Letter to the Editor

James V. Harte and Vitaliy Mykytiv*

A panhaemocytometric approach to COVID-19: a retrospective study on the importance of monocyte and neutrophil population data on Sysmex XN-series analysers

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To the Editor,

We read with interest the recent publication by Martens et al. [1] on the haemocytometric characteristics of patients with COVID-19. Martens et al. [1] reported strong associations between haematological cell population data (CPD) and SARS-CoV-2 infection, and herein we aim to support their findings and emphasize the need for a panhaemocytometric approach to monitoring COVID-19.

The haematology laboratory plays an important role in the prognostication of patients with COVID-19, but the existing literature predominately focuses on alternations in the lymphocyte population. Lymphocytopenia is a prominent feature of COVID-19 and meta-analysis has shown that a steady decline in the absolute lymphocyte count is associated with disease severity and mortality [2]. However, multi-lineage, morphological changes in circulating blood cells have been documented in patients with COVID-19 [3]. In addition, a recent study by Fahlberg et al. [4] highlighted that infiltration of the lungs by monocytes and neutrophils is a significant contributor to severe COVID-19 pathology, and that pulmonary accumulations could persist for up to four-weeks post-infection. Fahlberg et al. [4] postulate that enduring myelomonocytic infiltrates may partly explain the slow recovery from

COVID-19 seen in humans. This raises the question as to whether changes in monocyte or neutrophil populations precede severe pathophysiological outcomes which could enable clinicians to target patients at risk of clinical deterioration.

Technological innovations have allowed haematology analysers to generate quantitative CPD on the morphological and functional characteristics of circulating blood cells. As described by Martens et al. [1], the XN-series analysers (Sysmex, Kobe, Japan) can differentiate leukocytes according to their light scatter and fluorescence properties, providing information relating to intracellular complexity (SSC), nucleic acid content (SFL), and cellular size (FSC). In patients with COVID-19, Martens et al. [1] observed an increase in reactive, antibody-synthesizing, and high-fluorescence lymphocytes consistent with the lymphocyte-centric approach to COVID-19 monitoring in the literature [5]. However, Martens et al. [1] also noted significant changes in the haemocytometrics of all circulating blood cells in patients with COVID-19 who developed cytokine-storm syndrome, paying particular attention to the increased proportion of activated monocytes. This is clear evidence for a panhaemocytometric approach to COVID-19, as SARS-CoV-2 appears to affect the entire haematological system [6].

We conducted a retrospective study into the role of CPD at Cork University Hospital where the haemocytometrics of patients tested for COVID-19 were extracted from the laboratory information system. A total of 1,012 patients with haemocytometric data were tested for COVID-19 between 11th March and 28th May 2020; children under the age of 18 years (COVID-19 positive: 1; COVID-19 negative: 104) and patients with abnormal results suggestive of a haematological disorder (COVID-19 positive: 1; COVID-19 negative: 33) were subsequently excluded. As such, the final retrospective dataset consisted of 873 patients.

81 patients, with a mean age of 63 years (range: 22–90 years; 63.0% male), were diagnosed with COVID-19 by a

*Corresponding author: Dr. Vitaliy Mykytiv, Haematology Department, Cork University Hospital, Cork, Ireland, E-mail: Vitaliy.Mykytiv@hse.ie. <https://orcid.org/0000-0002-9937-4306>

James V. Harte, Haematology Department, Cork University Hospital, Cork, Ireland. <https://orcid.org/0000-0002-3817-3658>

positive real-time reverse-transcription polymerase chain reaction (rRT-PCR) for SARS-CoV-2; 792 patients, with a mean age of 62 years (range: 18–98 years; 52.0% male), tested negative during the same time period. Concurrent complete blood counts were performed on all patients tested for COVID-19 at initial presentation using XN10/XN20 modules (Sysmex, Kobe, Japan); the modules were routinely monitored and maintained according to the manufacturer's specifications.

Analysis of the dataset by the Shapiro–Wilk test revealed non-normally distributed values; accordingly, continuous variables were expressed as medians and interquartile ranges, and the statistical difference evaluated by the non-parametric Mann–Whitney U test. The

diagnostic utility of haemocytometric parameters was assessed by the area under receiver operating characteristic curves. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with GraphPad Prism 8 (version 9.00; GraphPad Software, San Diego, CA, USA).

In our cohort, patients with COVID-19 presented with distinct haemocytometric changes (Table 1), including the aforementioned lymphocytopenia with significant increases in lymphocyte complexity (LY-SSC; $p < 0.001$) and size (LY-FSC; $p < 0.001$). Eosinopenia and basopenia were significantly associated with COVID-19, as previously highlighted in the literature [7]. We also observed considerable alterations in the monocyte compartment of patients

Table 1: Median and interquartile range data for haemocytometric and cell population data in COVID-19-positive and COVID-19-negative.

Characteristics	COVID-19 positive (n=81)	COVID-19 negative (n=792)	p-Value
Complete blood count			
Leukocytes, $\times 10^9/L$	6.96 (5.59, 9.98)	8.43 (6.62, 11.33)	0.001
Neutrophils, $\times 10^9/L$	5.18 (3.73, 8.19)	5.94 (4.23, 8.86)	0.120
Lymphocytes, $\times 10^9/L$	0.87 (0.61, 1.41)	1.28 (0.81, 1.89)	<0.001
Monocytes, $\times 10^9/L$	0.53 (0.42, 0.75)	0.65 (0.48, 0.84)	0.010
Eosinophils, $\times 10^9/L$	0.01 (0.0, 0.08)	0.08 (0.03, 0.19)	<0.001
Basophils, $\times 10^9/L$	0.02 (0.01, 0.04)	0.04 (0.03, 0.06)	<0.001
IG, $\times 10^9/L$	0.04 (0.02, 0.09)	0.04 (0.02, 0.07)	0.324
IG, %	0.50 (0.40, 0.95)	0.40 (0.30, 0.70)	0.002
Platelets, $\times 10^9/L$	213 (166, 270)	238 (180, 285)	0.201
Cell ratios			
Neutrophil–lymphocyte	5.55 (3.58, 10.0)	4.52 (2.58, 8.68)	0.010
Lymphocyte–monocyte	1.62 (1.04, 2.36)	2.09 (1.22, 3.18)	0.004
Platelet–lymphocyte	252 (149, 357)	174 (115, 266)	<0.001
Cell population data			
NE-SSC, ch	155.8 (152.5, 158.1)	154.8 (151.9, 157.6)	0.058
NE-SFL, ch	51.3 (48.7, 53.8)	49.5 (47.7, 51.7)	<0.001
NE-FSC, ch	92.0 (89.7, 95.5)	92.7 (89.7, 95.5)	0.688
NE-SSC, width, ch	307.0 (298.5, 318.0)	308.0 (298.0, 319.0)	0.762
NE-SFL, width, ch	602.0 (571.5, 640.0)	615.0 (587.3, 648.0)	0.021
NE-FSC, width, ch	580.0 (536.0, 628.0)	584.5 (543.0, 637.0)	0.600
LY-SSC, ch	85.3 (83.6, 86.5)	84.2 (82.6, 85.5)	<0.001
LY-SFL, ch	75.0 (72.5, 77.8)	74.5 (71.5, 77.7)	0.185
LY-FSC, ch	62.7 (61.5, 64.1)	61.5 (60.3, 62.8)	<0.001
LY-SSC, width, ch	494.0 (459.0, 542.5)	476.0 (437.5, 521.0)	0.007
LY-SFL, width, ch	832.0 (754.5, 898.5)	880.0 (810.3, 953.0)	<0.001
LY-FSC, width, ch	499.0 (386.5, 546.0)	460.5 (389.3, 524.0)	0.167
HFLC, $\times 10^9/L$	0.02 (0.01, 0.03)	0.01 (0.00, 0.01)	<0.001
HFLC, % leukocytes	0.30 (0.10, 0.50)	0.00 (0.00, 0.10)	<0.001
MO-SSC, ch	126.6 (124.8, 128.8)	123.8 (122.2, 125.8)	<0.001
MO-SFL, ch	121.7 (114.0, 127.6)	117.5 (112.5, 122.6)	0.001
MO-FSC, ch	67.9 (64.7, 69.9)	68.3 (65.9, 70.3)	0.036
MO-SSC, width, ch	239.0 (220.0, 265.0)	251.0 (234.0, 277.0)	0.002
MO-SFL, width, ch	702.0 (649.0, 766.5)	689.0 (630.0, 747.8)	0.120
MO-FSC, width, ch	569.0 (522.0, 643.0)	552.0 (499.0, 612.0)	0.014

Data presented as median (interquartile range). IG, immature granulocytes; NE, neutrophils; SSC, side scatter; SFL, side fluorescence; FSC, forward scatter; LY, lymphocytes; HFLC, high-fluorescence lymphocytes, as a percentage of total leukocytes; MO, monocytes.

with COVID-19. The internal complexity of monocytes was shown to be significantly increased (MO-SSC; $p < 0.001$), with a reduction in the corresponding distribution width (MO-SSC, width; $p = 0.002$) and an increase in fluorescence intensity (MO-SFL; $p = 0.001$) that reflects monocyte reactivity and potentially a left-shift towards immaturity. Zhang et al. [8] previously reported a population of pro-inflammatory monocytes of unusual morphology in COVID-19, which were profoundly vacuolated and showed macrophage markers not typically seen under physiological conditions. This distinct monocyte population was exaggerated in patients requiring prolonged hospitalisation and intensive care [8], and our data suggests that these changes are readily detectable by routine haematology analysers at initial haemocytometric evaluation.

To further the diagnostic utility of monocyte CPD, the area under the curve of MO-SSC was calculated to be 0.76 (95% confidence interval: 0.71–0.81). With the exception of the absolute eosinophil count (AUC: 0.71; 95% confidence interval: 0.65, 0.77), the absolute basophil count (AUC: 0.72; 95% confidence interval: 0.66, 0.78) and percentage of high-fluorescence lymphocytes (AUC: 0.76; 95% confidence interval: 0.68, 0.82), no haemocytometric parameter approached the discriminatory power of MO-SSC.

Moreover, we wish to highlight a subtle but significant increase in neutrophil fluorescence intensity (NE-SFL; $p < 0.001$) in patients with COVID-19, indicative of increased reactivity or immaturity. Martens et al. [1] reported a similarly increased neutrophil fluorescence in COVID-19, particularly in patients with superimposed cytokine-storm syndrome. Longitudinal immune profiling of patients has demonstrated profound neutrophilia to be characteristic of late-stage severe disease [9] and these findings suggest that changes in the intracellular milieu of neutrophils may be early predictors of COVID-19 pathoprotection if serially monitored.

Collectively, these results support a transition towards a panhaemocytometric approach to COVID-19 monitoring; lymphopenia, eosinopenia and basopenia are observed at initial presentation; monocyte and lymphocyte CPD parameters appear to have similar discriminatory capabilities; and temporal changes in neutrophil CPD parameters may predict disease trajectory.

One of the strengths of this retrospective study is the large control population, which included all patients negative for COVID-19 at Cork University Hospital. However, there are limitations. First, this was a monocentric study of a small cohort of COVID-19 positive patients, with positivity rates in Ireland falling dramatically between June and August. Second, no clinical details were available for patients with and without COVID-19; as such, bias

cannot be fully excluded due to the lack of medical data, particularly on whether patients with low pre-test probabilities for COVID-19 were routinely tested.

In conclusion, COVID-19 is associated with morphological and functional alterations in blood cells from the early phases of disease, which are readily detectable by routine haematology analysers. Our findings support the data of Martens et al. [1], and we argue the usefulness of a panhaemocytometric approach to COVID-19. Further studies, including longitudinal profiling and multicentre validations, are now required to better understand the potential value of CPD – particularly monocyte and neutrophil data – in COVID-19.

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References

1. Martens RJ, Van Adrichem AJ, Mattheij NJ, Brouwer CG, Van Twist DJL, Broerse JJ, et al. Hemocytometric characteristics of COVID-19 patients with and without cytokine Storm syndrome on the Sysmex XN-10 hematology analyzer. *Clin Chem Lab Med* 2021; 59:783–93.
2. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. *J Intensive Care* 2020;8:36.
3. Lüke F, Orsó E, Kirsten J, Poeck H, Grube M, Wolff D, et al. Coronavirus disease 2019 induces multi-lineage, morphologic changes in peripheral blood cells. *EJH* 2020;1:376–83.
4. Fahlberg MD, Blair RV, Doyle-Meyers LA, Midkiff CC, Zenere G, Russell-Lodrigue KE, et al. Cellular events of acute, resolving or progressive COVID-19 in SARS-CoV-2 infected non-human primates. *Nat Commun* 2020;11:1–14.

5. Wang Z, He Y, Shu H, Wang P, Xing H, Zeng X, et al. High-fluorescent lymphocytes are increased in patients with COVID-19. *Br J Haematol* 2020;190:e76–8.
6. Linssen J, Ermens A, Berrevoets M, Seghezzi M, Previtali G, van der Sar-van der Brugge S, et al. A novel haemocytometric COVID -19 prognostic score developed and validated in an observational multicentre european hospital-based study. *Elife* 2020;9:1–37.
7. Rodriguez L, Pekkarinen PT, Lakshmikanth T, Tan Z, Consiglio CR, Pou C, et al. Systems-level immunomonitoring from acute to recovery phase of severe COVID-19. *Cell Rep Med* 2020;1. <https://doi.org/10.1016/j.xcrm.2020.100078>.
8. Zhang D, Guo R, Lei L, Liu H, Wang Y, Wang Y, et al. COVID-19 infection induces readily detectable morphologic and inflammation-related phenotypic changes in peripheral blood monocytes. *J Leukoc Biol* 2021;109:13–22.
9. Mann ER, Menon M, Knight SB, Konkel JE, Jagger C, Shaw TN, et al. Longitudinal immune profiling reveals key myeloid signatures associated with COVID-19. *Sci Immunol* 2020;5. <https://doi.org/10.1126/SCIIMMUNOL.ABD6197>.