Review

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Deciphering the role of monocyte and monocyte distribution width (MDW) in COVID-19: an updated systematic review and meta-analysis

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Abstract: The SARS-CoV-2 infection is characterized by both systemic and organ hyper-thromboinflammation, with a clinical course ranging from mild up-to critical systemic dysfunction and death. In patients with coronavirus disease 2019 (COVID-19) the monocyte/macrophage population is deeply involved as both trigger and target, assuming the value of useful diagnostic/prognostic marker of innate cellular immunity. Several studies correlated morphological and immunophenotypic alterations of circulating monocytes with clinical outcomes in COVID-19 patients, concluding that monocyte distribution width (MDW) may retain clinical value in stratifying the risk of disease worsening. Through an electronic search in Medline and Scopus we performed an updated literature review and meta-analysis aimed to explore the association between increased MDW levels and illness severity in COVID-19 patients, deciphering role(s) and function(s) of monocytes in the harmful network underlining SARS-CoV-2 infection. We found that significantly elevated MDW values were frequently present in COVID-19 patients who developed unfavorable clinical outcomes, compounded by a significant association between monocyte anisocytosis and SARS-CoV-2 outcomes. These findings suggest that blood MDW index and its scatter plot could represent useful routine laboratory tools for early identification of patients at higher risk of unfavorable COVID-19 and for monitoring the progression of viral infection, clinical outcomes, and therapeutic efficacy throughout hospitalization. According to this evidence, therapeutic decisions in patients with SARS-CoV-2 infection could benefit from monitoring MDW value, with administration of drugs limiting thrombo-inflammation due to monocyte hyper-activation in patients with severe/critical COVID-19 disease.

Keywords: COVID-19; histone; meta-analysis; monocyte; monocyte distribution width; NETosis; SARS-CoV-2; systematic review; thrombo-inflammation.

Daniela Ligi and Bruna Lo Sasso contributed equally to this work and share first authorship. Marcello Ciaccio, Giuseppe Lippi, Mario Plebani and Ferdinando Mannello share senior authorship.

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Introduction

Coronavirus disease 2019 (COVID-19) is the worldwide infectious viral disease etio-pathogenetically caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which mainly generates respiratory involvement, but may dramatically evolve up-to multi-organ failures (MOF) and death in some cases [1–4]. A plethora of studies has focused on the clinical manifestations of COVID-19 involving circulating blood cells characteristics and innate immunity, thus revealing a broad complexity of hematology findings in COVID-19. Interestingly, these studies shed light also

on the missing pieces of the intricate puzzle of COVID-19, providing information on landscape of involvement of both hematological and immunological cells (reviewed in [5, 6]).

Several studies focused on both morphological alterations and functional modifications of circulating monocytes, suggesting a crucial role and involvement of these important blood cells as both disease trigger and therapeutic target in progressive inflammation during COVID-19 infection [5, 7–10].

In keeping with prominent and promising laboratory features in both classic and viral sepsis, several recent studies on the diagnostic and prognostic value of routine hemocytometry markers revealed and underlined the clinical usefulness of monocyte distribution width (MDW) in COVID-19 [5, 11], emphasizing the role of this measure for distinguishing and stratifying the risk of developing critical illness and/or dying [12–22]. To this end, the innovative hematological parameter MDW, linked to heterogeneity of monocyte volume, depends on massive inflammatory activation, and has recently emerged as predictive factor of multiorgan dysfunction and increased risk of death in several physio-pathological conditions [11, 23–29]. MDW is both a Food and Drug Administration (FDA) and European Community (EC)-approved marker of early sepsis [24, 30], though recent evidence has emerged that its assessment may retain prognostic significance in COVID-19, as novel "viral sepsis" biomarker [13–16, 18, 20–22, 31, 32].

According to standard hematological procedures using the exclusive software of the UniCell DxH900 Hematology Analyzer (Beckman Coulter), MDW, which can be generated alongside differential counts of circulating leukocyte populations, is an innovative parameter mathematically based on the measure of specific cell volume index and standard deviation of volume distribution within the monocyte population, according to previously reported details [33, 34]. Noteworthy, MDW measures positional parameters with "VCS technology" (i.e., Volume, Conductivity, and Scatter), by means of three simultaneous and sindependent energy sources: direct current impedance to measure cell volume of all cell types; radio frequency opacity, to characterize conductivity for internal composition of each cell; a laser beam to measure light scatter for cytoplasmic granularity and nuclear structure. These data provide accurate and innovative information on volume, conductivity, and scatter parameters, thus allowing to detect morphologic changes in reactive/activated monocyte cells. This parameter improves and even outperforms the attainment of crucial details through classic optical microscopic evaluation of peripheral blood smears, providing also additional information on heterogeneity and modifications of monocyte volume upon massive inflammatory cascade.

We describe here the results of an updated systematic review and meta-analysis aimed to provide a pooled analysis of studies that have addressed the potential clinical utility of MDW in routine laboratory medicine, exploring the possible association between increased MDW levels, disease severity, and mortality in COVID-19 patients, including a comparison of MDW values between COVID-19 and non-COVID-19 patients.

Search strategies and selection criteria

We carried out an electronic search in Medline (PubMed interface) and Scopus, using the keywords "COVID-19" OR "SARS-CoV-2" AND "monocyte" OR "monocyte distribution width" OR "MDW", between 2019 and present time (i.e., latest search date: September 19, 2022), restricted to articles published in English. The reference list of all articles was also reviewed for identifying other potentially eligible studies, according to the protocol based on the transparent reporting of systematic reviews and meta-analysis (PRISMA).

The reference list of the documents included in our analysis was also scrutinized with forward and backward citation tracking, to detect other potentially eligible studies. All resulting items were screened (title, abstract and full text, when available or necessary) by three authors (DL, BLS and FM), to capture observational, cross-sectional, or prospective studies reporting data on MDW values at admission (or at the earliest time point during hospitalization) in COVID-19 patients with or without severe disease, as well as in non-survivors vs. survivors. Severe disease was clinically defined as patients needing intensive care unit (ICU) admission, mechanical (forced) ventilation, COVID-19 related hospitalization, pneumonia, or onset of critical symptoms and/or shock and/or presence of organ failure. All studies fulfilling these criteria were then included in a systematic literature review, also providing data for an updated meta-analysis. Disagreements between authors with respect to study eligibility were resolved by discussion and consensus among authors. Results of the review were organized into summary of finding tables. A pooled analysis was then performed, with estimation of weighted mean difference and 95% confidence interval (95% CI) of MDW values in subjects with or without SARS-CoV-2 infection. A random-effect model was used to adjust for potential heterogeneity emerging for the use of different threshold values and sampling time across

studies. Heterogeneity was assessed using chi-square test and I^2 statistics. The pooled analyses were performed with MetaXL, software version 5.3 (EpiGear Intl Pty Ltd). The software MetaXL calculates the pooled analyses by using mean, SD, and samples size of populations. For research studies providing median values, and interquartile ranges, the mean and SD have been calculated according to previously published methods [35].

All statistical tests were performed with GraphPad Prism 9.0. Values are expressed as mean ± standard deviations (SD). Unless otherwise specified, significant differences between groups were determined using one-way ANOVA followed by post-hoc test (i.e., Tukey's multiple comparison test, with a single pooled variance). p values <0.05 were considered significant.

Results

Study identification and characteristics

A total number of 424 studies were initially identified by our search criteria, 222 of which were excluded for duplication among the two databases, whilst 189 were also excluded because they failed to report usable MDW values. Thirteen studies, totaling 5,991 subjects/patients, were finally included in our systematic review and meta-analysis [13, 15–20, 22, 27, 32, 36–38]. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram is shown in Figure 1.

The selected studies were characterized as observational studies, prospective observational studies, or cross-sectional consecutive studies. All COVID-19 patients were hospitalized, and the diagnosis was confirmed by FDA-approved RNA testing (i.e., RT-PCR assays using nasopharyngeal swab, pharyngeal swab, bronchoalveolar lavage), and clinically confirmed by chest X-ray and/or CT-scan, according to guidelines and severity classification of the World Health Organization (WHO). Several studies also included non-COVID hospitalized patients, but all patients were compared to healthy, or COVID-19 negative control subjects analyzed in a routine diagnostic setting.

All MDW measurements were conducted using the *UniCell DxH900* or with *UniCell DxH800* [22, 32] hematology analyzers, on whole blood venous samples collected on K_2EDTA (where specified) and analyzed according to routine methods for cell blood count and determination of positional parameters, including MDW. All reported blood MDW values were measured upon hospital admission or at the earliest time point immediately after hospitalization. The present overview was exempted from Ethical Committee approval as not locally required for meta-analyses and did not receive any funding.

Narratively, among the plethora of studies focused on morphological anomalies of circulating blood cells in COVID-19 patients, some investigations using optical and electron microscopy and flow cytometry approaches clearly demonstrated that striking numeric and morphological white blood cell changes could be observed in COVID-19 patients. These reports highlighted pronounced multilineage aberrations in monocytes, mainly due to abnormal vacuolization and coalescing vacuoles, which were significantly associated with disease severity progression but were progressively lost upon remission [39–42].

A dysregulated host innate immune response, mainly due to altered balance of monocyte/macrophage activation amplified by specific cytokine pattern, is well known and established process during COVID-19(43). In particular, the presence in all phases of SARS-CoV-2 infection of both monocyte and macrophage populations (main players in the innate immunity, triggering systemic inflammation, procoagulant syndrome, and cytokine release syndrome) can act as a double-edged sword, ameliorating or exacerbating the viral infection, fostering multiorgan failure (MOF) and influencing severity and outcome of SARS-CoV-2 infection [5]. Accordingly, it has been widely demonstrated that increased levels of inflammatory monocytes (i.e., $CD14^+$ $CD16^+$ $IL\text{-}6^+$ $GM\text{-}CSF^+$ monocytes) lead to deleterious clinical manifestations and even acute mortality in COVID-19 infection through a pathogenic mechanism involving Th1 lymphocytes and inflammatory monocytes expressing IL-6 [9]. Notably, biomolecular pathways contributing to hyperactivation of monocyte-derived macrophages and hyperinflammation-hypercoagulation in COVID-19 have been described [5, 8].

All these studies paved the way for accurate analysis of volume, cytoplasmic inclusions, surface molecules-cluster of differentiation, and inflammation-related phenotypic changes of monocytes, early suggesting that MDW may be a possible additional biomarker for predicting disease severity and clinical course of COVID-19 infection [6, 7, 12].

The innovative role of MDW seems mostly linked to the heterogeneity of monocyte volume and inherently associated with a massive inflammatory reaction, thus becoming an attractive biomarker for predicting multi-organ dysfunction and increased mortality rate in several physio-pathological conditions, including Sepsis [11, 24, 30] and COVID-19 infections [14, 18].

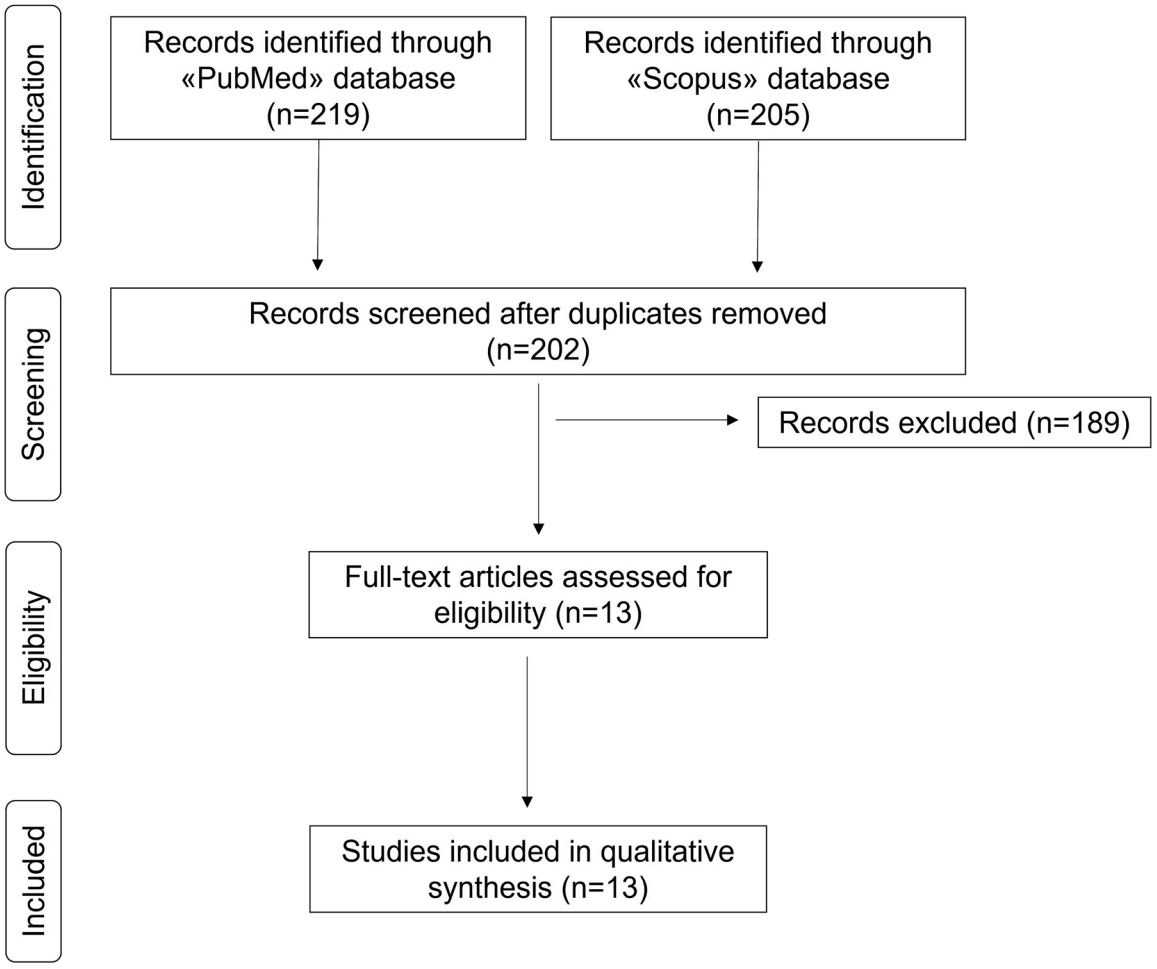

Figure 1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) diagram.

According to the PRISMA diagram (Figure 1), a total of thirteen articles were selected for our pooled analyses, totaling 5,991 subjects, 4,131 of whom were patients with SARS-CoV-2 infection (68.9%) [13, 15–20, 22, 27, 32, 36–38].

For better evaluation and comprehension of data complexity in the interplay between MDW and COVID-19, and to provide updated/improved meta-analysis, we divided the selected studies based on reported MDW values in patients with and without SARS-CoV-2 infection (n=5) [16, 17, 22, 27, 38] and MDW levels measured only in patients with COVID-19, without direct comparison with healthy or COVID-19 negative control subjects (n=8) [13, 15, 18–20, 32, 36, 37]. Finally, we also analyzed studies comparing MDW values between mild and severe clinical conditions or survivors vs. non-survivors (n=7) [13, 15, 16, 19, 20, 36, 37]. In these studies, the cut-off was set at a similar threshold, ranging from ≥20 [16] to ≥21 [19], even if some studies proposed a stratifying COVID-19 working model with "low, intermediate and high MDW values" (<20, 20–26.4 and >26.4, respectively) [14], and other described values >22 or >24 as strongly associated with unfavorable outcome in COVID-19 (i.e., non-survivor patients) [18, 32].

Table 1: MDW values in patients with and without SARS-CoV-2 infection.

Authors	Controls[a], n	COVID-19, n	MDW values (mean ± SD) COVID-19 (−) vs. COVID-19 (+)	p-Value
Ognibene et al. [16]	106	41	20.3 ± 3.3 vs. 27.3 ± 4.9	<0.005
Lin et al. [17]	141	9	21.8 ± 5.4 vs. 23.5 ± 2.1	0.096
Zeng et al. [22]	62	93	18.9 ± 2.0 vs. 22.1 ± 2.3	<0.0001
Piva et al. [27]	1,540	243	22.1 ± 3.3 vs. 26.2 ± 4.3	<0.001
Cusinato et al. [38]	11	15	16.0 ± 1.2 vs. 23.0 ± 4.3	<0.001

[a]Controls include both healthy subjects [38] and patients admitted to ED/ICU for other medical conditions, but negative for SARS-CoV2 infection [16, 17, 22, 27].

MDW index between patients with and without SARS-CoV-2 infection and monocyte morphology

As summarized in Table 1, MDW values in COVID-19 positive patients were always higher than those in control subjects (i.e., COVID-19 negative), demonstrating that the circulating monocyte population had a significantly changed heterogeneity of monocyte volume, conductivity, and scatter parameters in patients with SARS-CoV-2 infection.

The significantly increased values of MDW observed in COVID-19 patients by a routine hematological analyzer are directly correlated to the morphological changes observed by light microscopy in monocyte cells through May-Grunwald-Giemsa-stained blood smears, as depicted in Figure 2.

The morphological alterations of circulating monocytes observed by light microscopy in COVID-19 patients were also confirmed in studies performed through electron microscopy, demonstrating ultrastructural equivalent changes, larger monocytes, aberrant nuclei with clumped chromatin, prominent and diffuse coalescing vacuolization, which displayed a more atypical profile correlated with the disease outcome (e.g., ICU and non-survivors patients) [39–42]. Interestingly, despite monocytes/macrophages play a significant role in sustaining a hyperinflammatory response in SARS-CoV-2 infection [8, 9, 43], literature data suggested that not all COVID-19 patients display significant monocyte morphological changes [44]. These discrepancies may be explained by either the presence of different monocyte subsets with different functional characteristics in mild and severe disease states (e.g., higher percentage of nonclassical $CD14^+$ $CD16^+$ inflammatory monocytes enriched in peripheral blood of more severe COVID-19 patients) [9, 38, 42], or different amounts of peculiar monocyte inflammatory triggers in patients with severe COVID-19 disease (e.g., proinflammatory cytokines, histones, etc.) [45, 46].

Although the data characterizing the innate immune system and inflammatory/thrombotic status in patients with COVID-19 are emerging and *in itinere*, it is clear that hyperinflammation (the so called "cytokine storm"), coagulopathy and monocyte/macrophage represent an intricate network of pathways converging to foster disease severity and increasing the risk of death in patients with SARS-CoV-2 infection. A better understanding of laboratory biomarkers (including MDW) which may be helpful for improving risk stratification in COVID-19 patients (especially the risk of developing hyperinflammation/hypercoagulation) appears crucial for identifying and better characterizing the biomolecular basis of this disease, as well as for developing rationale-based clinical therapeutic strategies.

MDW values in SARS-CoV-2 positive patients with non-severe and severe illness/death

The analysis of literature data about MDW values in COVID-19 patients is summarized in Table 2, highlighting the difference in terms of clinical severity (mild vs. severe, 343 vs. 311, or survivors vs. non-survivors, 103 vs. 24, respectively).

A high significant difference was found in all studies in terms of clinical severity, thus suggesting a possible

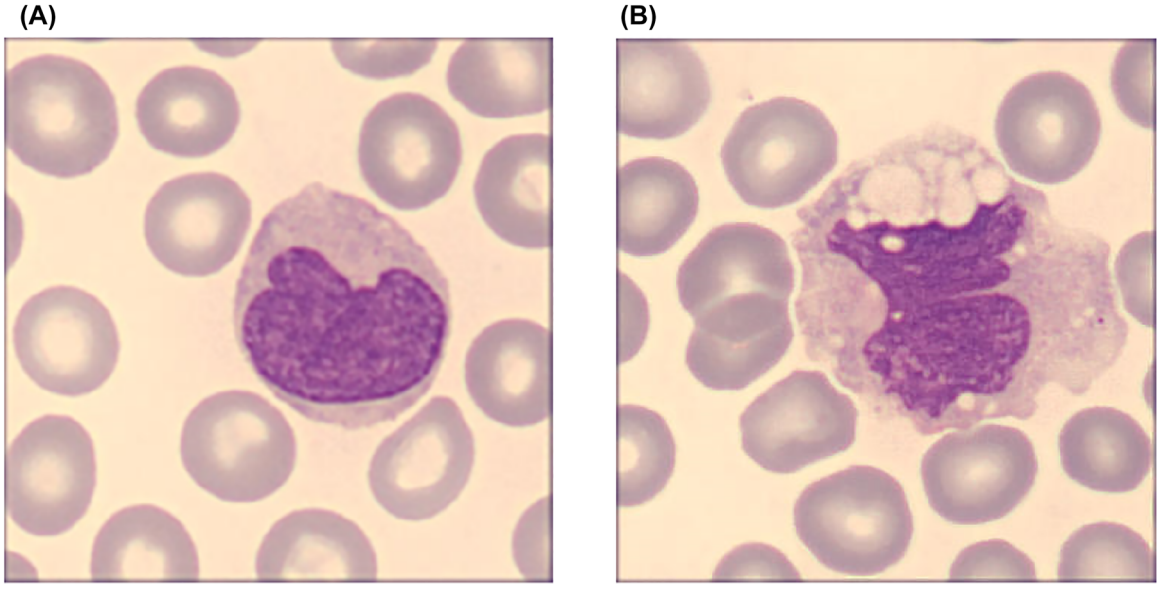

Figure 2: Light microscopy images of peripheral blood WBC representative of COVID-19 negative (A) and positive patients (B). Normal monocytes show only occasional and very small cytoplasmic vacuoles, compared to the atypical monocytes with large coalescing cytoplasmic vacuoles typically found in SARS-CoV-2 patients. (May-Grunwald-Giemsa, ×100) (personal unpublished observations).

Table 2: MDW values in COVID-19 patients with and without severe illness or mortality.

Authors	Study	COVID-19 positive (n; mean ± SD)		p-Value
		Mild vs. severe	Survivors vs. non-survivors	
Ognibene et al. [16]	Observational, prospective, single-center	n: 18 vs. 23 25.4 ± 3.6 vs. 28.8 ± 5.3		<0.005
Riva et al. [13]	Retrospective, observational, single-center		n: 71 vs. 16 20.5 ± 4.0 vs. 26.8 ± 4.8	<0.001
Stratan et al. [36]	Retrospective, single-center	n: 41 vs. 65 20.7 ± 2.9 vs. 24.5 ± 3.4		<0.05
Lorubbio et al. [15]	Retrospective, single-center		n: 32 vs. 8 26.2 ± 3.4 vs. 24.8 ± 3.0	<0.005
Lin et al. [19]	Retrospective, single-center	n: 72 vs. 48 23.5 ± 4.4 vs. 25.2 ± 4.6		0.0177
Hossain et al. [20]	Retrospective, single-center	n: 156 vs. 162 23.3 ± 3.7 vs. 25.7 ± 3.7		<0.0001
Kim et al. [37]	Retrospective, single-center	n: 56 vs. 13 21.6 ± 3.1 vs. 25.8 ± 3.9		<0.001

Of note, the data included from Kim et al. [37] are referred to patients with mechanical ventilation or high-flow nasal cannula oxygen therapy for the severe group and no oxygen therapy for the mild group, due to the absence of further details to classify the disease severity; the data included from Lin et al. [19] are referred to patients with a length of stay >14 days for the severe group and a LOS ≤14 days for the mild group, due to the absence of MDW details according to the disease severity as categorized into mild, moderate, severe and critical.

association between monocyte anisocytosis and biomolecular inflammatory activation, especially in those patients with metabolic, inflammatory, proteolytic, and thrombotic processes capable to trigger severe multiorgan pathologies and death [13, 15, 16, 19, 20, 36, 37].

In particular, Riva et al. [13] and Lorubbio et al. [15] classified COVID-19 patients according to the disease outcome, in survivors and non survivors; however, Riva et al. did not provide other information on the disease class in each group, whereas, Lorubbio et al. collected data from severe COVID-19 patients hospitalized for >4 days, and detailed that the 8 non survivors required high-flow nasal cannula O_2 therapy, and among the survivors 22 required no-invasive ventilation O_2 therapy, and 10 required high-flow nasal cannula O_2 therapy.

On the other hand, Stratan et al. [36] classified COVID-19 patients in moderate-severe disease (severe group) vs. mild disease/no pulmonary lesions (mild group); Ognibene et al. [16] classified COVID-19 patients in few symptomatic (mild group) and patients requiring hospitalization in high-intensity care unit (severe group); Kim et al. [37] referred to patients needing for mechanical ventilation or high-flow nasal cannula oxygen therapy (severe group) vs. patients no needing for oxygen therapy (mild group); Hossain et al. [20] referred to patients with or without respiratory failure (severe and mild group, respectively); Lin et al. [19] classified COVID-19 positive patients according to the length of stay >14 days (severe group) and a LOS ≤14 days (mild group).

Although the studies did not report clear indications on diagnostic cut-offs, the weighted mean value of MDW in COVID-19 patients with mild condition was 22.9 ± 3.6 (n=343), significantly lower than that in patients with severe COVID-19 (25.6 ± 3.9, n=311). The paucity of data describing MDW levels between survivors and non-survivors did not allow to make definitive conclusions, even with strong indication for a significant difference between the weighted mean MDW levels in survivors vs. non-survivors COVID-19 patients (22.3 ± 3.8 vs. 26.1 ± 4.2, n=127). Up-to now, only a recent study described graphical data on monocyte heterogeneity [47] and only a few reports provided images of altered/atypical monocytes circulating in blood in these COVID-19 patients [39–41]. Moreover, some studies described a significant relationship between MDW and inflammatory biomarkers (like C-reactive protein, ferritin, etc.) [13, 17, 19, 36, 37], but only few performed a comprehensive analysis of correlation among MDW and other laboratory biomarkers (full blood cell count, inflammation and coagulation biomarkers, and serum enzymes) [18, 36].

Actually, no data are available on possible MDW differences due to sex or age in COVID-19 patients, thus highlighting the need of designing additional studies aimed at evaluating if monocyte and MDW could be a novel part of gender medicine (e.g., sex-dependent number or histone sensitivity) or whether patient's age (e.g., young or old monocytes) may justify/explain the different sensitivity to biomolecular triggers (e.g., as in COVID-19 viral sepsis and classical bacterial/

fungal sepsis). Nonetheless, all these data support and emphasize the importance of MDW not only as additional routine hematological parameter and innovative prognostic biomarker in COVID-19, but also as promising test for monitoring clinical progression in patients with viral sepsis.

Meta-analyses of MDW values in COVID-19 patients

Among the 13 articles initially detected with our search criteria, 8 ought to be excluded because they did not provide usable MDW values in patients with and without COVID-19. A total of five studies, with 2.261 subjects (401 with COVID-19, 17.7%, range 2.2–60.6%) were finally included in our pooled analysis [16, 17, 22, 27, 38] (Table 3).

These studies underlined wide heterogeneity of subject characteristics, since they were comparing COVID-19 vs. upper respiratory tract infection [17], pauci-symptomatic vs. symptomatic patients [16], COVID-19 vs. sepsis and no-sepsis [27], and negative vs. positive COVID-19 patients [22].

Besides, COVID-19 patients were differently classified in each study. In particular, Ognibene et al. [16] classified patients in high-intensity care unit hospitalized vs. few symptomatic patients; Piva et al. [27] classified patients according to the presence or absence of sepsis of different origin (including SARS-CoV-2 infection), presenting to the ICU, and hospitalized for at least 24 h; Zeng et al. [22] collected data from COVID-19 hospitalized patients, with fever, dry cough, shortness of breath, fatigue, myalgias, and diarrhea as main symptoms; Cusinato et al. [38] recruited adult patients admitted to the emergency department with SARS-CoV-2 infection and healthy volunteers as controls; Lin et al. [17], enrolled consecutive patients admitted to the ED with fever, respiratory symptoms, or travel history and with suspected COVID-19.

Although in all five studies the cut-off was similar, our updated analysis revealed that MDW values were significantly higher in patients with COVID-19, confirming and improving a previous similar analysis [21]. As reported in the updated forest plot, we found consistent heterogeneity (as demonstrated by Higgins heterogeneity index I^2=88) with a Q Cochran test of 32.05; the analysis revealed also that MDW was 15.6% higher in COVID-19 patients than in those without infection (WMD 4.42, 95% CI, 3.05–5.78) (Figure 3).

Although the total number of papers available so far was low and lacked uniformity, we still performed a new pooled analysis of MDW in the subgroups of COVID-19 patients, comparing mild plus survivors vs. severe/critical plus non-survivors conditions; a total of 7 studies provided detailed information, with 781 COVID-19 patients, 446 with mild + survivors profile and 335 with severe/critical (n=311) + non-survivors conditions (n=24) (92.8 vs. 7.2%, respectively) [13, 15, 16, 19, 36, 37] (Table 4).

In all seven studies MDW values were higher in patients with severe/critical SARS-CoV-2 infection than in those with mild infection, as shown in Table 4. Despite relevant heterogeneity (as demonstrated by Higgins index of I^2=77, and a Q Cochran test of 25.99), and although the weighted MDW values displayed wide variability (ranging from –1.4 to 6.3), MDW in severe COVID-19 was 12.8% higher than in patients with mild infection (overall WMD 2.87, 95% CI, 1.53–4.20) (Figure 4).

We also performed a comparison of MDW values among the subgroups of COVID-19 positive patients, categorized as mild + survivors and severe/critical + nonsurvivors. As shown in Figure 5, we highlighted a significantly different MDW value between controls and patients with mild + survivor condition (p=0.01–0.05), as well as between mild + survivor vs. severe/critical + non-survivor COVID-19 patients (p=0.01–0.05); this difference became highly significant when comparing healthy control vs. severe/critical + non-survivor COVID-19 patients (p=0.0001–0.001).

Discussion

The scientific community has provided tremendous efforts against the harmful pandemic of SARS-CoV-2, focusing on identification of pathogenic biomolecular pathways and laboratory biomarkers with possible diagnostic and

Table 3: Main characteristics of the studies included in the pooled analysis of patients with and without COVID-19.

Authors	Setting	Sample size	MDW cut-off	COVID-19 infection	MDW values (without vs. with SARS-CoV-2 infection)	p-Value
Lin et al. [17]	Taiwan	150	≥20	9 (6%)	21.8 ± 5.4 vs. 23.5 ± 2.1	0.096
Ognibene et al. [16]	Italy	147	≥20	41 (27.9%)	20.3 ± 3.3 vs. 27.3 ± 4.9	<0.005
Piva et al. [27]	Italy	1.783	≥20.1	243 (13.6%)	22.0 ± 3.3 vs. 26.0 ± 4.3	<0.001
Zeng et al. [22]	China	155	≥20.1	93 (60.0%)	18.9 ± 2.0 vs. 22.1 ± 2.3	<0.0001
Cusinato et al. [38]	UK	26	≥20	15 (57.7%)	16.0 ± 1.2 vs. 23.0 ± 4.3	<0.001
Cumulative		**2.261**		**401 (17.7%)**	**21.8 ± 3.4 vs. 25.2 ± 3.9**	**<0.0001**

Bold values indicate the cumulative statistical data respect to the single studies reported.

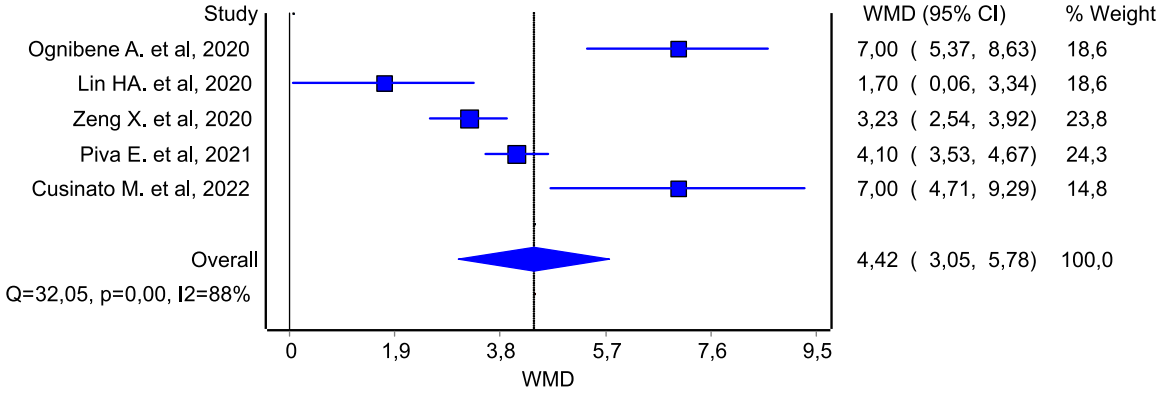

Figure 3: Forest plot of weighted mean difference and 95% confidence interval of MDW values in negative vs. positive COVID-19 patients.

Table 4: Main characteristics of the studies included in the pooled analysis of patients with mild and severe COVID-19 conditions.

Authors	Setting	Sample size	Severe/critical + non-survivors COVID-19	Mild + survivors COVID-19	MDW values (mild + survivors vs. severe/critical + non-survivors)
Ognibene et al. [16]	Italy	41	23 (56.1%)	18 (43.9%)	25.4 ± 3.6 vs. 28.8 ± 5.3
Riva et al. [13]	Italy	87	16 (18.4%)	71 (81.6%)	20.5 ± 4.0 vs. 26.8 ± 4.8
Stratan et al. [36]	Romania	106	65 (61.3%)	41 (38.7%)	20.7 ± 2.9 vs. 24.5 ± 3.4
Lorubbio et al. [15]	Italy	40	8 (20%)	32 (80%)	26.2 ± 3.4 vs. 24.8 ± 3.0
Lin et al. [19]	Taiwan	120	48 (40%)	72 (60%)	23.5 ± 4.4 vs. 25.2 ± 4.6
Hossain et al. [20]	USA	318	162 (50.9%)	156 (49.1%)	23.3 ± 3.7 vs. 25.7 ± 3.7
Kim et al. [37]	Korea	69	13 (18.8%)	56 (81.2%)	21.6 ± 3.1 vs. 25.8 ± 3.9
Cumulative		**781**	**335 (42.9%)**	**446 (57.1%)**	**22.7 ± 3.7 vs. 25.6 ± 3.9**

Bold values indicate the cumulative statistical data respect to the single studies reported.

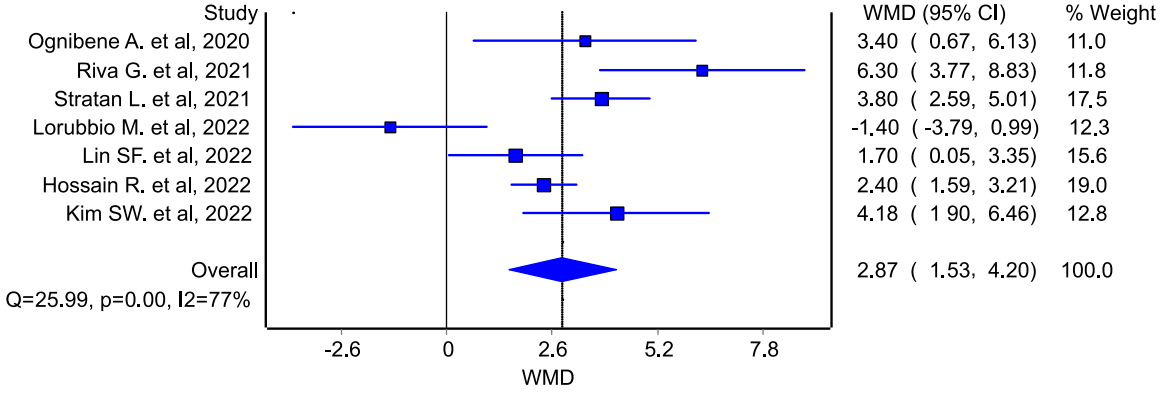

Figure 4: Forest plot of weighted mean difference and 95% confidence interval of MDW values in positive COVID-19 patients with mild + survivor and severe/critical + non-survivor conditions.

prognostic applications, in order triggers and targets for therapeutic approaches [2, 48, 49]. Among the various laboratory findings observed in COVID-19 [12, 48, 50, 51], several studies focused on extracellular traps of both neutrophils and monocytes (ETs) [52–54], which have now emerged as diagnostic and prognostic biomarkers COVID-19, since they are actively involved in both cytokine storm and coagulation dysfunctions [55–57].

As recently overviewed by Ligi et al. [58], extracellular histones (released as unmodified and/or citrullinated proteins from inflammatory activated leukocytes, in particular circulating monocytes) have gained momentum due to

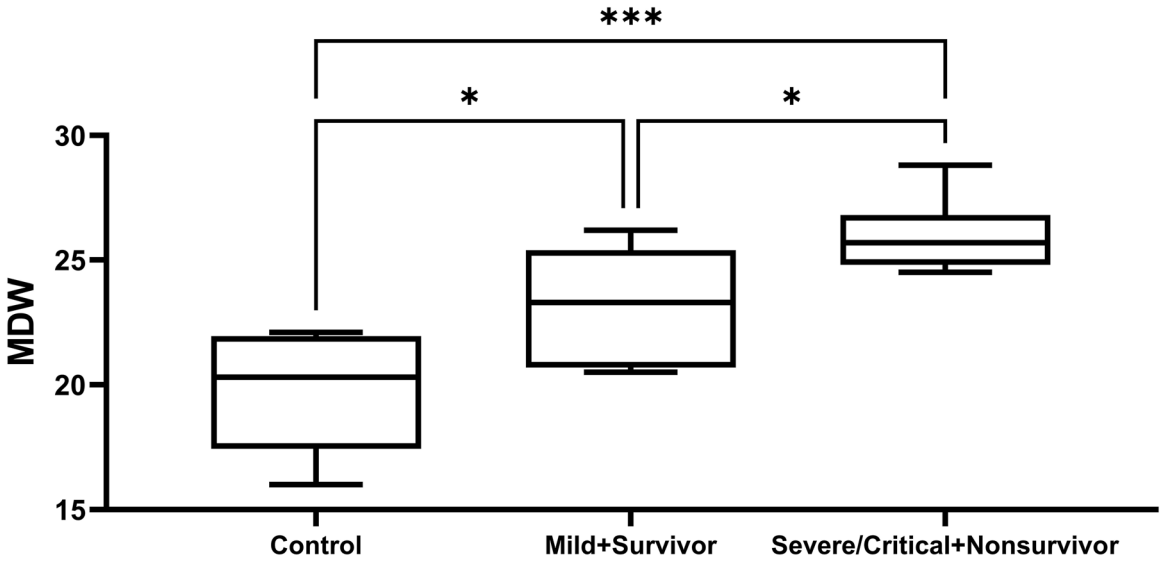

Figure 5: MDW values in positive COVID-19 patients with mild + survivor and severe/critical + non-survivor conditions (*: p=0.01–0.05; **: p=0.001–0.01; ***: p=0.0001–0.001).

their well-known role in fostering organ injuries and systemic diseases [59, 60], thus emerging as reliable diagnostic and/or prognostic markers of ETosis in SARS-CoV-2 infection [3, 52, 53, 61], due to their active role in COVID-19 related immunothrombosis and thromboinflammation [56] which are main causes of severe illness [45, 62]. Interestingly, early identification in COVID-19 of hyperhistonemia, which has been associated with higher mortality risk [46, 63], will predictably provide novel unexpected insights on whether circulating histone monitoring may be helpful in patients with SARS-CoV-2 infection [45].

We have recently shown that histone treatment of healthy whole blood produces a significant alteration of both monocyte morphology and MDW [47], demonstrating that histone play a crucial role in triggering profound and harmful, time- and dose-dependent modifications of circulating monocytes, likely those found in bacterial/fungal and viral (thus including SARS-CoV-2) sepsis.

The results of our systematic literature review demonstrate that COVID-19 patients have significantly higher MDW levels compared to healthy control subjects, and that patients with SARS-CoV-2 infection developing worse outcomes have higher MDW values than those with better outcomes. However, careful analysis of the included studies reveals a more complex and variable biochemical picture, due to the variability of the study population.

Although the SARS-CoV-2 pandemic is still ongoing and there is an impressive involvement of the scientific community to investigate from a biological, biochemical, molecular, and clinical perspective the different aspect of this life-threatening disease, the number of studies available so far on the innovative topic of MDW remains limited; the main limitation is mostly due to lack of uniformity about a cut-off for general population and subgroups of COVID-19 patients, other than an evident heterogeneity of leukocyte response (e.g., monocyte, neutrophil and lymphocyte) to the triggers of this infection.

Irrespective of these shortcomings, this systematic review and updated pooled analyses strongly suggest that MDW values may be higher in subjects with active SARS-CoV-2 infection than in those without, and that MDW may be a promising biomarker for distinguishing COVID-19 patients at major risk of unfavorable disease progression. Actually, no data are available on pharmacological agents that may be effective to down-regulate MDW values in COVID-19 patients, as well as therapeutic approaches restoring or at least improving monocyte functions and natural immunity, or even reverting the well-documented alterations of monocyte morphology and their harmful inflammatory activation that may frequently increase the risk of MOF and/or death.

Despite the technology beyond MDW assessment is unique, heterogeneous variability of patients' clinical data has emerged, though a substantial concordance has been reached that MDW plays an important role as additional diagnostic biomarker for predicting the risk of developing severe disease and/or death.

Notably, circulating monocytes were often found in normal values despite the clear heterogeneity of this

leukocyte population (i.e., monocyte anisocytosis), as highlighted by the MDW scatter plots. Noteworthy, MDW was found to be low in most control subjects (under the proposed cut-off of 20–21) and not significantly higher in mild/survivor COVID-19 patients. Patients who developed severe/critical COVID-19 illness and unfavourable clinical outcomes were identified and stratified as having higher risk of mortality based on MDW values [13, 15, 16, 19, 20, 36, 37] (Figure 5).

As reported by Lin et al. [17] and Kim et al. [37], MDW values were not significantly elevated in patients with COVID-19, even compared with those with upper respiratory tract infections and in patients with mechanical ventilation, according to the main cytopathic effects of SARS-CoV-2 infection on lung cells [64]. Interestingly, Polilli et al. [32], through a predictive models carried out on a retrospective study of 536 COVID-19 patients, showed that MDW values >22 and >25 was strongly predicted hospitalization and ICU admission. According to the well-known monocytes/macrophages involvement in immunopathogenesis of both systemic and organ (e.g., lung) hyperinflammatory manifestations (especially in COVID-19 infection), MDW values and scatter plots (alongside differential counts of circulating leukocytes) represent innovative routine and promising biomarker to reveal monocyte anisocytosis and their inflammatory activation and altered biomolecular functions. These crucial monocyte conditions and behaviors are in agreement with literature data about morphological, biochemical, cytometric and molecular modifications of monocytes in patients with COVID-19 [5, 8–11, 40, 41]. In particular, the role of circulating monocytes as well as their activation and different expansion of subsets (e.g., from classical to nonclassical and intermediate monocyte populations) has been widely discussed as primary innate immunity cells involved in SARS-CoV-2 disease severity and death correlated to the immune, inflammatory and thrombotic status of these patients [8, 9, 43, 65].

To this end, morpho-functional alterations of monocytes during COVID-19 have been not only related to dysregulated innate immunity and hyperinflammatory syndrome (mainly due to cytokine storm), but also to crucial release and activation in blood milieu and tissues of procoagulant triggers and pathways (e.g., tissue factor, platelet interaction inducing factor V and XIII release, D-dimer, etc.), thus linking hyperinflammation of monocytes to hypercoagulability clinical profile [6, 12, 36, 48, 66].

The hyperactivation of monocytes in COVID-19 patients also represents a further trigger to induce platelet activation, thus promoting coagulation dysfunctions. In fact, monocytes from patients with severe COVID-19 have been characterized to be more predisposed to bind platelets [67]. Platelets, as well, reciprocally activate monocytes in SARS-CoV-2 infection, promoting tissue factor (TF) expression and inflammatory cytokine release, exacerbating the vicious cycle of thrombo-inflammation [68].

Although the evidence on morpho-functional alterations of monocytes and significantly increased MDW levels reinforce the multifactorial pathogenesis of severe COVID-19 illness, on the other hand provides novel evidence on the significant role of monocytes and MDW scatters in SARS-CoV-2 viral sepsis, especially in COVID-19 patients with unfavorable clinical progression, paving the way for future perspectives of these hematological biomarkers in diagnostic algorithms [11, 32].

Noteworthy, there is still limited information about MDW evaluation in young COVID-19 patients, although some of them may be at risk of developing life-threatening complications and/or the multisystem inflammatory syndrome in children (MIS-C) [11, 69, 70]. Although children are often only mildly symptomatic or asymptomatic during acute SARS-CoV-2 infection, the emergence of novel SARS-CoV-2 lineages, the waning mRNA vaccine and natural immunity and vaccine hesitancy may have a serious impact on the number of young cases with severe illness [71, 72]. A stepwise approach for laboratory workup for diagnosing MIS-C has been advocated [69], though only recently MDW was found to be an additional hematological parameter reflecting monocyte anisocytosis [70].

Conclusions and future perspectives

The involvement and crucial functions of monocyte and its related marker MDW, and their impact on COVID-19 requires new strategies to address these changes in clinics and laboratory medicine. SARS-CoV-2 infection triggers significant organic injuries, especially mediated by thrombo-inflammation [73], which may lead patients to a higher risk of severe/critical conditions (up-to death) in the short term, whilst also fostering “chronic” complications and health consequences in the medium-long term due to accumulation of viral triggers [74]. Although the dynamics of these processes seem related to unresolved hyperinflammation of circulating monocytes [75], it will need further scrutiny for clarifying under which biological circumstances (e.g., clinical features, epigenetic and biomolecular hallmarks) accelerate or evolve toward multi-organ failure [3], and whether it may be partially or completely reversible [76].

The organic injury following SARS-CoV-2 infection has multifactorial causes, so that a combination of both “old and new” biomarkers [48, 50, 77, 78] may be more suited for risk prediction. The results of our systematic

literature review and updated meta-analysis suggest that monitoring MDW and morphological monocyte anisocytosis in patients with SARS-CoV-2 infection (upon admission and throughout hospitalization, especially in critical patients and/or those needing intensive care) may be seen as a useful tool for early identification of patients at higher risk of unfavorable disease evolution and predicting viral progression and complications. Moreover, according to recent observations on possible therapeutic approaches protecting cells and tissues (e.g., endothelial, pulmonary and cardiac cells) from inflammatory and thrombotic insults [45, 49, 79–81], future studies will be needed to identify both direct and indirect effects on cell activation and cytopathies of SARS-CoV-2 infection. A predictive/mechanistic scheme of how circulating COVID-19 virus may interplay with the innate immune response of circulating monocytes and trigger both inflammatory process and coagulative cascade mediated by monocyte alterations (mirrored by MDW index alterations) is summarily depicted in Figure 6.

Finally, further studies should be planned to define the role of MDW within diagnostic algorithms, and focused, in our opinions, to these main topics:

(1) On the basis of recent literature evidence suggesting different monocyte responses to COVID-19 and MIS-C according to the age of patients and monocyte subpopulations [82–84], it will be important to establish both ageing cohorts of COVID-patients and well-matched controls, identifying potential differences in monocyte response associated with ageing(pre-term, neonatal, infant/adolescent, adult, and aged subjects/patients) [85, 86].
(2) Large longitudinal studies are needed to accurately estimate gender differences of biological ageing of the blood monocyte cells, thus identifying sex- and age-dependent differences of some crucial clinical endpoints (e.g., survivors vs. non-survivors COVID-19 patients) [52, 87].
(3) Optimization of biological features of circulating biomarkers (e.g., subpopulations of monocytes; link between MDW and other laboratory biomarkers) for being used in routine clinical practice and for opening new frontiers in monitoring disease progression in those receiving pharmacological targeted therapies (e.g., heparin and heparinoids) [45, 49, 79–81, 88].

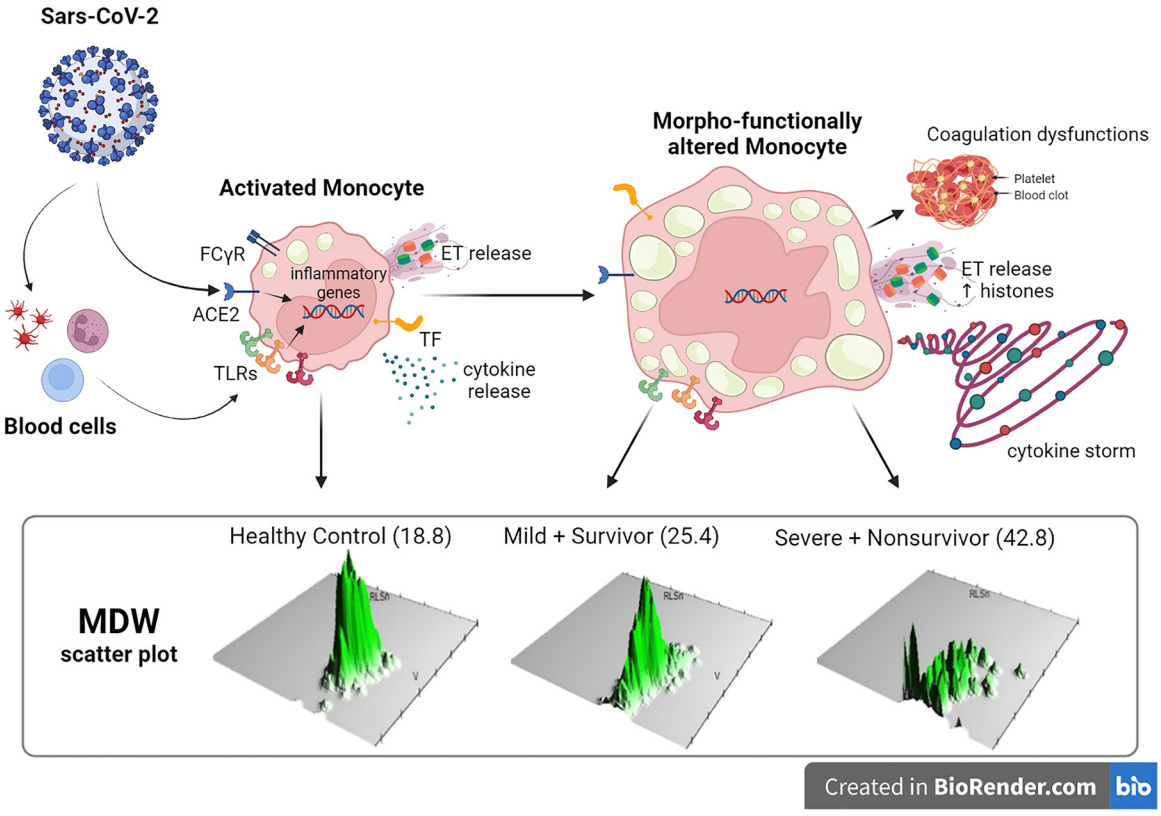

Figure 6: The key roles and functions of monocytes/macrophages and MDW index in the pathological inflammation in patients with mild and severe infection.

In conclusion, this systematic review and updated meta-analysis highlighted the MDW index (a parameter mirroring the changes in monocytes volume and heterogeneity in response to pro-inflammatory signals from COVID-19) as an additional, useful, innovative, and promising parameter for risk stratification that may serve, jointly with monocyte evaluation of morpho-functional alterations within a panel of hematologic/biochemical/immune biomarkers [48, 50, 77, 78], as reliable clinical predictor in COVID-19.

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