bjh short report

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.

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Introduction

The novel coronavirus SARS-CoV-2, responsible for COVID-19, confronts the health community with major challenges.[1] 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.[2–4] 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 ($RT\text{-}PCR^+$) 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.[3,5–7]

'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.[3,5] We excluded patients with symptoms for less than three days to overcome the 'grey-zone' of the chest CT. Patients with at least one $RT\text{-}PCR^+$ and/or CT^+ were considered as COVID-19-positive ($COVID\text{-}19^+$), whereas patients with $RT\text{-}PCR^-$ and CT^- were considered as COVID-19-negative ($COVID\text{-}19^-$). WDF and chest CT interpretations were blinded.

Once dichotomized ($COVID\text{-}19^{+/-}$), diagnostic performances of WDF were calculated.

All statistical analyses were performed using R version 3.6.1 (R Core Team 2019; https://www.r-project.org/). Patients' baseline characteristics were compared by non-parametric tests, either the exact Fisher's test for qualitative variables or the Kruskal–Wallis test for quantitative variables.

This study was conducted in accordance with the French CNIL (commission informatique et libertés) regulations.

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.[8,9] 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).[10]

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 $COVID\text{-}19^+$ patients versus $COVID\text{-}19^-$.[7,11–13]

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 $COVID\text{-}19^+$ and 91 (24%) were $COVID\text{-}19^-$. Among $COVID\text{-}19^+$ patients, 247 (85%) had $RT\text{-}PCR^+/CT^+$, 35 (12%) had $RT\text{-}PCR^-/CT^+$ and 8 (3%) had $RT\text{-}PCR^+/CT^-$.

Interestingly, 25 $COVID\text{-}19^+$ patients with WDF^- had a further CBC available, and the WDF became positive for 19 (76%) patients within 1–2 days. For the 15 $COVID\text{-}19^-$ 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.

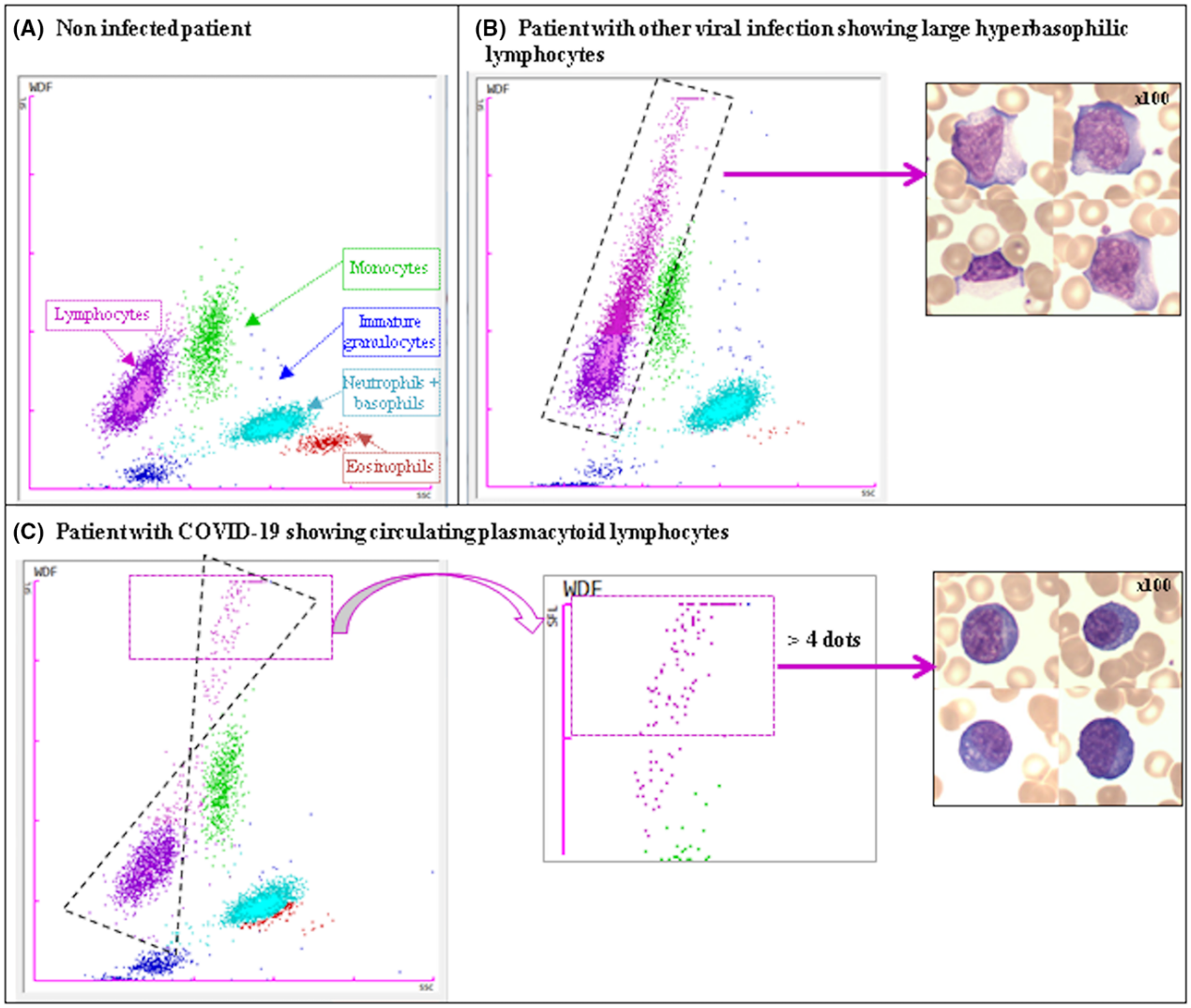

Fig 1. Atypical white blood cell scattergram of patients suspected of having COVID-19 and matching aspects on the blood smear. (A) Example of a normal WDF scattergram (White blood cell (WBC) Differential Fluorescence, XN3100 SYSMEX® (Sysmex Corporation) from a healthy patient (no or less than four dots in the upper graduation). After permeabilization of the leucocyte membrane and intracellular staining, the WDF channel can differentiate WBCs depending on their morphology (side scattered light, SSC, *x*-axis) and the content of RNA/DNA (side fluorescent light, SFL, *y*-axis). Each dot represents one analyzed cell. 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 (May–Grünwald–Giemsa staining, original magnification ×100). (B) Example of a WDF scattergram usually observed in case of other viral infections. This aspect consisted of a continuous cluster of lymphocytes and large hyperbasophilic lymphocytes as observed on the blood smear. (C) Example of an atypical aspect on the WDF scattergram of patients having COVID-19. This aspect consisted of a discontinuous cluster of lymphocytes characterized by the presence of more than four dots in the upper graduation of the scattergram ('sandglass' aspect), where plasmacytoid lymphocytes are usually plotted. This pattern reflects the presence of circulating plasmacytoid lymphocytes as observed from a careful analysis of blood smears from COVID-19 patients. [Colour figure can be viewed at wileyonlinelibrary.com]

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).

Table I. Characteristics of the cohort.

	Overall $n = 381$	COVID-19$^+$ $n = 290$	COVID-19$^-$ $n = 91$	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 (NS = 2)				
Symptoms frequently observed[7,9-11]				
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]				
Mean [range]	7·3 [3–30]	7·7 [3–30]	6·2 [3–30]	
Median	7	7	3	
Becoming				
Non-hospitalized	42 (11%)	19 (7%)	23 (25%)	
Pre-COVID unit[b]	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 status[c] (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, 10^9/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	
<1·10^9/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.

[a]Time interval since the onset of the first symptom.

[b]Temporary unit in expectation of RT-PCR results (<24 h).

[c]Median time interval for COVID-19 status includes the completion time of RT-PCR, chest CT and CBC (complete blood count).

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-19[14,15] 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

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 dichotomization 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.

Funding information

None reported.

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.

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