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Monocyte CD169 Expression as a Biomarker in the Early Diagnosis of Coronavirus Disease 2019

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We assessed the expression of CD169, a type I interferon-inducible receptor, on monocytes (monocyte CD169 [mCD169]) in 53 adult patients admitted to the hospital during the coronavirus disease 2019 (COVID-19) outbreak for a suspicion of severe acute respiratory syndrome coronavirus 2 infection. Monocyte CD169 was strongly overexpressed in 30 of 32 (93.7%) confirmed COVID-19 cases, compared with 3 of 21 (14.3%) patients in whom the diagnosis of COVID-19 was finally ruled out. Monocyte CD169 was associated with the plasma interferon-alpha level and thrombocytopenia. Monocyte CD169 testing may be helpful for the rapid triage of suspected COVID-19 patients during an outbreak.

Keywords. CD169; COVID-19; monocytes; SARS-CoV-2; viral infections.

Early identification of coronavirus disease 2019 (COVID-19) and prompt diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are critical for care and prognosis during outbreaks. It is also mandatory to avoid unnecessary and time-consuming interventions for ruling out COVID-19 in patients with serious respiratory conditions requiring appropriate care.

Type I interferons (IFNs) are important factors for homeostasis of the immune response and play a key role in antiviral immunity. A robust type-I IFN response is critical in the early

phase of SARS-CoV-2 infection to limit SARS-CoV-2 replication and avoid severe complications [1]. CD169, also known as sialoadhesin or Siglec-1, is constitutively expressed at low levels on monocytes, but its expression rises dramatically when monocytes become stimulated by IFN α and all other type I IFNs. Monocyte CD169 (mCD169) overexpression is associated with acute viral infections [2, 3]. Furthermore, a subset of CD169 lung-resident macrophages that have immune-regulatory functions and proliferate after influenza infection has recently been described [4].

In March 2020, Montpellier University Hospital reorganized its emergency service delivery in response to the COVID-19 outbreak to facilitate triage, diagnosis, and hospitalization of the patients presenting with suspected COVID-19. In this prospective observational study conducted during the COVID-19 outbreak, we evaluated mCD169 expression for the early identification of viral acute infection during SARS-CoV-2 epidemic in patients at hospital admission.

METHODS

This study was conducted by the University Hospital of Montpellier, during the peak of the COVID-19 epidemic between March 15 and April 5, 2020. It was approved by the accredited Institutional Review Board (IRB) No. 198711 (IRB-MPT_2020_03_202000426). Each day, the first 15 consecutive adult patients presenting with a suspicion of COVID-19 and hospitalized in the medical units dedicated to the diagnosis and early care of COVID-19 were included in the study after written consent. Of the 53 eligible patients, 32 were confirmed to be SARS-CoV-2 positive using a nasopharyngeal swab, whereas 21 tested negative. Among the confirmed cases, 18 patients had a second cytometry analysis before being discharged from the hospital. The median age of the patients was 64 years (interquartile range [IQR], 51–78.5 years) and 54.7% were male.

Ethical Approval Statement

All patients were included in the COVIDotheque cohort (ClinicalTrials.gov Identifier NCT04347850) and provided informed consent for the use of their data and clinical samples for the study. Institutional review board clearance for the scientific use of patient data has been granted by the Institution of Montpellier University Hospital and Ile de France III Ethical Committee (no. 2020-A00935-34).

Flow Cytometry

Ten microliters of ethylenediaminetetraacetic acid (EDTA) sample was simultaneously lysed with 500 μ L Versalyse lysing solution and stained with CD64-CD169/infections dried custom

mixture, composed of anti-CD169-phycoerythrin (clone 7-239) and anti-CD64-Pacific Blue (PB) (clone 22) ([Supplemental Figure 1](#)). Results were expressed as CD169 monocyte/lymphocyte fluorescence ratio and CD64 neutrophils/lymphocytes fluorescence.

A second panel was used containing CD45-FITC, CD8-ECD, CD3 PC5, CD4-RD1 (CYTO-STAT tetraCHROME), and CD38-PB. Samples were incubated at room temperature for 30 minutes in the dark. All products or custom products came from Beckman Coulter Inc. (Brea, CA). We acquired data on a 3-laser, 10-color Navios flow cytometer and analyzed data using Kaluza Software version 2.1 (both from Beckman Coulter Inc.).

Positive Controls and Thresholds

We assessed the expression of mCD169, nCD64, and CD38^{bright} on CD8 T cells in 30 healthy controls to establish a threshold based on the median + 3 standard deviations. The thresholds were as follows: 3.51 for the mCD169/mean fluorescence intensity (MFI) ratio, 4.59 for the neutrophil CD64 (nCD64)/MFI ratio, and 9.06% for CD38^{bright}. The nCD64 threshold was controlled on 10 clinical samples collected from patients with microbiologically confirmed bacterial infections (median = 8.44; IQR, 5.77–11.37) (data not shown).

Severe Acute Respiratory Syndrome Coronavirus 2 Ribonucleic Acid Testing

Severe acute respiratory syndrome coronavirus 2 ribonucleic acid (RNA) extraction from nasopharyngeal swabs (Sigma Virocult; Medical Wire Instrument, Corsham, UK) was done using the QIAamp Viral RNA Mini Kit on the QIA Symphony platform, following the manufacturer's instructions. Severe acute respiratory syndrome coronavirus 2 RNA was assessed using reverse-transcription polymerase chain reaction (RT-PCR) targeting RNA-dependent RNA polymerase as previously described.

Interferon- α Plasma Concentration

Plasma samples were stored at -80°C until processing. Interferon- α was quantified using a multiplex microsphere assay (Invitrogen Human Inflammation 20-plex ProcartaPlex Panel, Marne-La-Vallée, France) on a Luminex apparatus (MAGPIX; Thermo Fisher Scientific, Waltham, MA) following the manufacturer's instructions.

Serology

Plasma samples were tested for immunoglobulin (Ig)G antibodies directed against SARS-CoV-2 nucleocapsid using a CE-marked enzyme-linked immunosorbent assay (ID Screen SARS-CoV-2-N; ID.Vet, Montpellier, France) as previously described [5]. Each result is displayed as a ratio: S/P (sample/positive control) expressed in percentage (%): $S/P\% = (\text{optical density [OD] of sample} - \text{OD negative control}) / \text{OD positive}$

control—OD negative control) $\times 100$. The S/P $>40\%$ is positive and $<40\%$ is negative.

Statistical Analysis

Data were analyzed and illustrated using Excel 2016 (Microsoft Corp., Redmond, WA) and Prism 7 (GraphPad Software Inc., La Jolla, CA) software. To determine statistical significance between the 2 groups (ie, CD169 COVID⁺ vs COVID⁻), unpaired Student's 2-tailed *t* test or non-parametric Mann-Whitney test was applied, according to the distribution of the data set. Correlations were analyzed using Pearson or Spearman's rank test according to the normality of the data set. A $P < .05$ was considered statistically significant.

RESULTS

There was a limit of 15 patients enrolled per day due to the limited capacity to include patients in the study. Among 162 patients admitted in the COVID-19 emergency unit from March 15 to April 5, 2020, 53 (32.7%) were included and tested for mCD169 expression. Thirty-two of these patients tested positive for SARS-CoV-2 RNA, and 21 tested negative. Thirty patients with a confirmed diagnosis of COVID-19 had an mCD169 expression level above the positivity threshold (93.7%) ([Figure 1A](#)). In contrast, only 3 of the 21 patients (14.3%) with a negative SARS-CoV-2 PCR result had an mCD169 overexpression. The level of mCD169 was inversely correlated with the cycle time detection (CT) value of the SARS-CoV-2 PCR, suggesting that the immunological marker was slightly associated with the level of virus replication ($R^2 = 0.24$) ([Figure 1B](#)). At hospital admission, mCD169 was not associated with the duration, since the onset of symptoms in COVID-19-confirmed patients ([Supplemental Figure 2A](#)). Likewise, the level of mCD169 expression had no prognostic value because no differences were noted in patients who had to be admitted in intensive care units, patients with mild forms of COVID-19, or patients who died ([Figure 1B](#)). Interferon- α concentration was significantly higher in COVID-19-confirmed patients compared with COVID-19 negative patients ($P < .0001$) ([Figure 1D](#)). Overall, the mCD169 level was correlated with the concentration of IFN α in plasma ($r = 0.48$) ([Supplemental Figure 3F](#)). The mCD169 expression level decreased during hospitalization in 15 of 18 confirmed COVID-19 patients, alongside IFN α concentration ([Supplemental Figure 4](#)). Of note, the platelet count increased between the first and second time points in all of the confirmed COVID-19 patients.

The mCD169 level was also negatively correlated with the platelet count ($r = -0.45$) ([Supplemental Figure 3C](#)), but no correlation was found with the C-reactive protein (CRP) concentration, lymphocyte count, or neutrophil count ([Supplemental Figure 3A, B, and E](#)). To investigate whether mCD169 could complement serological testing, we retrospectively assessed anti-SARS-CoV-2 nucleocapsid IgG. Among the confirmed COVID-19 cases, 7 patients also tested positive for

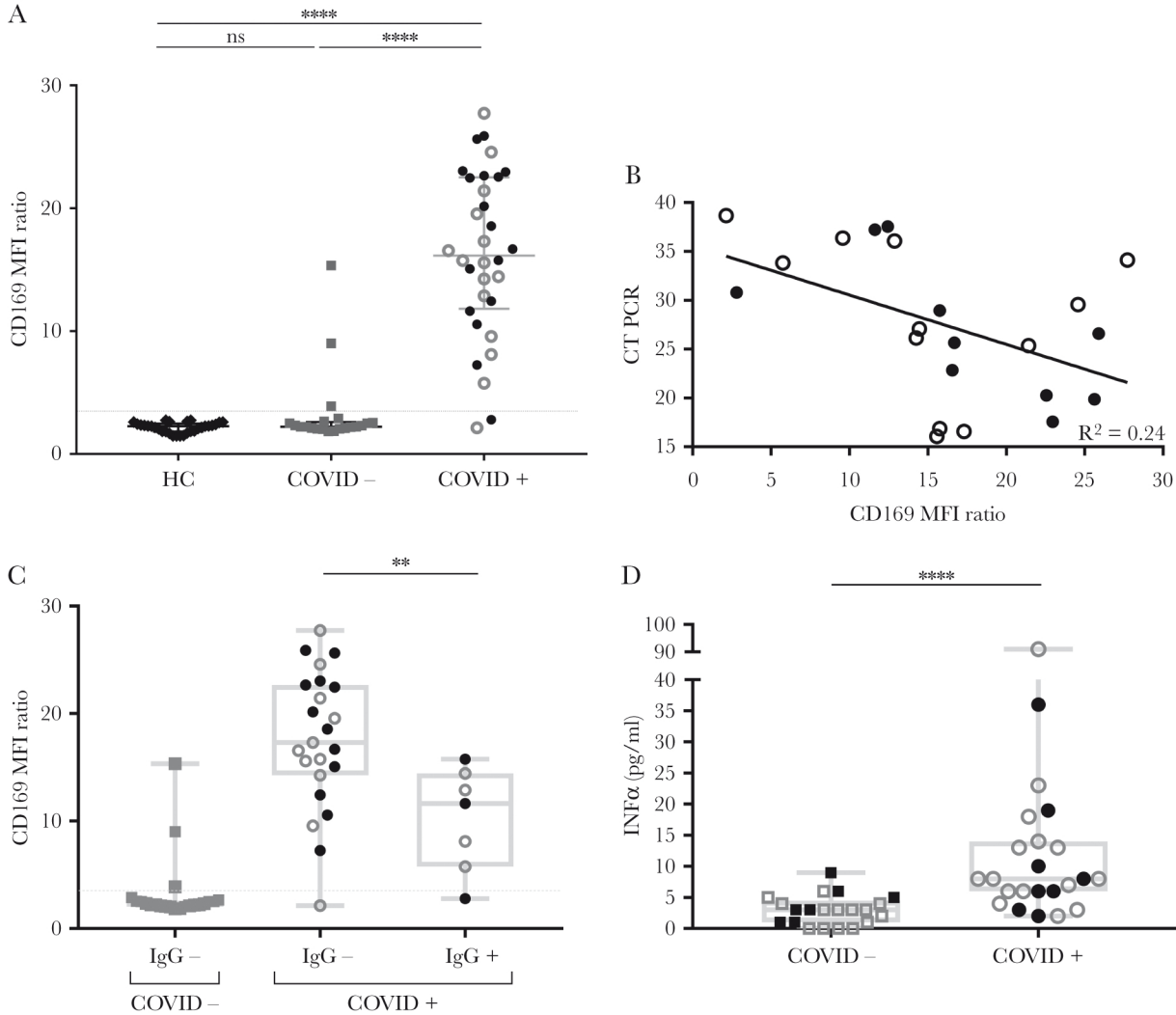


Figure 1. CD169 expression on monocytes in patients admitted in coronavirus disease 2019 (COVID-19) hospital units with a diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed or ruled out. (A) CD169 median of fluorescence intensity (MFI) ratio (CD169 on monocyte/CD169 on lymphocytes). Healthy controls are indicated by black diamonds; patients who tested negative for SARS-CoV-2 infection (COVID⁻) are indicated by open squares or black squares in patients with bacterial microbiologically confirmed infections; SARS-CoV-2 confirmed infection (COVID⁺) are open circles for mild and black circles for severe COVID-19. The threshold of CD169 MFI ratio is indicated by the dotted line: 3.51. ****, $P < .0001$. (B) Correlation between CD169 MFI ratio and cycle time detection (CT) value of the SARS-CoV-2 reverse-transcriptase real-time polymerase chain reaction in COVID-19-confirmed patients. (C) Interferon- α (INF α) plasma concentration at hospital admission in patients with a diagnosis of SARS-CoV-2 infection confirmed or ruled out. **, $P < 0.01$. (D) Expression of CD169 on monocyte according to serological status for anti-SARS-CoV-2 immunoglobulin G at hospital admission.

SARS-CoV-2 IgG at the time of hospital admission. These patients had a lower mCD169 level than patients who tested negative for anti-SARS-CoV-2 IgG ($P = .0084$), suggesting that high mCD169 may be associated with active SARS-CoV-2 infection, before seroconversion (Figure 1C).

Neutrophil CD64 (nCD64) was also assessed as a marker for systemic bacterial infection [3]. High nCD64 expression was observed in 7 of 21 (33.3%) SARS-CoV-2 PCR-negative patients (Supplemental Figure 2B). Bacterial infection was microbiologically confirmed for all but 1 of these patients (6 of 7). Four of 32 (12.5%) confirmed COVID-19 cases also had nCD64 expression above the threshold; 2 of them had confirmation of a bacterial infection. A higher median expression of CD38^{bright} on CD8⁺ T cells was also observed in the COVID-19-confirmed

group compared with the SARS-CoV-2 uninfected group (Supplemental Figure 2C). However, this marker has a limited capacity to identify COVID-19 cases since CD38^{bright} on CD8⁺ T cells was at a normal level in 11 COVID-19-confirmed cases (34.3%). Nevertheless, it shows a strong correlation with CD169 ($r = 0.62$) (Supplemental Figure 3D).

The test accuracy of mCD169 to predict COVID-19 infection during epidemic was studied by means of a receiver operating characteristic curve and compared with the CRP level (Figure 2). The sensitivity and specificity at the optimal operating point were 97% and 80%, respectively, with an area under the receiver operating characteristic curve (AUC) of 0.95. The CRP had a sensitivity and specificity of 94% and 33%, respectively, with an AUC of 0.58.

DISCUSSION

The rapid identification of COVID-19 is a major challenge for emergency units, especially when a hospital has to cope with 200 to 300 suspected cases but a lower number of true SARS-CoV-2 infections. Montpellier University Hospital has been strongly impacted by the COVID-19 crisis, although the region around Montpellier reported a relatively low proportion of COVID-19 cases during the first peak of the epidemic, which occurred in France between March and April 2020. Only a portion of patients suspected of COVID-19 had confirmation of the infection. For the remainder, the diagnosis of COVID-19 needed to be rapidly ruled out after hospital admission. Monocyte CD169 may be a sensitive marker for the rapid triage of patients suspected of having COVID-19. Our observation on mCD169 overexpression in SARS-CoV-2-infected patients has been confirmed in other studies [6–8]. Type I IFNs induce the transcription of CD169 messenger RNA. Amplification of CD169 monocytes has been observed in other viral infections such as Zika, untreated human immunodeficiency virus, and influenza [9–11]. Hence, mCD169 may be a broad marker of viral infections. In this study, inclusions were carried out in March–April, whereas the influenza epidemic ended in week 11 in France [12]. The value of this marker in a context of a COVID and influenza virus double epidemic need to be explored and compared.

Overexpression appeared very early during COVID-19, but the dynamic of mCD169 remains to be explored. Patients tested

negative for anti-SARS-CoV-2 had lower mCD169 compared with anti-SARS-CoV-2-positive patient. Likewise, a trend for a lower level of mCD169 was observed in patients with high CT value and low plasma IFN α concentration. These results suggest that the expression of the marker decreased over the course of the disease.

Severe acute respiratory syndrome coronavirus 2 infection elicits type I IFN production, considered as key contributor to early innate response against COVID-19 [1]. Later in the disease course, impaired type I IFN production could be a characteristic of severe disease. As IFN-stimulated genes, transcription of CD169 may be lower in severe COVID compared with middle forms of the disease. A trend for a lower mCD169 expression has been reported in COVID-19-confirmed patients hospitalized in the intensive care unit. In our study, mCD169 at hospital admission was consistent across severity groups. The same observation was done using blood transcriptomes [14] and monocyte analysis [15] in patients with moderate versus severe COVID-19 outcomes.

CONCLUSIONS

In conclusion, mCD169 is strongly overexpressed on monocytes during the early phase of the SARS-CoV-2 infection, and it remains elevated in patients admitted to the hospital during the second week after the onset of symptoms. Alongside RT-PCR and serological testing, mCD169 may contribute to preserving the medical capacities of emergency departments by favoring the rapid orientation of patients with possible COVID-19. The value of leukocytes activation markers, including mCD169 and nCD64, in the diagnosis of acute infection needs to be evaluated during viral outbreaks in clinical studies. The development of fully automated tests for these markers may be crucial to prepare for future epidemics.

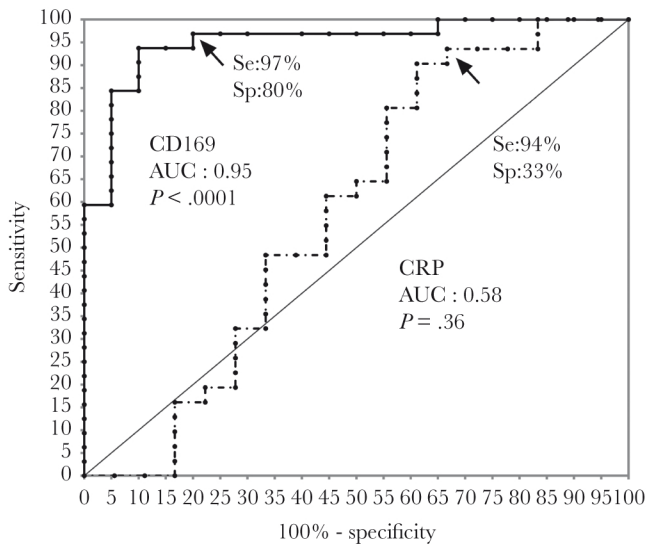


Figure 2. Receiver operating characteristic curve of CD169 median of fluorescence intensity (MFI) ratio and C-reactive protein (CRP) level for accuracy to predict coronavirus disease 2019 (COVID-19) infection. (1) Black line: COVID-19-positive and COVID-19-negative patients tested for CD169 MFI ratio. Area under the receiver operating characteristic curve (AUC) = 0.95; $P < .0001$; 95% confidence interval, 0.894–1.009. (2) Dotted line: COVID-19-positive and COVID-19-negative patients tested for CRP. The AUC of CRP = 0.58; $P = .36$; 95% confidence interval, 0.39–0.76.

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Notes

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Author contributions. A.-S. B. planned and supervised laboratory testing, analyzed the data, and wrote the manuscript. A. M., D. M., A. B., and V. L. M. managed patients and evaluated clinical data. A. P., L. M., E. N., K. B., S. D., V. F., and C. B. performed laboratory testing. F. M. wrote the manuscript. M.-C. P. evaluated clinical data and supervised statistical analysis. P. V. d. P. designed the study and wrote the manuscript. E. T. designed and conceptualized the study, analyzed the data, and wrote the manuscript.

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Potential conflicts of interest. F. M. and P. B. are Beckman Coulter employees. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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