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A rapid, easy, and scalable whole blood monocyte CD169 assay for outpatient screening during SARS-CoV-2 outbreak, and potentially other emerging disease outbreaks

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Abstract

Objective: The COVID-19 corona virus disease outbreak is globally challenging health systems and societies. Its diagnosis relies on molecular methods, with drawbacks revealed by mass screening. Upregulation of neutrophil CD64 or monocyte CD169 has been abundantly reported as markers of bacterial or acute viral infection, respectively. We evaluated the sensitivity of an easy, one-step whole blood flow cytometry assay to measure these markers within 10 min, as a potential screening test for COVID-19 patients.

Methods: Patients ($n = 177$) with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were tested on 10 µL blood and results were compared with reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR).

Results: We observed 98% and 100% sensitivity in early-stage ($n = 52$) and asymptomatic patients ($n = 9$), respectively. Late-stage patients, who presented for a second control RT-qPCR, were negative for both assays in most cases. Conversely, neutrophil CD64 expression was unchanged in 75% of cases, without significant differences between groups.

Conclusion: Monocyte CD169 evaluation was highly sensitive for detecting SARS-CoV-2 infection in first-presentation patients; and it returns to basal level upon infection clearance. The potential ease of fingerprick collection, minimal time-to-result, and low cost rank this biomarker measurement as a potential viral disease screening tool, including COVID-19. When the virus prevalence in the tested population is usually low (1%–10%), such an approach could increase the testing capacity 10 to 100-fold, with the same limited molecular testing resources, which could focus on confirmation purposes only.

Keywords

COVID-19, SARS-CoV-2, CD169, flow cytometry, monocytes, CD64, neutrophils, screening test, emerging disease, outbreak

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19), a pandemic still expanding 2 years after emergence.[1] Even with massive vaccination, the control of COVID-19 requires targeted detection, protection, distancing, and isolation. To selectively isolate subjects with confirmed infection, a policy of massive diagnostic testing has been put in place

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in many countries. Ideally, this should allow any person to receive a prompt assessment at the time of symptom onset or contact tracing. This analysis is essentially based on molecular tests (reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR)) that detect viral RNA in a sample taken from the back of the nose or throat. Despite being the gold standard, RT-qPCR has limitations.

The sensitivity is not optimal,[2] mainly due to sampling quality and to the delay between contamination and colonization of the upper ear, nose, and throat area. Patients may receive a false negative result putting those around them at risk.

Although RT-qPCR is a fairly fast technique (one to a few hours), laboratory throughput due to massive screening volumes may lead to significant delays in sampling, processing, and result delivery. This may jeopardize distancing policies or hinder social and professional activity.

Deep nasal swabbing is unpleasant for the patient and puts the sampler at risk of infection.

Finally, RT-qPCR remains expensive. As a result, while massive testing is required for the fight against the pandemic, considerable efforts are underway in the search for efficient diagnostic techniques for mass testing strategies.

New point-of-care molecular methods reduce the turnaround time to less than 30 min. Although several methods are commercially available, performance inconsistency and relatively low sensitivity and positive predictive values for detection of ongoing infection hamper their potential contribution to the management of the COVID-19 outbreak.[3–6]

Antigenic tests of viral proteins are cheaper than RT-qPCR, faster, and some can be performed on salivary samples that are easier to access and provide for home testing. However, their sensitivity is variable, especially when saliva is used instead of a nasal swab.[7,8] On the other hand, serological tests do not determine current viral load, are positive at least 1 week postinfection, and lack sensitivity.[9,10]

In this context, harnessing early immune markers of leukocyte activation is a promising alternative. Leukocytes rapidly detect and respond to infection with secreted and surface activation molecules. We and others have previously reported that acute viral infections induce the appearance of CD169 (Siglec-1, sialoadhesin) at the surface of blood monocytes.[11,12] Monocyte CD169 expression is upregulated by type 1 interferons,[13] produced by locally attacked tissues, and is found in all circulating blood monocytes, allowing its detection in minimal blood volumes such as a drop of blood at the fingertip. CD169 upregulation has been found in patients with HIV,[14] EBV,[15] RSV, and influenza,[16,17] CMV,[18] dengue,[19,20] Zika,[21] noroviruses,[22] and Lassa and Marburg viruses.[23] Altogether, this shows that all viruses studied so far are detected, at least during the acute phase. Transcriptomic and mass cytometry studies have identified CD169 as a relevant biomarker for COVID-19.[24,25] The first clinical evaluation by flow cytometry in the COVID-19 context showed that CD169 was a potential SARS-CoV-2 infection marker, with upregulation levels much higher than those induced by any other virus tested so far.[26,27]

Having developed a rapid (10–15 min), easy, and affordable assay to measure monocyte CD169 upregulation in a few microliters of blood, applicable to fingerprick samples,[28] we set out to assess its efficacy in a cohort of COVID-19-confirmed patients, with SARS-CoV-2 RT-qPCR as the reference method. This assay evaluated in parallel two other immune markers: upregulation of CD64 on neutrophils, which is widely used as an indicator of bacterial infection,[29] and expression of human leukocyte antigen-DR (HLA-DR) isotype on monocytes, which reflects the general state of the immune system[30] which is increased when activated by a pathogen (viral or bacterial), and decreased if the immune system is "exhausted" by a severe infection (e.g., sepsis).

Materials and methods

Ethics statement

This retrospective observational study involved human subjects and was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). It was reviewed and approved by the local ethics committee (reference number PADS20-334). According to French law, the patients were informed and they retained the right to oppose the use of their anonymized medical data for research purposes, but formal consent was not required for this non-interventional study.

Samples

This study was conducted in the immunology laboratory of the IHU Méditerranée Infection (University Hospitals of Marseille, Assistance Publique – Hôpitaux de Marseille, Marseille, France). We included leftover samples from patients aged 16 or older with RT-qPCR-confirmed SARS-CoV-2 infection (at least one positive SARS-CoV-2 RT-qPCR in nasopharyngeal swabs or tracheal aspiration).

To determine the sample size, we assumed the COVID-19 incidence rate of 50% with a margin error of 10% and a confidence level of 95%, according to previous estimation from local registry. The minimum sample size needed to estimate the true population proportion was 97. Thus 177 leftover samples were consecutively collected within 2 days separated by a 1-week interval (92 and 85 samples, respectively). Patients were subsequently sorted into four groups: RT-qPCR+ patients who presented at an early stage of the disease, within 14 days after symptoms onset (group 1: 52 patients), RT-qPCR+ asymptomatic patients (group 2: 9 patients) since by definition they cannot be included in any group, RT-qPCR+ patients at a later stage of the disease, with more than 14 days after symptom onset (group 3: 5 patients), and patients who had become RT-qPCR negative at

Table 1. Demography of the cohort study.

Value (±1 SD)	SARS-CoV-2 RT-PCR positive early stage	SARS-CoV-2 RT-PCR positive asymptomatic	SARS-CoV-2 RT-PCR positive late stage	SARS-CoV-2 RT-PCR negative	*p*-Value
n	52	9	5	111	
Age, year	44.6 (26.5–62.6)	47.7 (31.3–64)	48.4 (31.4–65.4)	45.2 (29.6–60.7)	0.7
Sex, male, *n* (%)	24 (46%)	5 (56%)	1 (20%)	50 (45%)	0.6142
Time from symptom onset	5 (3–7)	NA	20 (13–26)	18 (10–27)	<0.0001
SARS-CoV-2 RT-PCR Ct value	27 (22–31)	27 (21–33)	31 (28–33)	NA	0.1614

Early stage is defined as samples obtained within 14 first days from the symptom onset. Late stage is defined as samples obtained after 14 days of first symptoms onset. Data are expressed as medians and interquartile ranges (IQR) unless otherwise specified. Ct, cycle threshold.

the time of the blood sampling (mostly patients retested at a later time point (group 4: 111 patients)).

Demographic, clinical, and laboratory data including date of onset of SARS-CoV-2-related symptoms were collected for each patient retrospectively from electronical medical records (Table 1). Twenty-five samples of healthy blood donors (HBD), obtained from the national blood bank, served as further controls (Convention N°7828, "Etablissement Français du Sang," Marseille, France). The national blood bank obtains written consent from donors and distributes control tubes accordingly.

Flow cytometry

Antibodies and reagents were from Beckman Coulter Life Sciences (Marseille, France). Excess EDTA-treated samples were maintained at room temperature (15°C–25°C) for a maximum of 24 h prior to flow cytometry investigations. The three specific antibodies were premixed in a ready-to-use cocktail (prototype of the IOTest Myeloid Activation CD169-PE/HLA-DR-APC/CD64-PacBlue Antibody Cocktail). The cocktail was then premixed with 0.5 mL of VersaLyse red blood cell lysing solution (Beckman Coulter Life Sciences), and 10 µL of blood were added in the reaction tube. After 10 min incubation at room temperature, the samples were analyzed with a three-laser Navios flow cytometer (acquisition time: 1 min) (Beckman Coulter Life Sciences).

Data analysis and statistics

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 (IQR) and were compared with the Wilcoxon test equivalent to Mann–Whitney *U* test.

Flow cytometry data files were analyzed using the Kaluza software, version 2.1 (Beckman Coulter Life Sciences). Leukocytes were gated using side scatter (SSC) and CD64 expression as lymphocytes (low SSC CD64−), monocytes (intermediate SSC CD64+), and neutrophils (high SSC), prior to the analysis of CD169, CD64, and HLA-DR level of expression. The CD169 index was calculated as the ratio of monocyte CD169 expression (signal) and lymphocyte CD169 expression (background). Similarly, the CD64 index was calculated as the ratio of neutrophil and lymphocyte signal to background. Data were exported to JMP 14.2.0 software (SAS, Cary, NC, USA) for statistical analysis. Median comparison between groups was performed via Kruskal–Wallis test then Wilcoxon rank sum tests, equivalent to Mann–Whitney tests. Correlations were established with Pearson correlation coefficient (r) and determination coefficient (r^2). A two-sided p-value <0.05 was considered statistically significant.

Results

Using RT-qPCR as the gold standard, 177 patients who had tested positive for SARS-CoV-2 at least once were included. One hundred twenty-three were new cases and the other 54 were old cases retested since they returned for monitoring their viral load. Overall, at the time of blood sampling, 66 patients out of 177 were positive for SARS-CoV-2 RT-qPCR and 111 turned out to be negative at this time point. Among positives, 91% (60/66) were detected by a positive blood CD169 index, using the previously established threshold of 3.5.[26]

We reasoned that a triage assay would mostly encounter patients at an early stage of the disease, or asymptomatic patients. Thus, we sorted the cohort into four groups according to time since the onset of symptoms (Table 1).

Group 1: Fifty-two patients were RT-qPCR+ and at an early stage of the disease (less than 14 days after symptom onset). They were detected by the CD169 index with 98% sensitivity (51/52) (Figure 1).

Group 2: Nine patients were RT-qPCR+ and asymptomatic. They were detected by the CD169 index with 100% sensitivity (9/9).

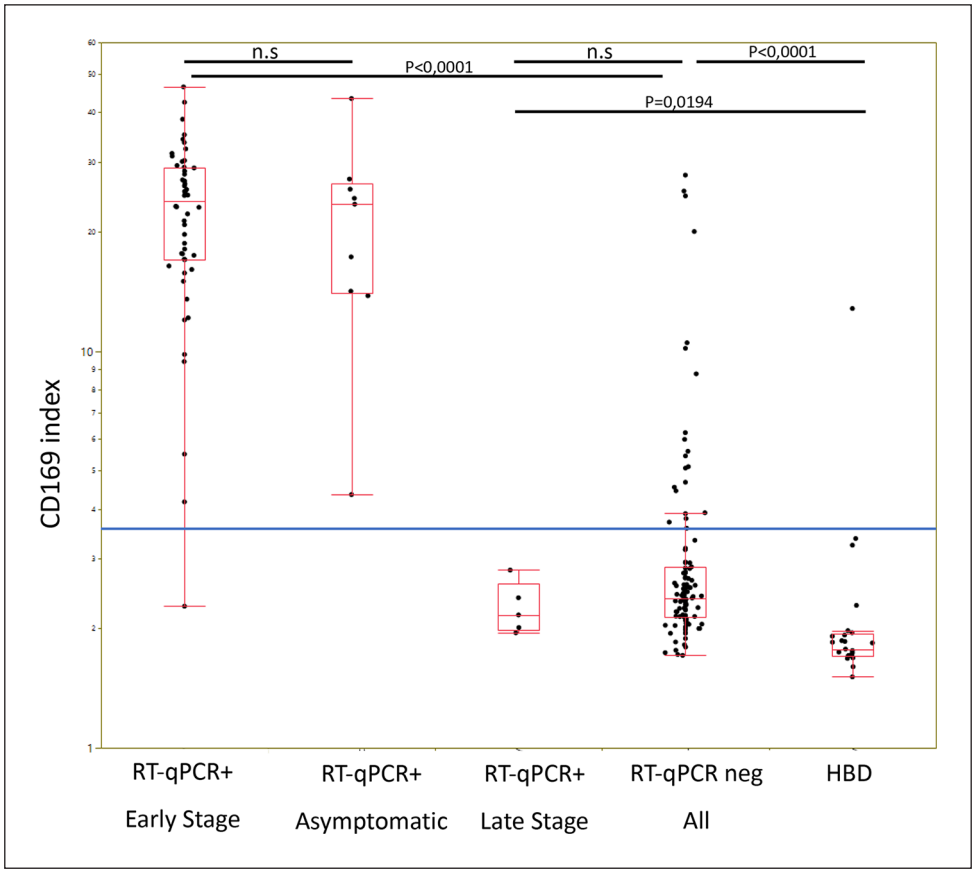

Figure 1. Expression of monocyte CD169 in COVID-19 patients.
Box plots summarizing the differential CD169 expression on blood monocytes in four COVID-19 groups and control group (HBD). Data were compared with Kruskal–Wallis test then each group pairs were compared upon Wilcoxon test. Blue line: Threshold at 3.5.

Group 3: Only five patients were RT-qPCR+ at a late stage of the disease (14–48 days after symptom onset), and they were not detected by the CD169 index (0/5).

Group 4: One hundred eleven patients that were negative for the RT-qPCR at the time of the study, among them 21 did show a CD169 index positivity, indicating a specificity of 81%.

As a further control, we included in the study 25 HBD, who were all negative but one (Figure 1).

CD169 expression was significantly higher in groups 1 and 2 (early stage and asymptomatic) as compared to other groups ($p \leqslant 0.001$). Review of medical records for the only patient exhibiting a CD169 index below the threshold but a positive nasopharyngeal RT-qPCR showed very low and decreasing RNA quantities (cycle threshold (Ct) at 33.5 and 34.5, respectively, 48 and 24 h earlier), suggesting a near complete viral clearance.

In group 3 (late stage), CD169 was negative indicating that the biomarker returns to baseline levels after 2–3 weeks, even if viral clearance is not complete according to RT-qPCR.

In group 4 (negative RT-qPCR), CD169 was observed in 21 cases, at a lower level than in groups 1–2, indicating either a persistence of the marker after viral RNA clearance, another infection, or pointing to a potential false negative RT-qPCR result.

CD169 level and RT-qPCR Ct were weakly but significantly correlated, with the six CD169 negative patients ranking among those with the highest RT-qPCR Ct, that is, the lowest RNA quantities (Figure 2).

Neutrophil CD64 expression, a marker of bacterial infection, was unchanged in 75% of the cases and weakly upregulated in 25% of the cases (45/177). Within this cohort of outpatients presenting with mild disease, HLA-DR was expressed at normal or slightly increased levels, an expected finding as opposed to the decrease usually observed in severe cases[31] (Figure 3).

Discussion

The rapid whole blood assay of CD169 upregulation yielded a detection rate of 98% and 100% in early-stage (2 weeks) and asymptomatic patients respectively. Since the only false

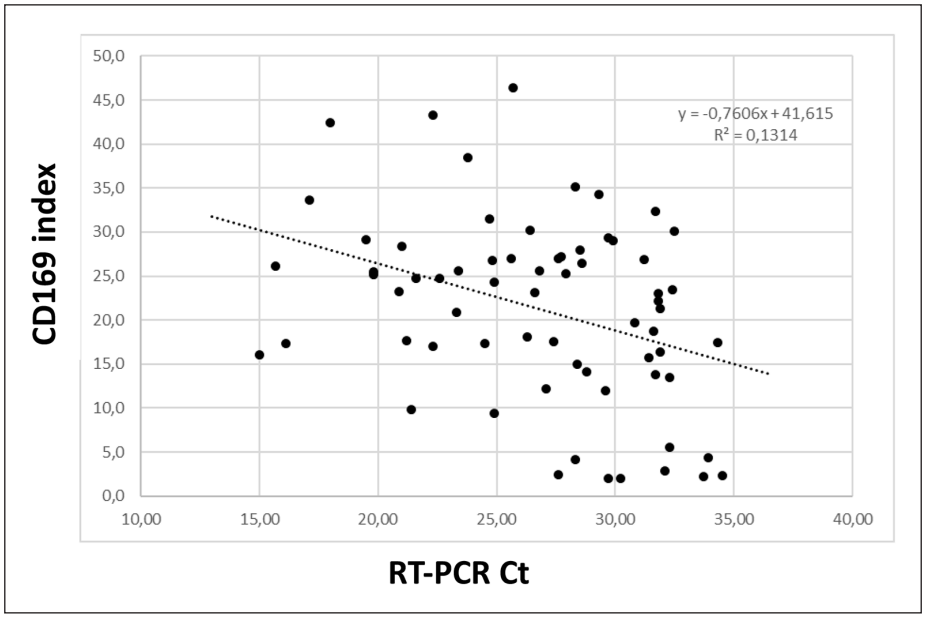

Figure 2. Correlation plot between SARS-CoV-2 RT-qPCR Ct and CD169 index.
Upregulation of monocyte CD169, expressed as CD169 index of monocyte-to-lymphocyte CD169 expression, was inversely correlated with SARS-CoV-2 Ct, itself inversely correlated with the patient's viral load. Ct, cycle threshold.

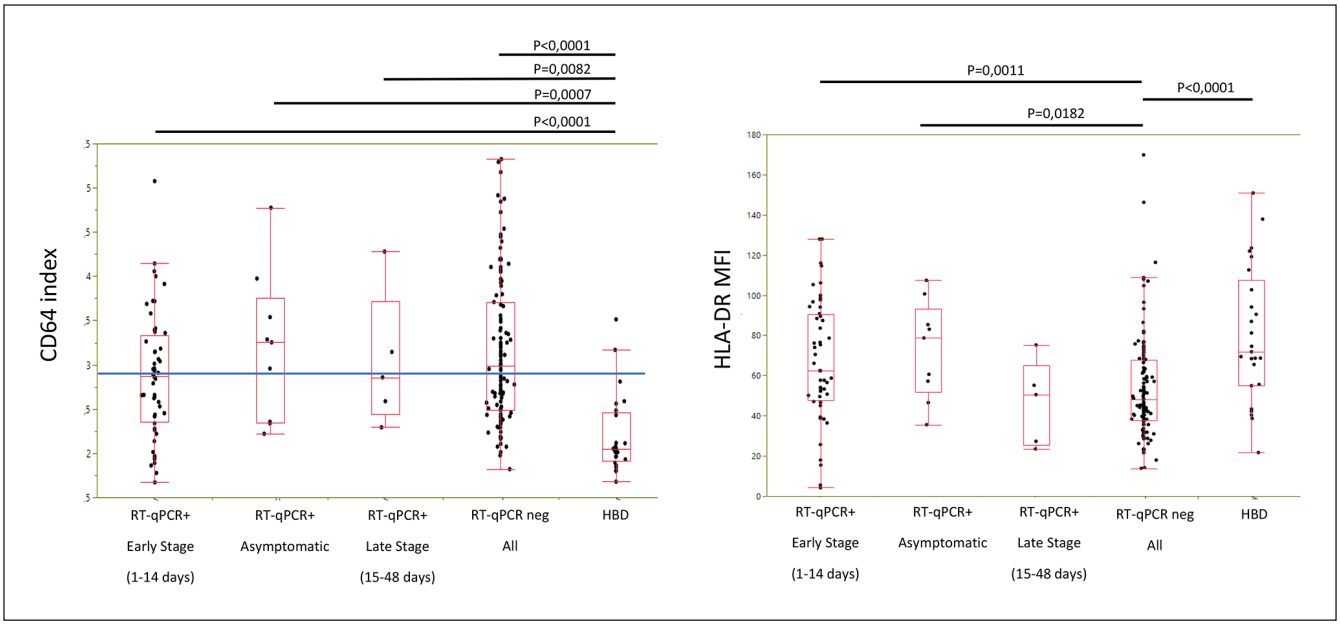

Figure 3. Expression of neutrophil CD64 and monocyte HLA-DR in COVID-19 patients.
Box plots summarizing the differential CD64 expression on blood neutrophils and HLA-DR expression on blood monocytes in four COVID-19 groups and control group (HBD). Data were compared with Kruskal–Wallis test then each group pairs were compared upon Wilcoxon test. Blue line: CD64 threshold at 4.

negative case was sampled 8 days after symptom onset, and had a very low and decreasing viral load, one could also consider a 100% sensitivity within the first week after symptom onset, thus a performance equal to the specific RT-qPCR. These results are superior to other rapid methods, such as antigenic tests, usually described with lower sensitivity than RT-qPCR.

It is remarkable that asymptomatic patients, who made up 14% of the RT-qPCR+ cases, expressed CD169 at the same level as patients experiencing symptoms. These findings

give pathophysiological insights, suggesting that a systemic response orchestrating infection control takes place despite the absence of symptoms, rather than a locally limited control of the infection (mucosal and/or tissue immunity).

As expected, monocyte CD169 upregulation was also observed in the control cohorts: in 1 out of 25 HBD (4%), and in 21 out of the 111 RT-qPCR negative patients enrolled as COVID-19 cases (19%). This could be easily explained by the broad specificity of this biomarker which can detect any acute viral infection tested so far.[14–23] We cannot rule out this possibility, both for the 21 patients in the cohort, and for the single case from the control cohort. Still, the significantly higher proportion within the COVID cohort suggests that at least some of them are real SARS-CoV-2 infections, undetected by RT-qPCR. It would not be surprising that the immune reaction mediating the CD169 expression would not perfectly parallel the viral load detectable by RT-qPCR, both at the end of the infection and at the beginning. In support of this, two recent studies have found similar performances for CD169 in hospitalized patients, sensitivity and specificity being beyond 90%, and discrepancies with PCR being explained by late sampling and potential false negative RT-qPCR.[32,33] In line with this idea, we found in the medical records a few cases where RT-qPCR tests were still positive at a later sampling time point, in contradiction to the results of the study (pointing to a false negative RT-qPCR at the time of the study). Also, it is now well established that the performance of RT-qPCR tests is not optimal, mainly limited by the quality of sampling.[2] We conclude from these results that the CD169 detects the early COVID cases, both symptomatic and asymptomatic, with a sensitivity at least as high as RT-qPCR, and then returns to baseline within 2–3 weeks as do the RT-qPCR results.

These characteristics are those expected for a screening test: the highest possible sensitivity within the early phase, and as few as possible false positive within the general population, provided no other acute viral infection is taking place. Data obtained in the present study demonstrate that a testing strategy leveraging monocyte CD169 upregulation as a triage test could be designed. Taking into account a positivity rate of RT-qPCR tests ranging from 1% to 10%, it appears that 90%–99% of these expensive and uncomfortable tests are done for no reason. Monocyte CD169 screening could help prioritize true positives, thereby opening the perspective of decreasing the number of RT-qPCR tests currently run on the health system by a factor of 10–100. Alternatively, it could increase the testing capacities by the same magnitude and open new opportunities.

Taken together, the monocyte CD169 assay displays advantages for routine implementation, including (i) high sensitivity for the detection of early-stage and asymptomatic patients; (ii) 10–15 min time-to-result granting an immediate response during patients' presence; (iii) readily available technology supported by flow cytometers available in most clinical laboratories; (iv) finger prick or venous blood sampling, short technician time, affordable reagents (a few dollars per test), and simple logistics chain.

This strategy would be ideally suited in areas where a zero-cases strategy is implemented, and where anyone entering the area needs to be rapidly and efficiently tested. It has been argued that biomarkers like CD169 are nonspecific and therefore may not be used as diagnostic tools. Nonspecificity is real but does not reduces their potential as rapid and easy screening tools, coupled with a rapid diagnostic tool used to confirm the result. Of course, in case the polymerase chain reaction (PCR) result would turn out to be negative, it would suggest another viral infection that would probably need to be investigated for an optimal patient care. This could also help detecting new outbreak or new variants invisible to "too-specific" tools. The positive result in the control cohort from the blood bank also raises questions about the utility of such a test as a screening tool with broader detection capabilities than current specific tests.

Also, one may consider the probability of the next epidemics, where specific RT-qPCR tests will again require months to be developed. Such "nonspecific" tools may offer during this window a reasonable help at triaging "virally infected" from healthy people. Of course, it is not known which pathogen will cause the next pandemic, nor whether it will increase CD169 or CD64 expression, but given the large number of acute infections correlated with those markers so far,[14–22] there is a good chance such an assay would work as a first line detection tool, and thus improve our preparedness.

The main strengths of this study are its outpatient design. Previous reports enrolled only ICU or emergency unit patients. However, this paper has some limitations that need to be addressed. First, venous blood leftover has been used instead or real fingerprick sampling. Even if we have strong indications that these are equivalent, it remains to be formally proven. Second, a full characterization of other potential infections occurring in the patients and controls would be helpful to refine the specificity and sensitivity estimates. Finally, the size of the subgroups is limited and can only serve as a determination cohort. Therefore, these results need to be confirmed in a larger and independent cohort for validation prior to generalization.

Conclusion

Detection of monocyte CD169 would allow the identification of SARS-CoV-2 suspect patients for referral to RT-qPCR with no sensitivity loss. Combined neutrophil CD64 for bacterial infections, and monocyte HLA-DR for immune status would help identify those suffering from other diseases or at risk for more severe forms. Therefore these three markers would help for a better management of COVID-19 and non-COVID-19 cases.

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Author contributions

JV, TM, and PEM designed the study. MoM, IAB, and FM conducted experiments and acquired data. MaM and HTD supervised clinical procedures. MoM collected and analyzed demographic, clinical, and laboratory data. PB and FM provided antibody panels and PEM and IA the flow cytometry platform. MoM, FM, and JMB analyzed experimental data. FM, MoM, JV, and JMB wrote the manuscript. JLM revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: TM, JMB, IAB, PB, and FM are employees of Beckman Coulter Life Sciences, IAB and PB are recipient of a PhD grant from the ANRT (National Agency for Research and Technology). JV has received speaker and consultancy fees from Meda Pharma, Mylan, Novartis, Sanofi, Thermo Fisher, outside this work. The other authors declare no conflict of interest related to this work.

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Informed consent

Informed consent was not sought for the present study because according to French law, the patients were informed and retained the right to oppose the use of their anonymized medical data for research purposes, but formal consent was not required for this non-interventional study using only blood leftover. Twenty-five samples of healthy blood donors (HBD), obtained from the national blood bank, served as further controls (Convention N°7828, "Etablissement Français du Sang," Marseille, France). The national blood bank obtains written consent from donors and distributes control tubes accordingly.

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