Rapid screening of COVID-19 patients using white blood cell scattergrams, a study on 381 patients

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Summary

Complementary tools are warranted to increase the sensitivity of the initial testing for COVID-19. We identified a specific ‘sandglass’ aspect on the white blood cell scattergram of COVID-19 patients reflecting the presence of circulating plasmacytoid lymphocytes. Patients were dichotomized as COVID-19-positive or -negative based on reverse transcriptase polymerase chain reaction (RT-PCR) and chest computed tomography (CT) scan results. Sensitivity and specificity of the ‘sandglass’ aspect were 85.9% and 83.5% respectively. The positive predictive value was 94.3%. Our findings provide a non-invasive and simple tool to quickly categorize symptomatic patients as either COVID-19-probable or -improbable especially when RT-PCR and/or chest CT are not rapidly available.

Keywords: COVID-19, SARS-Cov-2, coronavirus, plasmacytoid lymphocytes, white blood cells scattergram.
Introduction

The novel coronavirus SARS-CoV-2, responsible for COVID-19, confronts the health community with major challenges. Early diagnosis of COVID-19 is crucial for the optimal management of infected patients to control viral spread. The standard test for COVID-19 remains the reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral RNA from clinical samples. RT-PCR is specific but lacks sensitivity. Complementary tools are warranted to increase the sensitivity of the initial testing of COVID-19 patients.

Complete blood count (CBC) is a routine test during initial biological assessment of patients. CBC analyzers such as SYSMEX® (Sysmex Corporation, Kobe, Japan), provide a white blood cell (WBC) differential fluorescence (WDF) scattergram, displaying a classification of WBCs based on their morphology and their intracellular components. Each type of leucocytes is always displayed in the same area. The different clusters of leucocytes displayed on the WDF scattergram match with the visual examination by optical microscopy.

During this outbreak, we have noticed a recurrent atypical aspect on the WDF scattergrams of COVID-19 patients. We therefore decided to evaluate the sensitivity and specificity of our finding in order to propose WDF as a screening tool for COVID-19.

Methods

Patients admitted at Versailles Hospital suspected of having COVID-19 were eligible if symptoms were present for three or more days and if RT-PCR and a chest CT were performed (Figure S1).

A complete blood count was performed using an XN3100 analyzer (SYSMEX® (Sysmex Corporation). WDF analyses were assessed blindly by two readers. Presence of the new pattern was considered WDF-positive (WDF+), all other patterns were considered negative (WDF-). Blood cell morphology was assessed by microscopy (Fig 1 and Figure S2).

RNA was extracted from clinical samples obtained via upper or lower respiratory tract swabs or aspirates. RT-PCR assays were performed on Applied Biosystems® analyzers (Foster City, CA, USA), following the National Reference Center protocol (Pasteur Institute). Results were concluded as positive (RT-PCR+) if amplification of SARS-CoV-2 cDNA was observed after 40 cycles.

Chest CT scans were performed on General Electric® scanners (Boston, MA, USA) and classified as typical (CT+) or not (CT-) for COVID-19 according to the published definition.

Index test’ was the WDF pattern on the CBC performed at admission time, whereas the ‘reference test’ was a diagnostic algorithm combining RT-PCR and CT results, as recommended by recent studies.

Results

We noticed a recurrent atypical aspect on the WDF scattergram of COVID-19 patients. This aspect, named the ‘sand-glass’ pattern, consisted of a discontinuous cluster of lymphocytes characterized by the presence of more than four dots in the upper graduation of the scattergram, where plasmacytoid lymphocytes are usually plotted. This observation was reinforced by the presence of circulating plasmacytoid lymphocytes on blood smears from patients with COVID-19, whereas large hyperbasophilic lymphocytes, normally seen in other viral infections, were absent (Fig 1). The four-dots threshold was derived from the receiver operating characteristic (ROC) curve to maximize the weighted Youden index (Figure S3).

We then retrospectively analyzed 381 WDF scattergrams from symptomatic adults admitted at Versailles Hospital from March 16th to April 5th 2020 [Median age: 61 years (18–99), sex ratio M/F: 1.47]. Complete characteristics of patients are reported in Table 1.

In summary, 57% (216/381) of the patients were hospitalized including 36 patients (9%) immediately admitted to the intensive care unit for an acute respiratory distress syndrome. Loss of smell/taste (33/290) and lymphopenia (159/290) were largely reported in COVID-19+ patients versus COVID-19–. The COVID-19 status confirmation was available within one day for 353/381 (93%) patients (range: 0–3 days). Of the 381 patients studied, 290 (76%) were COVID-19+ and 91 (24%) were COVID-19–. Among COVID-19+ patients, 247 (85%) had RT-PCR+/CT+, 35 (12%) had RT-PCR-/CT+ and 8 (3%) had RT-PCR-/CT-.

Interestingly, 25 COVID-19+ patients with WDF+ had a further CBC available, and the WDF became positive for 19 (76%) patients within 1–2 days. For the 15 COVID-19– patients with WDF+, a diagnosis of clinically documented pneumonia (10/15) or dyspnoea (3/15), flu-like syndrome (1/15), or vaso-occlusive crisis (1/15) was finally made.
Using the COVID-19 group as reference, we validated the performance of the WDF ‘sandglass’ pattern as a screening tool for COVID-19. The ROC curve was plotted and showed good discriminative performances of WDF with an area under the curve of 0.870 (95% CI: 0.830–0.910; Figure S3). Using the four-dots threshold, the diagnostic performances were: sensitivity: 85.9% (95% CI: 81.3–89.7), specificity: 83.5% (95% CI: 74.3–90.5), positive predictive value (PPV): 94.3% (95% CI: 90.8–96.8), negative predictive value (NPV): 65.0% (95% CI: 55.6–73.5), positive likelihood ratio: 5.2 (95% CI: 3.3–8.3), and negative likelihood ratio: 0.17 (95% CI: 0.13–0.23).

We then applied our test to a validation cohort of 170 WDF scattergrams from patients infected with a well-defined pathogen (85 SARS-CoV-2, 54 influenza virus, 19 Epstein–Barr virus, 8 Mycoplasma pneumoniae and 4 parvovirus B19) and found a sensitivity to distinguish COVID-19 versus other infections of 88.2% (95% CI: 79.4–94.2) and a specificity of 83.5% (95% CI: 73.9–90.7).
Discussion

We report here a specific and original ‘sandglass’ aspect on the WDF scattergram of COVID-19 patients. We hypothesize that this pattern reflects the presence of circulating plasmacytoid lymphocytes as observed from our careful blood smears examination of COVID-19 patients.8,9 Circulating plasmacytoid lymphocytes, absent in healthy people, have previously been reported in COVID-1914,15 and deserve further immunological exploration. We showed that WDF is a highly reliable screening test to detect COVID-19 patients with 85/9% sensitivity and 83/5% specificity. It remains a simple, rapid, inexpensive and non-invasive method. Due to COVID-19-associated lymphopenia,7,11,12 WDF analysis appears more accurate than

Table I. Characteristics of the cohort.

|                         | Overall | COVID-19+ | COVID-19− |
|-------------------------|---------|-----------|-----------|
|                         | n = 381 | n = 290   | n = 91    | P values |
| Demographics            |         |           |           |
| Median of age/Range (years) | 61 [18–99] | 62 [21–99] | 57 [18–94] | 0.33     |
| <50 years, No. (%)      | 102 (27%) | 66 (23%)  | 36 (40%)  |          |
| ≥70 years, No. (%)      | 131 (34%) | 100 (35%) | 31 (34%)  |          |
| Male (No.)/Female (No.)| 227/154 | 185/105   | 42/49     | 0.003    |
| Clinical features       |         |           |           |
| Fever                   | 296 (78%) | 243 (84%) | 53 (58%)  | <0.001   |
| Cough                   | 246 (65%) | 194 (67%) | 52 (57%)  | 0.10     |
| Dyspnoea                | 240 (63%) | 186 (64%) | 54 (59%)  | 0.46     |
| ARDS                    | 37 (10%)  | 31 (11%)  | 6 (7%)    | 0.31     |
| Loss of smell or taste  | 36 (9%)   | 33 (11%)  | 3 (3%)    | 0.023    |
| Confusion               | 11 (3%)   | 8 (3%)    | 3 (3%)    | 0.79     |
| Headache                | 50 (13%)  | 39 (13%)  | 11 (12%)  | 0.86     |
| Chest pain              | 42 (11%)  | 24 (8%)   | 18 (20%)  | 0.004    |
| Asthenia                | 150 (39%) | 128 (44%) | 22 (24%)  | <0.001   |
| Flu-like syndrome       | 103 (27%) | 86 (30%)  | 17 (19%)  | 0.043    |
| Digestive disorders     | 79 (21%)  | 62 (21%)  | 17 (19%)  | 0.66     |
| Duration of symptoms at admission time (days)a | 7.3 [3–30] | 7.7 [3–30] | 6.2 [3–30] |          |
| Becoming                |         |           |           |
| Non-hospitalized        | 42 (11%)  | 19 (7%)   | 23 (25%)  |          |
| Pre-COVID unitb         | 121 (32%) | 88 (30%)  | 33 (36%)  |          |
| Hospitalized            | 216 (57%) | 182 (63%) | 34 (37%)  |          |
| Among ICU               | 36 (9%)   | 30 (10%)  | 6 (7%)    |          |
| Biological features     |         |           |           |
| Median time interval for COVID-19 statusc (range, days) | 1 [0–3] | 1 [0–2] | 1 [0–3] |          |
| RT-PCR+                 | 255 (67%) | 255 (88%) | 0 (0%)    | <0.001   |
| RT-PCR−                 | 126 (33%) | 35 (12%)  | 91 (100%) |          |
| Chest CT+               | 282 (74%) | 282 (97%) | 0 (0%)    | <0.001   |
| Chest CT−               | 99 (26%)  | 8 (3%)    | 91 (100%) |          |
| WDF+                    | 264 (69%) | 249 (86%) | 15 (17%)  | <0.001   |
| WDF−                    | 117 (31%) | 41 (14%)  | 76 (84%)  |          |
| Lymphocyte count, 10⁹/l |         |           |           |
| Mean [range]            | 1.19 [0.08–4.90] | 1.03 [0.08–4.22] | 1.70 [0.14–4.90] | <0.001   |
| Median                  | 1.01     | 0.96      | 1.05      |          |
| <1.0·10⁹/l, No. (%)     | 186 (49%) | 159 (55%) | 27 (30%)  | <0.001   |

Patient’s baseline characteristics were compared by non-parametric tests, either the exact Fisher’s test (qualitative) or the Kruskal-Wallis test (quantitative variables).

ARDS, Acute Respiratory Distress Syndrome; ICU, Intensive Care Unit; No., Number of patients; NS, Not Specified; WDF White blood cell Differential Fluorescence scattergram (XN3100, SYSMEX®); RT-PCR, Reverse Transcriptase Polymerase Chain Reaction; CT, Computed Tomography.

aTime interval since the onset of the first symptom.

bTemporary unit in expectation of RT-PCR results (<24 h).

cMedian time interval for COVID-19 status includes the completion time of RT-PCR, chest CT and CBC (complete blood count).
blood smear examination. If confirmed, detection of circulating plasmacytoid lymphocytes can be a useful alternative for centres where WDF is not available.

Our study, however, presents some limitations: first, it is a monocentric study carried out using a specified type of CBC analyzers. However, SYSMEX® analyzers (Sysmex Corporation) are largely available in clinical institutions all over the world. This report may allow other laboratories and hospitals to confirm our results and provide multicentric data. Second, in order to exclude undetermined cases and reduce potentially wrong dichotimization resulting from early negative CT, 5–7 we excluded early symptomatic patients. Thus, prevalence of COVID-19 cases was higher than in the general population for which RT-PCR was required, and therefore PPV may be overestimated while NPV underestimated.

Based on this retrospective study, we conclude that WDF analysis can be implemented during the SARS-CoV-2 pandemic to quickly categorize symptomatic patients as either COVID-19-probable or -improbable, depending on the presence of the plasmacytoid lymphocytes cluster on their scattergram.

Finally, given that CBC is available within a few minutes, the ‘sandglass’ WDF pattern may be a valuable tool assisting clinicians to pilot the medical management of symptomatic patients suspected of having COVID-19 at time of admission in hospitals.

This simple tool may be of particular importance: (i) when RT-PCR and/or chest CT are not rapidly available; (ii) to decide to repeat the RT-PCR; (iii) in addition to other diagnostic tools such as chest CT; and (iv) for patients for whom the diagnosis was not initially suspected.

We are now conducting a prospective study with a validation cohort to derive a new algorithm combining RT-PCR, chest CT and WDF in order to facilitate the initial management of symptomatic patients suspected of having COVID-19.

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Author contributions
JO conceptualized, designed the study and wrote the first draft. JO, MT, FD, RF, CF, DB and VR analyzed data. JL provided the statistical analysis. All authors provided critical revision of the manuscript.

Conflicts of interest
The authors declare no competing financial interests.

Data sharing
All data and materials used in this work are available upon reasonable request to the corresponding author.

Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Flow chart of the study.
Fig S2. Analysis of white blood cell scattergram and categorization of patients suspected of having COVID-19.
Fig S3. Receiver operating characteristic curve.

References
1. WHO/Europe | Coronavirus disease (COVID-19) outbreak [Internet]. [cited 2020 Apr 25]. Available from: http://www.euro.who.int/en/health-topics/health-emergencies冠状病毒-covid-19
2. Ai T, Yang Z, Hou H, Zhan C, Chen G, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. 2020;296:200642.
3. Kim H, Hong H, Yoon SH. Diagnostic performance of CT and reverse transcriptase-polymerase chain reaction for coronavirus disease 2019: a meta-analysis. Radiology. 2020;17:201345.
4. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv. 2020. https://doi.org/10.1101/2020.02.11.20021493
5. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020;20(4):425–34.
6. Bai HX, Hsieh B, Xiong Z, Halsey K, Choi JW, Tran TML, et al. Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT. Radiology. 2020;10:200823.
7. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323(11):1061.
8. van Mirre E, Vridlink GI, Tjon-a-Tsz N, Hendriks H, de Kievet W, ten Boekel E. Sensitivity and specificity of the high fluorescent lymphocyte count-gate on the Sysmex XE-5000 hematology analyzer for detection of peripheral plasma cells. Clin Chem Lab Med. 2011;49(4):685–8.
9. Linssen J, Jennissen V, Vildmann J, Reisinger E, Schindler J, Malchau G, et al. Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/secretion cells using the automated routine hematology analyzer XE-2100. Cytometry B Clin Cytom. 2007;72(3):157–66.
10. Perkins NJ, Schisterman EF. The inconsistency of “optimal” cutpoints obtained using two criteria based on the receiver operating characteristic curve. Am J Epidemiol. 2006;163(7):670–5.
11. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet Lond Engl. 2020;395(10223):507–13.
12. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China. Lancet Lond Engl. 2020;395(10223):497–506.
13. Giacomelli A, Pezzati L, Conti F, Bernacchia D, Siano M, Oreni L, et al. Self-reported olfactory and taste disorders in patients with severe acute respiratory coronavirus 2 infection: a cross-sectional study. Clin Infect Dis. [cited 2020 Apr 20]; Available from: https://academic.oup.com/cid/article/doi/10.1093/cid/ciaa330/5811989
14. Foldes D, Hinton R, Arami S, Bain BJ. Plasmacytoid lymphocytes in SARS-CoV-2 infection (Covid-19). Am J Hematol. 2020;95(7):861–862. https://doi.org/10.1002/ajh.25834
15. Chong VCL, Lim KGE, Fan BE, Chan SSW, Ong KH, Kuperan P. Reactive lymphocytes in patients with COVID-19. Br J Haematol. 2020;189(5):844. [cited 2020 May 18]; Available from: https://onlinelibrary.wiley.com/doi/ab s/10.1111/bjh.16690

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