Monocyte CD169 expression as a biomarker in the early diagnosis of COVID-19

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We assessed the expression of CD169, a type I interferon-inducible receptor, on monocytes at hospital admission. mCD169 is strongly overexpressed during the early phase of the SARS-CoV-2 infection, and remains elevated during the second week following the onset of symptoms.

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Abstract:

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

Keywords: SARS-CoV-2, Covid-19, CD169, Monocytes, viral infections

Background

Early identification of COVID-19 and prompt diagnosis of 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 (IFN) 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 IFNα 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 in order 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 15 March and 5 April 2020. It was approved by the accredited Institutional Review Board N 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, while 21 tested negative (Sup. Table 1). 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 (IQR, 51–78.5 years) and 54.7% were male (Sup. Table 2).

Flow cytometry

10 µl of EDTA sample was simultaneously lysed with 500 µL of Versalyse lysing solution and stained with CD64-CD169/infections dried custom mixture, composed of anti-CD169-phycoerythrin (PE) (clone 7-239) and anti-CD64-Pacific Blue (PB) (clone 22) (Sup.Fig.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 on a 3-laser, 10-color Navios flow cytometer and analyzed using Kaluza Software version 2.1 (both from Beckman Coulter Brea, CA 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 + 3SD. The thresholds were: 3.51 for the mCD169 MFI ratio, 4.59 for the nCD64 MFI, and 9.06% for $CD38^{bright}$. The nCD64 threshold was controlled on 10 clinical samples collected from patients with microbiologically confirmed bacterial infections (median (IQR) = 8.44; (5.77-11.37), data not shown).

SARS-CoV-2 RNA testing

SARS-CoV-2 RNA extraction from nasopharyngeal swabs (Sigma Virocult, Medical Wire Instrument, Corsham, UK) was done using the QIAamp Viral RNA Mini Kit on the QIAsymphony platform, following the manufacturer's instructions. SARS-CoV-2 RNA was assessed using a RT-PCR targeting RNA-dependent RNA polymerase (RdRp) as previously described.

Interferon alpha plasma concentration

Plasma samples were stored at − 80 °C until processing. Interferon alpha (IFN-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, Massachusetts, USA) following manufacturer's instructions.

Serology

Plasma samples were tested for IgG antibodies directed against SARS-CoV-2 nucleocapsid using a CE-marked ELISA (ID.Vet, ID screen® SARS-CoV-2-N, Montpellier, France) as previously described[5]. Each result is displayed as a ratio: S/P (Sample/positive control) expressed in percentile (%): S/P % = (Optic density (OD) of sample – OD negative control) / OD positive control – OD negative control) x 100. S/P > 40% is positive and < 40% is negative.

Statistical analysis

Data were analysed and illustrated using Excel 2016 (Microsoft Corp, Redmond, Washington) and Prism 7 (GraphPad Software Inc, La Jolla, California) software. To determine statistical significance between the two groups (ie. CD169 COVID+ vs COVID -), unpaired Student's two-tailed t-test or non-parametric Mann-Whitney test was applied, according to the distribution of the data set. Correlations were analysed using Pearson or Spearman's rank test according of the normality of the data set.

A *p* value of <0.05 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 15 March to 05 April 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 a mCD169 expression level above the positivity threshold (93.7%) (Fig.1.A). In contrast, only three of the 21 patients (14.3%) with a negative SARS-CoV-2 PCR result had a mCD169 overexpression. The level of mCD169 was inversely correlated with the CT (Cycle time detection) 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) (Fig.1.B). At hospital admission, mCD169 was not associated with the duration, since the onset, of symptoms in COVID-19 confirmed patients (Sup.Fig.2.A). Likewise, the level of mCD169 expression had no prognostic value as 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 (Fig.1.B). IFNα concentration was significantly higher in COVID-19 confirmed patients compared to COVID-19 negative patients. (p<0.0001) (Fig.1.D). Overall, the mCD169 level was correlated with the concentration of IFNα in plasma (r=0.48) (Sup.Fig.3.F). The mCD169 expression level decreased in 15 out 18 confirmed COVID-19 patients, alongside IFNα, retested before they were discharged from the hospital (Sup.Fig.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) (Sup.Fig.3.C), but no correlation was found with the CRP concentration, lymphocyte count or neutrophil count

(Sup.Fig.3. A.B.E). To investigate whether mCD169 could complement serological testing, we retrospectively assessed anti-SARS-CoV-2 nucleocapsid IgG. Among the confirmed COVID-19 cases, seven patients also tested positive for 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=0.0084), suggesting that high mCD169 may be associated with active SARS-CoV-2 infection, before seroconversion (Fig.1.C).

Neutrophil CD64 (nCD64) was also assessed as a marker for systemic bacterial infection[3]. High nCD64 expression was observed in seven out of 21 (33.3%) SARS-CoV2 PCR negative patients (Sup.Fig.2.B). Bacterial infection was microbiologically confirmed for all but one of these patients (6/7). Four out of 32 (12.5%) confirmed COVID-19 cases also had nCD64 expression above the threshold; two of them had confirmation of a bacterial infection. A higher median expression of $CD38^{bright}$ on CD8+ T-cells also was observed in the COVID-19 confirmed group compared to the SARS-CoV2 uninfected group (Sup.Fig.2.C). However, this marker has a limited capacity to identified 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) (Sup.Fig.3.D).

The test accuracy of mCD169 to predict COVID-19 infection during epidemic was studied by means of a receiver operating characteristic curve (ROC curve) and compared to the CRP level (Fig.2). The sensitivity and specificity at the optimal operating point were 97% and 80%, respectively, with an AUC of 0.95. 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 part 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 following hospital admission. mCD169 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[6,7,8]. Type I IFNs induce the transcription of CD169 mRNA. Amplification of CD169 monocytes has been observed in other viral infections such as Zika, untreated HIV, and influenza [9, 10,11]. 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 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 to 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 alpha concentration. These results suggest that the expression of the marker decreased over the course of the disease.

SARS-CoV-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 (ISGs), transcription of CD169 may be lower in severe COVID compared to middle forms of the disease. A trend for a lower mCD169 expression has been reported in COVID-19 confirmed patients hospitalized in intensive care unit [13]. 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.

In conclusion, mCD169 is strongly overexpressed on monocytes during the early phase of the SARS-CoV-2 infection, and remains elevated in patients admitted to the hospital during the second week following 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.

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Contributions

A.S.B. planned and supervised laboratory testing, analyzed the data and wrote the manuscript. A.M, D.M, A.B, V.L.M managed patients and evaluated clinical data. A.P, L.M., E.M, 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.Vd.P. designed the study and wrote the manuscript. E.T. designed and conceptualized the study, analyzed the data and wrote the manuscript.

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 Institutional of Montpellier University Hospital and Ile de France III ethical committee (n°2020-A00935-34).

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Figure 1. CD169 expression on monocytes in patients admitted in COVID-19 hospital units with a diagnosis of SARS-CoV-2 infection confirmed or ruled out

A. CD169 Median of fluorescence intensity (MFI) ratio (CD169 on monocyte / CD169 on lymphocytes). Healthy controls (HC) are indicated by black diamonds; patients 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<0.0001 ; *p<0.05.

B. Correlation between CD169 MFI ratio and CT value of the SARS-CoV-2 reverse-transcriptase- real-time polymerase chain reaction (CT-PCR) in COVID-19 confirmed patients.

C. Interferon alpha plasma concentration at hospital admission in patients with a diagnosis of SARS-CoV-2 infection confirmed or ruled out.

D. Expression of CD169 on monocyte according to serological status for anti-SARS-CoV-2 IgG at hospital admission.

Figure 2. Receiver operating characteristic curve (ROC curve) of CD169 MFI ratio and CRP level for accuracy to predict COVID-19 infection. 1) Black line: COVID-19 positive and negative patients tested for CD169 MFI ratio. Area under the receiver operating characteristic curve (AUC) = 0.95; p < 0.0001; 95% confidence interval [0.894 to 1.009]. 2) Dotted line: COVID-19 positive and negative patients tested for CRP. AUC of CRP= 0.58; p = 0.36; 95% confidence interval [0.39 to 0.76].

Figure.1.A

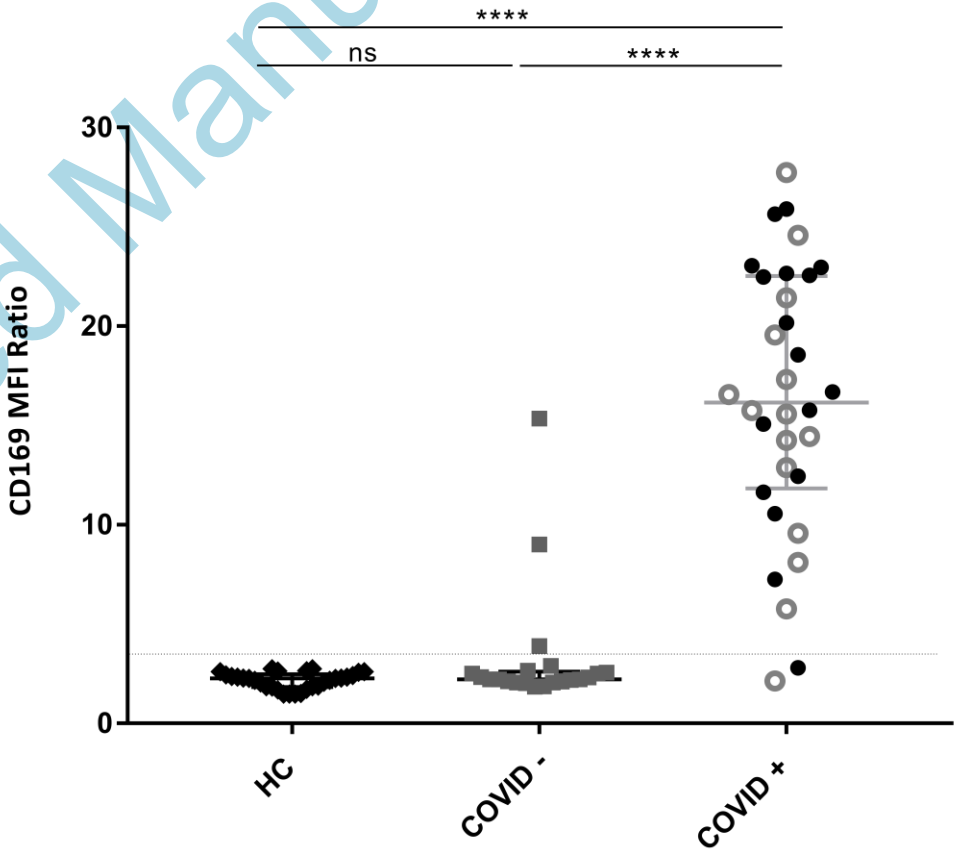

Figure.1.B

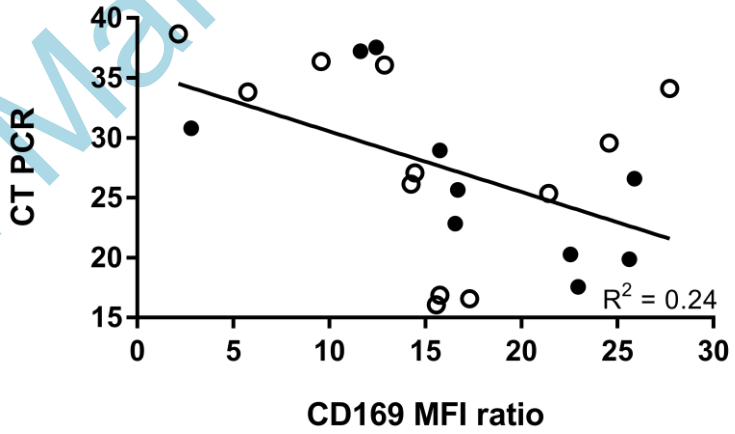

Figure.1.C

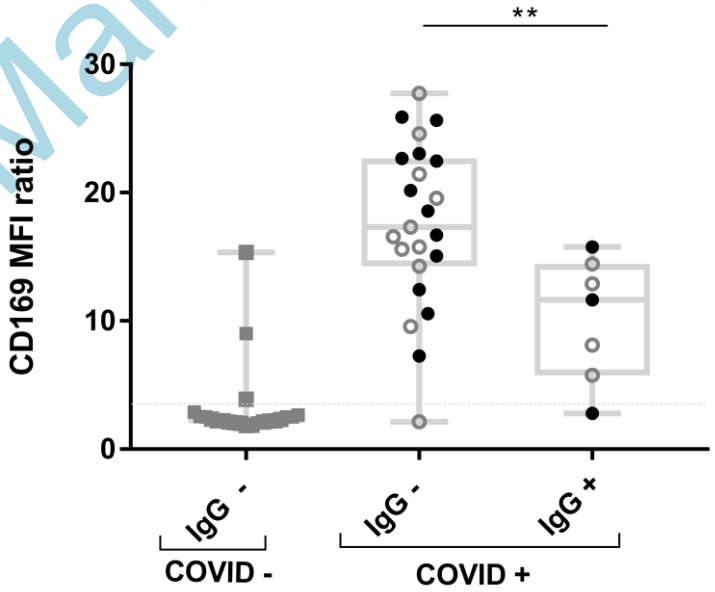

Figure.1.D

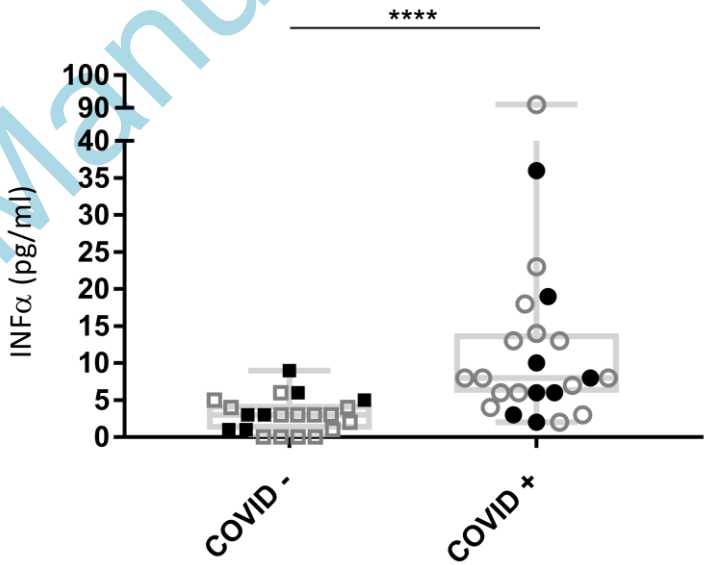

Figure 2