Original Research Article

MONOCYTOSIS / MONOCYTIC DYSPLASIA: Testing A New Diagnostic Tool Using Sysmex XN Analyzer Parameters.

ABSTRACT

BACKGROUND: In a medical laboratory, monocytosis is very common, often reactive, but may be related to a hematological malignancy, particularly Chronic myelomonocytic leukemia (CMML).

CMML is considered a diagnosis of elimination given the frequency of reactive monocytosis.This increases the number of blood smears (BS) performed without diagnostic interest.The objective of this study is to test and validate on our population a score established from the parameters of SYSMEX XN analyzers, to differentiate patients with monocytic dysplasia - which may suggest a hematological malignancy, in particular CMML -, from patients with reactive monocytosis ,and reduce the number of unnecessary BS.

METHODS: Patients aged ≥18 years with monocytosis>1G/L and>10% leukocyte formula were included. BS were performed manually with the objective of confirming monocytosis and looking for signs of monocyticdysplasia.The calculation of the monodysplasia score (MS) was performed using parameters derived from the SYSMEX XN9100 analyzer.

RESULTS: During the study period 263 patients were collected with a sex-ratio of 1.4. The mean monocyte value was 1.49 G/L.The MS was <0.160 in 242 patients, and >0.160 in 21 patients of which 12 showed signs of monocytic dysplasia on BS.The MS showed 100% sensitivity and 96.41% specificity with a negative predictive value at 100% and a positive predictive value at 57.14%.

CONCLUSION: The effectiveness of MS has been confirmed since 2018 in a validation cohort as well as in a multicenter cohort in France,having shown high sensitivity and specificity. The diagnosis of CMML remains difficult, so the use of MS helps to select patients most suspected of malignancy and will reduce the number of unnecessary BS.

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KEYWORDS: Monocytosis, Chronic myelomonocytic leukemia, Monodysplasia score, Blood smear

INTRODUCTION

World Health Organization defines monocytosisis as an absolute monocyte count >1 G / L with monocytes accounting for > 10% of leukocytes (1)

Monocytosis is frequently discovered in daily medical laboratory practice and can be caused by a wide variety of neoplastic and non-neoplastic conditions. The blood smear is the first step to distinguish between these two major entities in patients with a monocyte count ≥ 1 G/L and $>$ 10% of the white blood cell count (WBC).(2)

Reactive monocytosis is the most common, found in various conditions: infection (parasitic, bacterial, etc.), chronic inflammatory pathology (autoimmune disease, cancer, etc.), other hemopathy (acute myelomonocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma) (3–5). While neoplastic monocytosis - also called "clonal" - is more serious and requires awareness to allow early diagnosis and thus optimal management.

Among the entities included in the 2017 WHO classification, the main haematological malignancy, monocytosis can be related to is probably myelodysplastic/myeloproliferative (MDS/MPN) neoplasm (1,6,7). Chronic myelomonocytic leukemia (CMML) is the prototypical myeloid neoplasm, associating monocytosisans cytological dysplasia (8,9).

CMML is an acquired clonal hemopathy of the hematopoietic stem cell (CSH) (10) characterized by a proliferative side involving a malignant proliferation; essentially monocytic(11), and a dysplastic side characterized by a proliferation of abnormal myeloid progenitors leading to a premature apoptosis.(3). Its biological diagnosis is based on both positive and negative criteria, including: the presence of persistent peripheral monocytosis $\geq$1 G / L representing $\geq$ 10% of the total number of white blood cells, dysplasia affecting at least one lineage in the blood or bone marrow, percentage of blasts less than 20% in blood or bone marrow, absence of the Philadelphia chromosome or of the BCR-ABL1 fusion gene and absence of the rearrangement of PDGFR α or β (platelet-derived growth factor receptor alpha or beta (12), (13).

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To date, the diagnosis of CMML remains difficult to establish (14) , it is a diagnosis of exclusion due to the frequency of reactive monocytoses(1,15).

In this context, a recent study proposes the use of an orientation score called "monoscore" - established from the parameters of the Sysmex analyzer - as a new diagnostic tool from the first complete blood count (CBC), allowing the the selection of patients with monocyte dysplasia, which may correspond to a hematological malignancy, particularly CMML.

OBJECTIVES:

The objective of our work is to test and validate this score, established from the parameters of SYSMEX XN ANALYSERS to differentiate patients with monocytic dysplasia - which may suggest a hematological malignancy, in particular CMML -, from patients with reactive monocytosis, and thus reduce the number of unnecessary blood smears in case of monocytosis.

MATERIALS AND METHODS:

This is a prospective and analytical study carried out over a period of 3 months from December 21 to February 21, 2020, at the hematology laboratory of the University Hospital Center 'IBN ROCHD' in Casablanca. We included a total of 263 patients aged over 18 years, all sexes combined, with a monocytosis> 1G/L and >10% of the leukocyte formula (FL).

Peripheral blood samples were collected in plastic tubes containing the tri-potassium salt of ethylenediaminetetraacetic acid (K3-EDTA) then sent - within an hour of the sample - to the hematologylaboratory.

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The complete blood count with *Differential* (*CBC* with *DIFF*) analyses were performed on SysmexXN 9100® hematology analyzers. In the DIFF channel, XN analyzers provide a leukocyte formula (LF) including polymorphonuclear neutrophils (Ne), polymorphonucleareosinophils (Eo), polymorphonuclear basophils (Ba), lymphocytes (Ly), monocytes (Mo) and immature granulocytes (IG) by flow cytometry (CMF) after specific labeling of RNA with a fluorochrome. First, the blood sample is labeled with a fluorochrome that specifically binds to nucleic acids. Then, it is illuminated by the beam of a laser which allows the separation of cells into distinct populations. Thus, each of the cells will be identified by its structure such as the size of the nucleus or the granulations (X axis corresponding to the SSC "side scatter"), its fluorescence (Y axis corresponding to the SFL "side fluorescence light") and its size (Z axis corresponding to FSC "forward scatter")

Finally, cells with similar physical and chemical properties form a similar population on a graph called a scattergram. The analyzer allows for each population the measurement of the median position on the three axes (X, Y, Z), as well as their dispersion, which is the result of a ratio between the width of the cell cloud and the value of the median position of the latter **(Figure 1)**(2)**.**

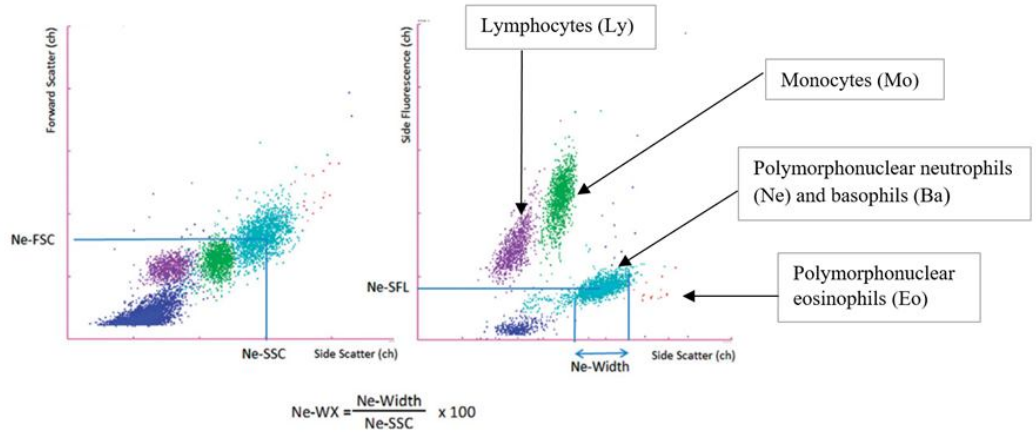

Fig. 1.Illustration of the calculation of Ne-WX* according to Schillinger F et al. (2)
Ne-WX: Neutrophil dispersion value on the X axis*

The data were collected and analyzed by the EXCEL software version 2016. The calculation of the Monodysplasia score (MS) was done using 3 parameters: Neutrophils / Monocytes (Ne / Mo) ratio, absolute value of monocytes (Mo) and the NeWX parameter, according to this equation:

[1/(1 + e-(-11.623 + 0.026 x NeWX - 1.385 x Ne/Mo + 2.714 x Mo))]. The calculation of MS was carried out by the EXCEL software version 2016

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For the interpretation of the monodysplasia score (MS) we have a cut-off value of 0.160, so for a MS > 0.160 the blood smear is mandatory for research of dysplasia, while for a MS ≤ 0.160 the blood smear is not necessary, and monocytosis is probably reactive. To check the validity of this score, we performed blood smears with microscopic study for all samples.

Blood smears were performed manually and stained with May-GrünwaldGiemsa (MGG) and then analyzed under a Leica® optical microscope by more than 2 expert observers: Professor of hematology-oncology, experienced laboratory technician and 2 biologists.

The objective of the cytological study was to confirm the monocytosis, identify the monocytic aspect (normal or dysplastic), look for blasts and signs of dysgranulopoiesis; mainly observed in Neutrophils (degranulation, abnormal condensation of chromatin, presence of spicules, abnormal nuclear segmentation).

RESULTS:

During the study period, 263 adult patients were collected, of which 155 were men (59%) and 108 were women (41%) with a sex ratio of 1.4.

Within the university hospital center, 21% of samples were received from the hematology department, 14% from intensive care units, 6% from each of the pulmonology and cardiology departments, 4% from each of the gastrology ; urology; plastic surgery and visceral surgery departments, with a lower percentage for the rest of the departments.

Regarding the analysis of complete blood counts (CBC), the median value of white blood cells was 10,33 G/L [3,05-32,10], 1,33 G/L [1-5,02] for monocytes and 7,08 G/L [1,28-65,70] for neutrophiles **(Table I).** Different aspects of scattergrams were obtained on the screen of ourSysmexXN 9100® analyzer, the most representative are shown in **Figure 2.**

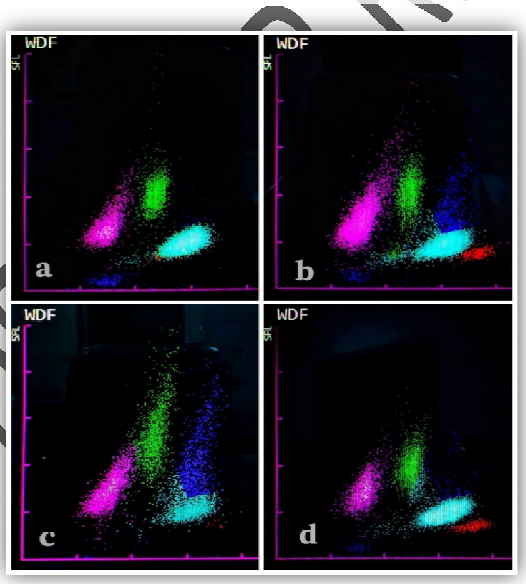

Fig. 2.Position of different cell populations on white blood cell differential scatterplot by Sysmex XN 9100®,hematology analyzer of our laboratory.
**Patients a – b – c – d respectively had monocytes (G/L) at: 1,69 – 1,54 – 1,86 – 2,40.*

Table I. Analysis of basic patient characteristics. *WBC* : white blood cell count*

	Median value	Minimum value	Maximum value	Mean value	Standard deviation
Age	45	19	90	46,7	16,8
WBC* G/L	10,3	3,05	32,1	11,2	4,2
Monocytes %	12,6	10	36,1	14,1	4,4
Monocytes G/L (Mo)	1,3	1	5,02	1,5	0,5
Neutrophils G/L (Ne)	7,08	1,28	65,7	7,9	5,4
Ne/Mo	5,2	1,2	54,6	5,3	3,7
Ne-WX	309	209	536	315,3	30,1

For the othercelllineages, 200 patients (i.e. 76%) had no associatedabnormalities, 51 hadanemia (i.e. 19%), sevenhadthrombocytopenia (i.e. 2.6%), and five hadthrombocytopeniawithanemia (i.e. 1.9%).

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Monodysplasia score was <0.160 in 242 patients (i.e. 92%), and > 0.160 in 21 patients (i.e. 8%). Among these, **12 patients presented signs of monocytic dysplasia** on blood smears (12/21 i.e. **57%**), and 9 patients had normal blood smears (9/21 i.e. 43%). Different aspects of dysplastic monocytes are found, includingnuclear and cytoplasmicabnormalities, shown in **Figure 3.**

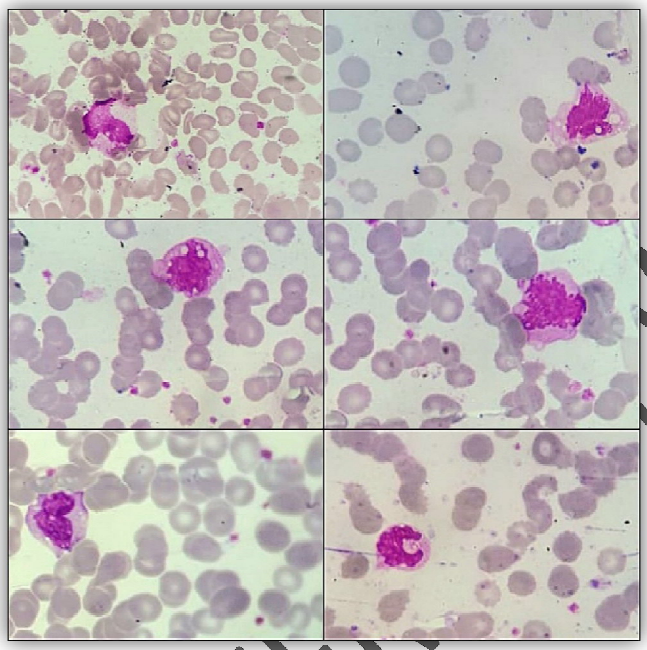

Fig. 3.Dysplastic monocytes found on the blood smears studied.

Statistical analysis of our results allowed us to have a sensitivity of 100% and a specificity of 96.41% for the monodysplasia score **(Table II).** The Youden's index (sensitivity + (specificity -1)) is 0.96 (≈1), and theYule's Q coefficient is equal to one, which confirms thatthere is a perfect association between monocytic dysplasia and a MS > 0,160, and thus the myelodysplasia score constitutes an effective diagnostic tool in the case of monocytosis.

Table II. Statistical analysis of Monodysplasia Score (MS) results

	Dysplasia	*Normal Blood Smear*	
MS > 0,160	True positives : 12	False positives : 9	**PPV = 57,14%**
MS ≤ 0,160	False negatives : 0	True negatives : 242	**NPV = 100%**
	SS = 100%	**Sp = 96,41%**	

PPV = Positive predictive value, NPV = Negative predictive value, SS = Sensitivity, Sp = Specificity

DISCUSSION:

In a medical laboratory, monocytosis ≥ 1G / L are very common, and are often reactive (1,15). The slide examination under a microscope in case of any monocytosis generates a very large number of blood smears. Thus the ISLH (International Society for Laboratory Hematology) and the GFHC (Groupe Francophone d'HématologieCellulaire) (16) have recommended a blood smear when the number of monocytes exceeds 1.5 G / L in the first CBC, or when monocytosis persists for more than 30 days in adult patients, to avoid unnecessary excessive smear examinations, reducing their rate to 1.2% (17).

Based on this threshold, hematological malignancies ; in particular CMML with a monocyte level less than 1.5 G / L but greater than 1 G/L would not be controlled on a blood smear and thus risk to be underdiagnosed.

In a recent multicenter study (learning cohort) (2), using the structural parameters of Sysmex XN ™ analyzers, a "mono-dysplasia score" (MS) also called "Monoscore" was establishedhere

This score integrated three parameters : - the neutrophils / monocytes ratio (Ne/Mo), - neutrophil structural dispersion (Ne-WX) and the absolute monocyte count (Mo), and was calculated for any monocytosis ≥ 1 G / L and ≥ 10% of leukocytes, according to the following equation:

[1/(1 + e-(-11.623 + 0.026 x NeWX - 1.385 x Ne/Mo + 2.714 x Mo))]

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At the end of thisstudy - for users of Sysmex XN analyzers - a new strategyusing the MS waspproposed for the examination of bloodsmears in patients withmonocytosis ≥ 1 G / L and ≥ 10% of leukocytes: - If MS> 0.160 = bloodsmearshouldbeperformed for cytologicalanalysis of monocytes and for dysplasia. - If MS ≤ 0.160 = probablyreactivemonocytosis, and bloodsmearis not necessary.

Based on the recommendations of the GFHC (16) (blood smear to be performed if monocytes ≥ 1.5 G / L) Schillinger et al (2) had 14.1% cases of reactive monocytosis generating unnecessary blood smears ,against only 2.2% using the MS. In addition, 13.1% cases of CMML with a monocytosis<1.5 G / L, would not have benefited from a blood smear, against only 2 (3.3%) with the MS .In our study, the number of cases was 263, using the MS we had 9 false positives (MS> 0.160 with a normal blood smear) i.e. 3.4%, and no false negatives **(Table III).**

Table III. Comparison of « false negative » and « false positive » rates using GFHC recommendations, versus MS.

		False negatives	*False positives*
Schillinger et al	*Using GFHC recommendations*	13,1%	14,1%
	Using Monodysplasia Score	3,3%	2,2%
Our study	*Using Monodysplasia Score*	0%	3,4%

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The effectiveness of Mondysplasia score and high sensitivity (92.3%) and specificity (93.6%), were confirmed since 2018 in a validation cohort (2) of more than 1809 samples of adult patients with monocytosis, as well as in a French multicenter cohort study (Paris, Besançon and Caen) (18).

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In our study based on data of our population, we found a sensitivity 100% and 96.41% specificity thus reinforcing the added value of this Monoscore in the diagnostic **orientation** in front of a monocytosis.

Unlike the morphological study on blood smears, this new approach using the "monoscore" is fully standardized, immediate and independent of the operator (18).

Lately, a Monocytosis Workflow Optimisation (MWO) (19), has been introduced by Sysmex as a concept designed for samples with monocytosis, to optimise the workflow and improve CMML detection. It combines the 'mono-dysplasia score', the monocyte counts and information from the WBC scattergram to recommend samples for microscopic examination.

A recent Turkish study in 2020 (20), aimed to examine the contribution of MWO rule set. They concluded that using the MWO rule set and abnormal lymphocyte/blast indicators together is more effective, as the unecessary blood smear rate decreased significantly and interestingly, none of the patients with hematological malignancies were missed.

CONCLUSION:

In front of a monocytosis it is essential not to miss a malignant hemopathy and in particular a CMML which has many serious complications, particularly infectious and hemorrhagic, and to delay its transformation into acute leukemia.

The benefit of the Monodysplasia score would be to reduce the number of unnecessary blood smears in any monocytosis, and to select patients most suspected of malignancy in particular CMML. This Monoscore will therefore help the biologist to be more relevant in his diagnostic approach, and to target the examination of the smears on the confirmation of signs of dysplasia.

There are other tools in the diagnostic approach of CMML such as flow cytometry, which allows quantification of monocyte subsets in the diagnosis and monitoring of CMML. Other studies will be of interest to compare the effectiveness of these two diagnostic tools.

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