 (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics of the Study Population

Thirty patients with COVID-19 pneumonia were included and compared with 18 healthy controls. The characteristics of the patients are presented in **Table 1**, and the flowchart of the study is displayed in **eFigure 4** (http://links.lww.com/CCX/B98). Most of the COVID-19 patients (93%) received early dexamethasone therapy, and eight (27%) also received, at a

later stage, methylprednisolone therapy. Seven patients (23%) were treated with a unique dose of the IL-6 receptor blocker tocilizumab. In five patients (17%), a treatment by azithromycin was initiated prior to the hospital admission but was discontinued within 3 days (1–5 d) after introduction. None of the patients had received antiviral agents or hydroxychloroquine.

Acute Immune COVID-19 Signature

Of the 38 flow cytometry parameters evaluated at hospital admission, 24 significantly discriminated COVID-19 patients from controls (**eFig. 5*A***, http://links.lww.com/CCX/B98). The COVID-19 patients had lower membrane expression of human leukocyte antigen-DR isotype (HLA-DR) on monocytes (mHLA-DR) but also decreased count of basophils, eosinophils, total lymphocytes, CD56+ natural killer cells, natural killer T-cells, total T-cells, CD8+ T-cells, CD4+ T-cells, total monocytes, and nonclassical monocytes. Simultaneously, the expression of several membrane proteins was upregulated: CD169 on monocytes; CD64 on monocytes and neutrophils; CD274 on monocytes, neutrophils, and eosinophils; CD62L on neutrophils, eosinophils, basophils, and monocytes; and CD11b on basophils, monocytes, and neutrophils. Further, PCA showed heterogeneity within COVID-19 patients (**eFig. 6**, http://links.lww.com/CCX/B98), and hierarchical clustering identified two subgroups with distinct proportion of respiratory support: 59% of patients received MV in subcluster A, whereas 86% never received MV in subcluster B (**Fig. 1**).

Flow Cytometry Parameters at Hospital Admission and the Need for Mechanical Ventilation

Fourteen patients received MV during the hospital stay, and two of them were already supported by MV for less than 24 hours at the time of first sampling. The demographic characteristics and laboratory indices of COVID-19 patients who received MV or not (non-MV) are presented in **Table 2**. The discriminating value of 48 parameters (including 38 cytometry markers, five laboratory indices, and five demographic variables) was assessed with the response screening platform (**eFig. 5*B***, http://links.lww.com/CCX/B98). Four parameters significantly discriminated MV from non-MV patients: the membrane expression of CD4 and mHLA-DR on monocytes (mCD4, mHLA-DR), the CD8+ T-cell count, and the LDH (**Fig. 2**; and **eFig. 7**, http://links.lww.com/CCX/B98). The AUC of each parameter was 0.87, 0.87, 0.79, and 0.79, respectively (**eFig. 8**, http://links.lww.com/CCX/B98). In multivariate stepwise logistic regression analysis, only mCD4 (p = 0.032) and CD8+ T-cell count (p = 0.026) remained associated with the need for MV (**Table 3**). The model combining these two variables provided an AUC of 0.967 (95% CI, 0.83–0.99) (**eFig. 9**, http://links.lww.com/CCX/B98). When excluding the two patients who already received MV at inclusion, univariate and multivariate analyses yielded similar findings than those obtained in the whole population with an AUC of the combined model of 0.964.

Kinetics of Flow Cytometry Parameters Over 28 Days

Among the 38 flow cytometry parameters investigated in COVID-19 patients, 26 exhibited significant variation when compared with controls. The kinetics of some parameters also differ between MV and non-MV patients.

The profile of mCD4 expression clearly diverged within the acute phase (0–10 d) of the infection. Compared with controls, mCD4 was significantly increased in patients who did not require MV but conversely was marginally reduced in MV patients (p = 0.062). Over the study period, mCD4 remained upregulated in non-MV patients and downregulated in MV patients, and the level of expression decreased in the most severe cases, including all nonsurvivors (**Fig. 3**, ***A*** and ***B***). The membrane expression of mHLA-DR followed a different pattern from mCD4. During the acute phase, mHLA-DR was reduced in all COVID-19 patients, but the greatest reduction was observed in MV patients. Over the study period, mHLA-DR recovered in non-MV patients, whereas it remained downregulated in the most severe cases including all nonsurvivors (**eFig. 10**, http://links.lww.com/CCX/B98). The kinetics of CD8+ T-cell count followed a similar pattern than mHLA-DR with a greater reduction during the acute phase and a delayed recovery in MV patients. The time courses of the other flow cytometry parameters are displayed in **eFigure 11, *A*** and ***B*** (http://links.lww.com/CCX/B98).

TABLE 1.
Demographic and Clinical Characteristics of the Study Population

Variables, Units	COVID-19	Control
n	30	18
Age, yr	61 (50–71)	44 (34–53)
Sex, male, *n* (%)	26 (87)	14 (70)
Body mass index, kg/m²	30 (26–32)	25 (23–27)
Comorbidities, *n* (%)		
Diabetes	12 (40)	0
Hypertension	15 (50)	0
Chronic pulmonary diseases	3 (10)	0
Chronic heart diseases	5 (17)	0
Chronic liver diseases	0	0
Chronic renal failure	0	0
Active cancer	0	0
Immunosuppression	0	0
Time from first symptom to hospital admission, d	8 (6–10)	-
Time from hospital admission to inclusion, d	1 (1–2)	-
Simplified Acute Physiologic Score II, at hospital admission	24 (18–35)	-
Sequential Organ Failure Assessment score, at inclusion	4 (2–4)	
Immune therapies during the stay		
Early corticosteroid therapy (dexamethasone), *n* (%)	28 (93)	-
Time from hospital admission to treatment, d	0 (0–1)	-
Rescue corticosteroid therapy (methylprednisolone), *n* (%)	8 (27)	-
Time from hospital admission to treatment, d	13 (11–15)	-
Interleukin-6 receptor blocker (tocilizumab), *n* (%)	7 (23)	-
Time from hospital admission to treatment, d	1 (1–2)	-
Main outcomes		
ICU admission during the stay, *n* (%)	21 (70)	-
Length of hospital stay, d	14 (8–29)	-
Hospital mortality, *n* (%)	4 (13)	-
Maximal respiratory and organ support during the stay		
Oxygen (WHO-CPS grade 5), *n* (%)	9 (30)	-
High-flow oxygen therapy or noninvasive ventilation (WHO-CPS grade 6), *n* (%)	7 (23)	-
MV (WHO-CPS grade 7–9), *n* (%)	14 (47)	-
Length of MV, d	15 (8–28)	-
Ratio of Pa_{O_2} to Fi_{O_2} on the day of MV initiation, mm Hg/%	99 (88–133)	-
Positive end-expiratory airway pressure on the day of MV initiation, cm H_2O	14 (12–15)	-
Prone position during MV, *n* (%)	12 (86)	-
Venovenous extracorporeal membrane oxygenation, *n* (%)	3 (10)	-
Norepinephrine, *n* (%)	10 (33)	-
Renal replacement therapy, *n* (%)	3 (10)	-

MV = mechanical ventilation, WHO-CPS = World Health Organization Clinical Progression Scale.
Data are expressed as median (interquartile range, 25–75%) unless otherwise specified.

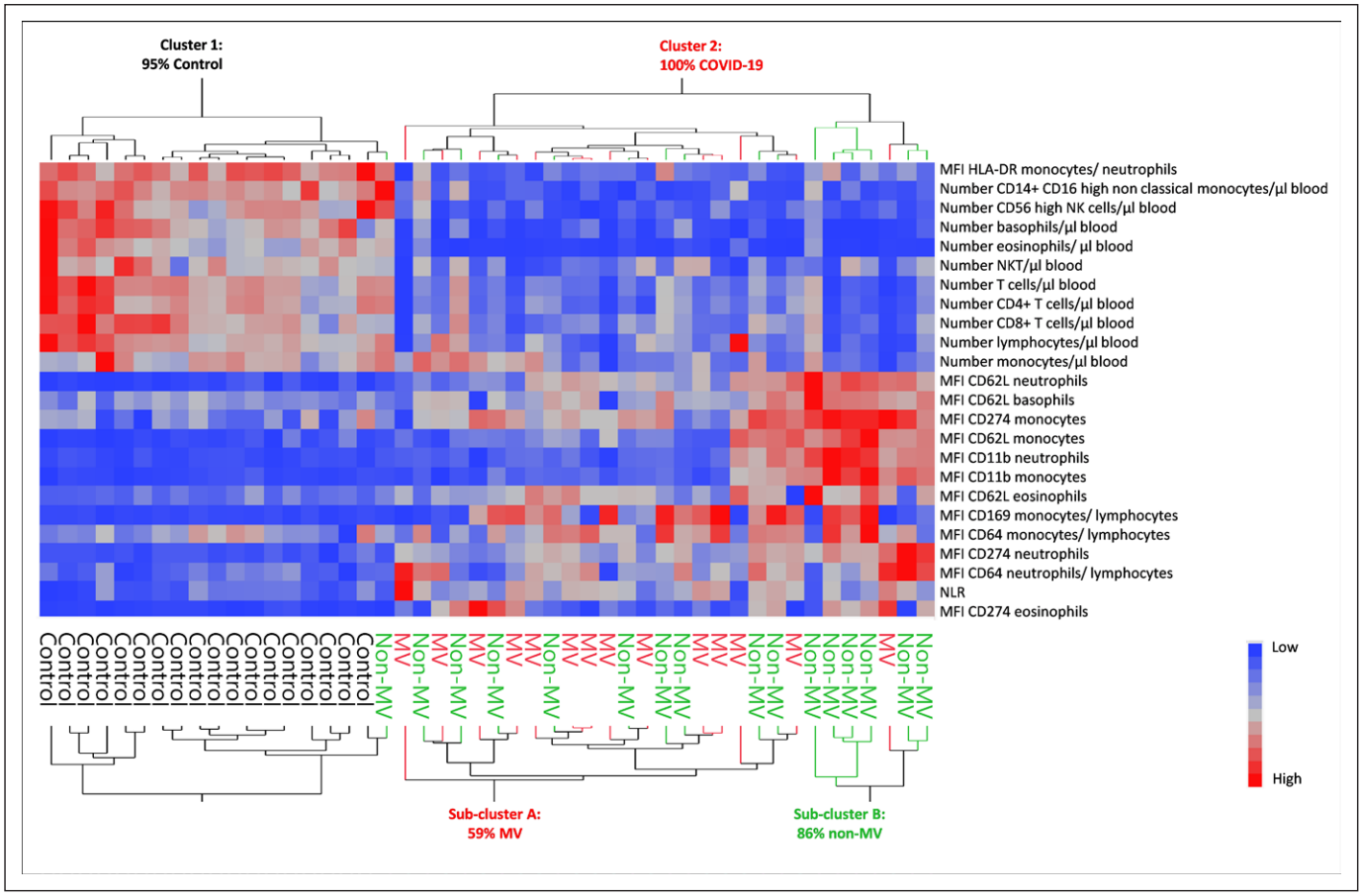


Figure 1. Hierarchical clustering at hospital admission by the discriminant flow cytometry parameters. Only the parameters with significant false discovery rate *p* value (≤ 0.05) are represented. The population was first separated into two clusters differentiating the COVID-19 patients from the controls. Among the COVID-19 patients, the population was further separated into two subclusters: 59% of patients received mechanical ventilation during the stay in subcluster A, whereas 86% of patients never received mechanical ventilation in subcluster B. HLA-DR = human leukocyte antigen-DR isotype, MFI = median of the fluorescence intensity, NK = natural killer, NKT = natural killer T.

Flow Cytometry Parameters and the Severity of Organ Failure

The correlation of the parameters that discriminated MV from non-MV patients (mCD4, mHLA-DR, CD8+ T-cell count, and LDH) was determined first with the type of respiratory support as assessed by the WHO-CPS (i.e., a surrogate of the severity of the respiratory failure), and then with the severity of organ failure as assessed by the SOFA score. These analyses were performed on the whole dataset of measurements over the study period. Among these four biomarkers, mHLA-DR and mCD4 exhibited the most robust correlations. The mHLA-DR had higher correlation with the WHO-CPS (r^2=0.53) and the SOFA (r^2=0.34) than the mCD4 (r^2=0.39 and r^2=0.29, respectively) (**eFig. 12**, http://links.lww.com/CCX/B98).

DISCUSSION

In this prospective cohort of hospitalized patients with COVID-19 pneumonia, using a one-step standardized flow cytometry method and three commercially available immune panels, we identified an immune COVID-19 signature that discriminates COVID-19 patients from healthy controls. Most importantly, we also discovered a combination of two immune markers (low CD4 on monocytes and low CD8+ T-cell count) predicting at hospital admission the subsequent need for MV.

Most clinical studies have focused on identifying risk factors of death among hospitalized COVID-19 patients, but few have specifically addressed the risk factors of MV. These include older age, comorbidities, higher severity score, increased LDH and D-dimer levels, and reduced CD4+ and CD8+ T-cell count (4,

TABLE 2.
Characteristics of the COVID-19 Patients According to the Need for Mechanical Ventilation During the Hospital Stay

Variables, Units		Non-MV	*p*
n	14	16	
Age, yr	69.5 (56–72)	53 (42–68)	0.032
Sex, male, *n* (%)	12 (86)	14 (88)	0.888
Body mass index, kg/m²	30 (29–33)	28 (25–30)	0.058
Comorbidities, *n (%)*			
Diabetes	7 (50)	5 (31)	0.304
Hypertension	9 (64)	6 (38)	0.15
Chronic pulmonary diseases	1 (7)	2 (13)	0.631
Chronic heart diseases	4 (29)	1 (6)	0.107
Time from first symptom to hospital admission, d	8 (6–10)	8 (6–10)	0.752
Time from first symptom to inclusion, d	9 (7–11)	9 (8–11)	0.883
Sequential Organ Failure Assessment score, at inclusion	4 (2–6)	2 (2–3)	0.022
Immune therapies during the stay, *n* (%)			
Early corticosteroid therapy (dexamethasone)	14 (100)	14 (88)	0.178
Corticosteroid therapy prior first sample	12 (86)	11 (69)	0.281
IL-6 receptor blocker therapy	5 (36)	2 (13)	0.14
IL-6 receptor blocker prior first sample	1 (7)	1 (6)	0.923
Laboratory tests at inclusion (normal values)			
Leukocytes, $\times 10^9$/L (4–11)	9.7 (7.7–12.5)	7.9 (5.4–10.1)	0.042
Neutrophils, $\times 10^9$/L (1.8–6.6)	8.8 (6.8–11.4)	6.5 (4.3–8.5)	0.02
Lymphocytes, $\times 10^9$/L (1.2–3.9)	0.54 (0.42–0.78)	0.86 (0.62–1.08)	0.05
Neutrophils/lymphocytes ratio	14.5 (9.5–21.5)	7.1 (4.8–9.5)	0.002
Monocytes, $\times 10^9$/L (0.2–0.8)	0.39 (0.22–0.63)	0.36 (0.29–0.6)	0.546
Platelets, $\times 10^9$/L (160–390)	230 (193–276)	250 (190–287)	0.647
D-dimer, µg/mL (< 0.5[a])	1.31 (0.96–2.15)	1.1 (0.59–1.76)	0.198
Fibrinogen, g/L (2–4)	7.4 (6.8–7.8)	7 (6.6–7.6)	0.406
C-reactive protein, mg/L (< 5)	192 (172–286)	148 (109–203)	0.146
Ferritin, µg/L (20–250)	1,253 (811–1,794)	969 (651–1,389)	0.17
SGOT, U/L (<50)	56 (44–67)	46 (32–93)	0.574
Lactate dehydrogenase, U/L (< 248)	591 (513–643)	420 (330–475)	0.006
Creatinine, µmol/L (59–104)	80 (67–102)	60 (52–76)	0.008
High-sensitivity troponin, ng/L (< 19.8)	13.8 (6.8–25.7)	16.5 (8.9–25.2)	0.862

IL = interleukin, MV = mechanical ventilation, SGOT = serum glutamic oxaloacetic transaminase.
[a]Normal values of D-dimer depend on age above 50 yr and should be adjusted according to the following formula: 0.5 + (age × 0.01).
Data are expressed as median (interquartile range, 25–75%) unless otherwise specified.

25, 34). In this study, we used the response screening platform to directly compare the association of laboratory indices, demographic characteristics, and flow cytometry-related parameters with the need for MV. After adjustment for multiple comparisons, we identified four discriminants between MV and non-MV patients: three were obtained by flow cytometry analysis, and one was a laboratory biomarker of end-organ damage (LDH) known to be associated with the severity of the disease (35). The multivariate logistic

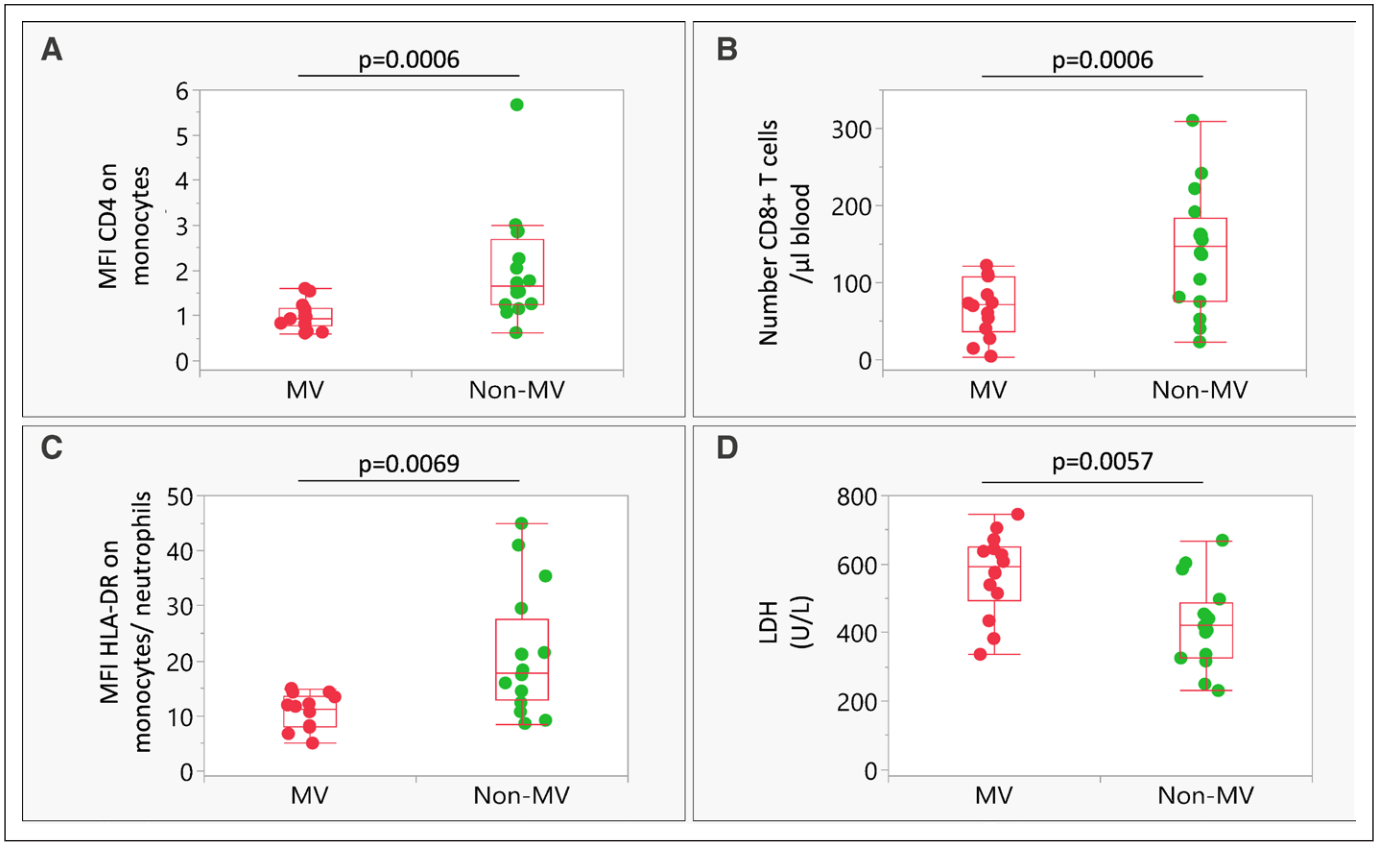


Figure 2. Box plot of the parameters at hospital admission, which discriminated the COVID-19 patients requiring or not mechanical ventilation (MV) during the hospital stay. Only the parameters with significant false discovery rate *p* value (≤0.05) are represented. The two groups of patients (MV and non-MV) were compared with the Mann-Whitney *U* test. **A**, The membrane expression of CD4 on monocytes (median of the fluorescence intensity [MFI]). **B**, CD8+ T-cell count (per µL of blood). **C**, The ratio of the expression of human leukocyte antigen-DR isotype (HLA-DR) on monocytes to neutrophils (signal to noise). **D**, Lactate dehydrogenase (LDH) plasma level (UI/L).

TABLE 3.
Final Multivariate Logistic Regression Model of Parameters Associated With the Need for Mechanical Ventilation During the Hospital Stay Among COVID-19 Patients

Parameter	Estimate	Standard Estimate	Wald X^2	Pr > X^2	OR (95% CI)
Intercept	10.174	4.005	6.452	0.011	-
CD4 on monocytes	−4.746	2.214	4.593	0.032	0.009 (0.0001–0.667)
T-CD8+ cell count	−0.044	0.0198	4.943	0.026	0.957 (0.921–0.995)

OR = odds ratio.

regression analysis identified collinearities between variables and finally outputted a two-factor model that provided high predictive value.

In adaptive immunity, CD8+ and CD4+ T-cells play an essential role in controlling viral infection trough a cytotoxic activity on virus-infected cells and the release of effector cytokines. During the acute phase of SARS-CoV-2 infection, these cells undergo quantitative and qualitative (activation/exhaustion phenotypes) changes, particularly in the severe form of the disease (36–39). The reduced peripheral T-cell count seems related to Fas-dependent apoptosis in the spleen and hilar lymph nodes but not to viral invasion of lymphocytes in autopsied COVID-19 patients (40). In this study, we confirmed the peripheral decrease of both CD4+ and CD8+ T-cells, which was much more pronounced in MV patients. Furthermore, we identified the CD8+ T-cell count as an independent risk factor of MV. This finding strengthened the relevance of this cell subset, which has been already identified as an

independent risk factor of death in a prospective cohort of hospitalized COVID-19 patients (37).

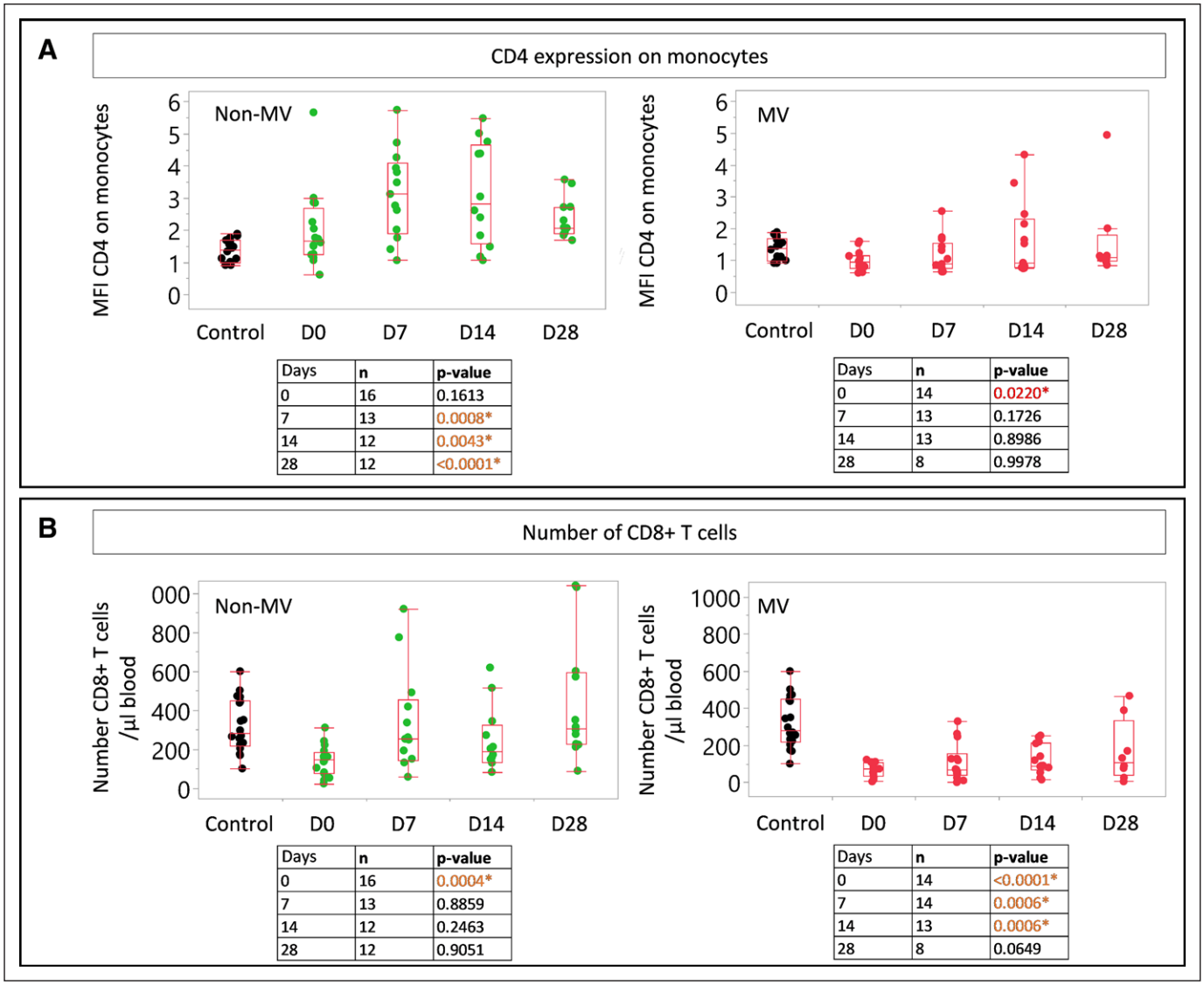
Interestingly, we and others had previously noticed low CD4 staining on the circulating monocytes of severe COVID-19 patients (27, 41). CD4 is expressed at the membrane surface of all human peripheral monocytes, yet its function on these cells is not fully understood and poorly studied (42). The CD4 molecule is a membrane-bound glycoprotein, member of the immunoglobulin receptor family known to be expressed on T-cells and to interact with major histocompatibility complex class II (MHC-II), interleukin-16, and HIV gp120 (43). Recently, it has been proposed that the binding of mCD4 with the MHC-II of activated endothelial cells triggers monocyte activation and differentiation into macrophages, resulting in cytokine secretion and phagocytosis (44). In this study, we reported two patterns of mCD4 expression at hospital admission: upregulation in non-MV patients and downregulation in MV patients. This finding suggests that mCD4 contributes to the successful response of the patient against SARS-CoV-2 infection, and failure to upregulate mCD4 may worsen the outcome. The regulation of CD4 expression on the surface of monocytes could be related to transcriptional or posttranscriptional mechanisms. The mechanisms by which CD4 expression is altered by SARS-CoV-2 infection need to be elucidated in future investigations.

The membrane expression of mHLA-DR mediates key functions of innate immunity including antigen presentation. In clinical settings, the decrease in mHLA-DR indicates cell exhaustion, and this biomarker is used to quantify the severity of immunosuppression. In critically ill patients, mHLA-DR is commonly decreased on the day following the ICU admission, irrespective of the type of the initial injury (e.g., sepsis, trauma, or surgery) (45). However, the persistence in some patients of a low level of mHLA-DR at the end of the first week is associated with increased risk of secondary infections (45). In this study, mHLA-DR was downregulated at hospital admission in most of the COVID-19 patients, but the reduction was greater in the most severe cases (i.e., those who will require MV). Although most of non-MV patients normalized the mHLA-DR level within 21–30 days of symptom onset, MV patients in contrast had persistent downregulation over the study period. Thus, the prolonged acquired immunosuppression experienced by MV patients may contribute both to hamper the SARS-CoV-2 viral clearance (46) and to increase the rate of nosocomial infections as previously reported in this population (47, 48).

The present study also brings new insights into the sequential implications of 38 immune markers, which were monitored up to 40 days after symptoms onset. Some parameters were either upregulated or downregulated within the acute phase (0–10 d) before returning to baseline levels. Here, we confirm the intense but transient upregulation of CD169 on monocytes in most of the COVID-19 patients (49). Conversely, some parameters remained unaltered during the acute phase but presented variations within the second part of the course of the disease. However, the pattern of expression of these "second phase" markers could have been influenced by the occurrence of secondary infections or medications and should be interpreted with caution.

This study has some limitations. First, the healthy controls had no major comorbidities, were free from steroids, and were younger than the COVID-19 patients. Therefore, the immunophenotypic differences observed between these two populations might not be exclusively related to the COVID-19. In addition, we cannot exclude that some of the immunophenotypic differences between groups could be related to race, ethnicity, or socioeconomic background imbalances as we did not evaluate these parameters. Further, our population of COVID-19 patients was mainly constituted by male (87%), a proportion quite high but close to the upper range of cohort studies on hospitalized COVID-19 patients (5, 50–52).

Second, most of the COVID-19 patients (93%) received corticosteroids during their stay, and this therapy was initiated before inclusion in 77% of them. Corticosteroids are known to decrease the level of inflammatory markers (53) and may have altered the immune response against SARS-CoV-2 infection. Nonetheless, the proportion of COVID-19 patients who received a first dose of corticosteroid therapy prior inclusion was similar between the MV and non-MV groups. Therefore, it is plausible that the absolute values of cytometry and laboratory markers have been shifted downward by the corticosteroids therapy, but it is unlikely that it has contributed to the differences observed between the MV and non-MV patients. Similarly, IL-6 receptor blocker (tocilizumab) may interfere with immune marker expression (54); however,



Days	n	p-value
0	16	0.1613
7	13	0.0008*
14	12	0.0043*
28	12	<0.0001*

Days	n	p-value
0	14	0.0220*
7	13	0.1726
14	13	0.8986
28	8	0.9978

Days	n	p-value
0	16	0.0004*
7	13	0.8859
14	12	0.2463
28	12	0.9051

Days	n	p-value
0	14	<0.0001*
7	14	0.0006*
14	13	0.0006*
28	8	0.0649

Figure 3. Kinetics of the membrane expression of CD4 on monocytes and the CD8+ T-cell count among the COVID-19 patients requiring or not mechanical ventilation (MV) during the hospital stay. Data from COVID-19 patients were compared at each time point with those from healthy controls using the Steel test. **A**, CD4 expression on monocytes (median of the fluorescence intensity [MFI]). **B**, CD8+ T-cell count.

the proportion of patients who received tocilizumab was similar between the COVID-19 subgroups.

Third, two COVID-19 patients among the fourteen who received MV during their stay were already supported by MV at the time of first sampling; however, we found similar results when repeating the analyses without these two patients.

Finally, this cohort included a limited number of patients and served only as a determination cohort. In addition, the predictive performance of the final multivariate model might be overestimated due to the restrictive approach of variables selection. Therefore, these results need to be validated in a large multicentric cohort study prior to be generalized.

CONCLUSIONS

In this prospective cohort study of patients hospitalized with COVID-19 pneumonia, using a one-step standardized flow cytometry method, we reported an immune signature, which discriminates COVID-19 patients from controls. Most importantly, we identified the low membrane expression of CD4 on monocytes and the low CD8+ T-cell count upon hospital admission as parameters independently associated with the need for MV. The association of these two parameters provides a high predictive value. The immune phenotypic signature described in this study is valid in COVID-19-treated patients as most of them

did receive steroids or even IL-6 receptor blocker tocilizumab.

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