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Vanessa Nivaggioni[1] ; Edwin Van Mire [2] ; Julie Brousseau [3] ; Marie Loosveld [1 4]

1- Laboratoire d'Hématologie, Hôpital de la Timone, Assistance Publique – Hôpitaux de Marseille, Marseille France
2- CERTE Medical Diagnostics and Advice, Division clinical chemistry, Groningen, The Netherlands
3- AP-HP, service d'Hématologie Biologique, Hôpital R. Debré, Paris, France.
4- CNRS, INSERM, CIML, Aix Marseille University, Marseille, France.

In daily laboratory practice, the use of automated haematology analysers delivers reliable results, but no analyser can determine properly red blood cell (RBC) morphological abnormalities and cyto-morphological examination of a blood smear is necessary to interpret automated abnormal results for unknown patients. The decision to examine a blood smear is triggered by one or more alarms generated by the analyser or quantitative and/or qualitative criteria decided by the laboratory performing the analysis. These criteria are not always relevant; hence, the interest to use several RBC and reticulocyte parameters to improve the specificity and sensitivity of microscopy examination. We recently established a decision tree using RBC and reticulocyte parameters from the SYSMEX XN-10[1] to distinguish between patients with hereditary RBC disease from iron deficiency anaemia and other patients. This algorithm also uses the increased MCHC management algorithm embedded in the *Extended* IPU of XN analysers previously published by Berda-Haddad et al.[2] This concept manages patients with mean corpuscular haemoglobin concentration (MCHC) >365 g/L by the alternative use of optical parameters provided by the reticulocyte channel of XN analysers, the optical red blood cells count (RBC-O) and the optical haemoglobin calculation (HGB-O) and calculates an erythropoietic score called 'RBC Score' involving reticulocytes and fragments to judge spurious erythropoiesis. As shown previously, this two-step decision tree can differentiate

TABLE 1 Median, minimum and maximum values for analysed RBC and reticulocyte parameters in groups with MCHC >365 g/L

MCHC >365 g/L				
	OTHERS (n = 68)	**SCD (n = 24)**	**HS (n = 14)**	**SAO (n = 18)**
Age	51 (15; 91)	29 (16; 50)	33 (16; 46)	50 (15; 98)
Sexe (F/M)	39/29	18/6	8/6	4/14
RBC (T/L)	3.73 (0.93; 5.75)	2.56 (1.53; 4.37)	4.28 (3.30; 5.55)	4.37 (3.29; 5.06)
HGB (g/L)	121 (29; 187)	90 (51; 127)	138 (97; 164)	**146 (108; 157)**
MCV (fL)	86.9 (73.9; 112.1)	91.3 (72.9; 117.0)	86.1 (79.5; 91.9)	86.3 (79.2; 91.6)
Micro% (%)	2.5 (0.5; 23.0)	5.5 (0.3; 19.9)	3.3 (1.7; 17.2)	2.8 (1.5; 10.1)
RDW-SD (fL)	45.5 (38.8; 63.9)	53.9 (41.3; 71.1)	49.1 (39.3; 61.3)	44.3 (39.6; 48.7)
MCH (pg)	32.1 (27.3; 41.7)	33.6 (26.6; 43.0)	32.1 (29.0; 34.2)	32.3 (29.1; 34.9)
MCHC (g/L)	370 (366; 387)	369 (366; 376)	374 (366; 383)	374 (367; 382)
NRBC% (%)	0.0 (0.0;7.4)	1.0 (0.0; 10.5)	0.0 (0.0; 0.1)	0.0 (0.0; 0.4)
RET# (T/L)	0.072 (0.009; 0.379)	0.199 (0.064; 0.468)	0.211 (0.073; 0.483)	**0.032 (0.010; 0.082)**
IRF% (%)	10.8 (0.0; 49.6)	30.0 (10.0; 42.8)	9.7 (6.1; 16.0)	14.4 (6.0; 36.8)
FRC% (%)	0.3 (0.0; 2.6)	1.1 (0.1; 5.9)	0.1 (0.0; 0.7)	0.5 (0.3; 1.0)
HYPO-He% (%)	0.2 (0.0; 4.6)	1.7 (0.2; 10.5)	0.3 (0.1; 2.5)	20.5 (9.5; 38.7)
RBC-O (T/L)	3.81 (0.90; 5.73)	2.51 (1.55; 4.36)	4.24 (3.36; 5.58)	4.29 (3.20; 5.04)
HGB-O (g/L)	123 (27; 189)	84 (47; 123)	133 (101; 161)	93 (64; 109)
RBC SCORE	0.013 (0.001; 1.000)	0.918 (0.149; 1.000)	0.560 (0.009; 1.000)	**0.004 (0.001; 0.059)**
Delta RBC-O vs RBC (%)	0.3 (−6.0; 9.4)	0.2 (−4,8; 3,4)	−0.5 (−3.4; 3.9)	−0.7 (−5.6; 2.8)
Delta Hb-O vs Hb (%)	−1.3 (−5.1; 6.3)	−7.7 (−19.7; −2.4)	−2.4 (−7.6; 4.1)	**−35.4 (−40.7; −28.6)**
Ratio Hypo-He%/ Micro%	0.10 (0.00; 0.86)	0.46 (0.11; 1.33)	0.07 (0.00; 0.32)	**6.72 (2.78; 11.87)**

Abbreviations: HS, hereditary spherocytosis; OTHERS, other patients who did not have a RBC disease or iron deficiencyanaemia; SAO, southern Asian ovalocytosis; SCD, sickle cell disease. Parameter abbreviations: FRC%, fragments; HGB, photometric haemoglobin; HGB-O, optical haemoglobin; hYPO-He%, percentage of hypochromic cells; IRF%, immature reticulocyte fraction; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; Micro%, percentage of microcytes; NRBC%, nucleated RBC; RBC, red blood cells; RBC-O, optical RBC; RDW-SD, RBC distribution width; RET#, reticulocyte count. Bold values show the typical SAO profile.

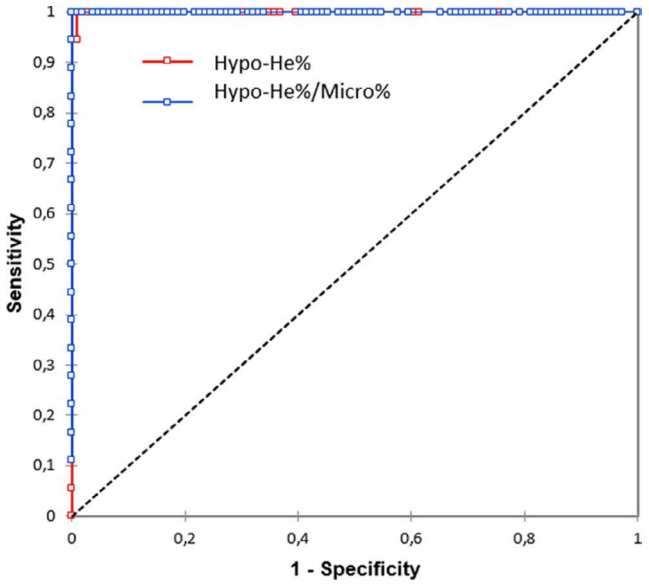

FIGURE 1 ROC curves established for hypo-He% and hypo-He%/micro% to discriminate SAO from other patients with MCHC >365 g/L. AUC, respectively, 0.999 [0.998-1.000] and 1.000 [1.000-1.000]. Abbreviations: 'micro%': percentage of microcytes, 'hYPO-He%': percentage of hypochromic cellsa

between several causes of anaemia and red blood cell abnormalities, both acquired and constitutional. However, patients presenting with SAO are misclassified as patients with no RBC disease. Indeed, these patients show a severe difference in haemoglobin, whereby HGB-O appears much lower than photometric haemoglobin and require a haemoglobin interference check with a clear plasma. This can lead to confusion amongst users in deciding which haemoglobin value to correctly report. Our study aimed to complete this established decision tree to improve the detection of SAO patients.

As a first attempt, we collected patients presenting with SAO and analysed the results for all parameters issued by the XN-analyser. Blood samples were collected in EDTA K3 and complete blood counts (RBC and reticulocytes) were performed using Sysmex XN-10 (Sysmex Corporation™) analysers. The analysis of SAO adult patients from Marseille University Hospital (AP-HM, France), Robert Debré hospital (AP-HP, France) and CERTE (Groningen, The Netherlands) demonstrated that these patients present with almost normal standard RBC parameters. They present no anaemia and no microcytosis but an increased MCHC which is in most cases >365 g/L (18/22) with a median value of 374 g/L [337;382] (data not shown).

In a second step, we retrospectively analysed a heterogeneous group of patients presenting an MCHC >365 g/L to highlight the difference in RBC and reticulocyte parameters between SAO, red blood cell disease patients and other patients. A total of 124 patients presenting with an increased MCHC, in which the 18 SAO from the first analysis were included, were divided into 4 different groups: sickle cell disease "SCD" (n = 24), hereditary spherocytosis "HS" (n = 14), Southern Asian Ovalocytosis 'SAO' (n = 18) and other patients who did not present an RBC disease 'OTHERS' (n = 68). High-pressure liquid chromatography on a Bio-Rad Variant™ II analyser, capillary electrophoresis on SEBIA® Capillarys 2 and molecular diagnosis were, if necessary, used to make a diagnosis of haemoglobinopathy. Diagnosis of HS was obtained with a positive eosin-5-maleimide binding test (EMA test) according to morphology and clinical and biological history. All patients with SAO have typical 'theta cells' on blood films and a positive EMA test or a characteristic profile on the ektacytometric curve.

Red blood cell parameters were analysed as quantitative variables and summarized as median and range (Table 1). All analyses were performed using XLSTAT 2020 from Microsoft Corporation TM (Version 2020.3.1). The results of the complete RBC count were analysed in this cohort and summarized in Table 1. In the group 'SAO', the median value of MCHC was similar to the other groups (374 g/L [367-382]). Haemoglobin (HGB) was in most cases normal (146 g/L [108-157]) and optical haemoglobin (HGB-O) was systematically lower than photometric haemoglobin (94 g/L [77-109]). The RBC score remained low with a median value of 0.004 [0.001-0.059] in contrast to 'SCD' (0.918 [0.149-1.000]) and 'HS' (0.560 [0.009-1.000]). Hypo-He% was very high (22.5% [9.5-38.7]), whilst the micro% is low and similar to the other groups 2.8% [1.5-10.1]. The ROC curve for SAO diagnosis with the hypo-He% parameter shows an area under the curve equal to 0.999 [0.998-1.000]. The association of hypo-He% with micro% in the form of a ratio shows a very clear distinction between SAO and the other groups, particularly the red blood cell disease groups. The area under the curve rises to 1 [1.000-1.000] when using the ratio hypo-He%/micro% (Figure 1). The optimal threshold is then equal to 1.5. Thanks to these new findings, we suggest modifying the RBC algorithm previously described.[1] The sample is considered as a suspect for RBC disease if MCHC >365g/L and either presents a high RBC score (>0.15) or a ratio hypo-He%/micro% greater than 1.5 (Figure 2).

Overall, the hypo-He%/micro% ratio demonstrates excellent sensitivity and specificity which allowed us to well classify 100% of the SAO patients with a spurious increased MCHC. Of note, this ratio remains excellent for SAO detection even if MCHC is normal. This additional step does not generate any additional cost since the reticulocytes count is done automatically when a patient has an elevated MCHC. Even if the pathology is uncommon, its detection and correct classification are very important. As demonstrated by Berda et al[2] in the context of RBC disease, the optical measurement of RBC can lead to an underestimation of HGB-O due to cell abnormalities and as such should be considered only in case of evidence of haemolysis. Reporting a correct haemoglobin result is fundamental for clinical management of patients and the risk of reporting false anaemia needs to be managed and prevented in all cases and this is now properly managed with this proposed amendment to the RBC algorithm and CBC-O application. The hypo-He%/micro% ratio will then automatically identify SAO patients with perfect accuracy offering a new capability in the management of hereditary RBC disease in routine practice. Some limitations of our study can be underlined. The number of SAO is not very high and our population does not take into account possible overlaps between the different pathologies described here. In addition, it would be important to verify the

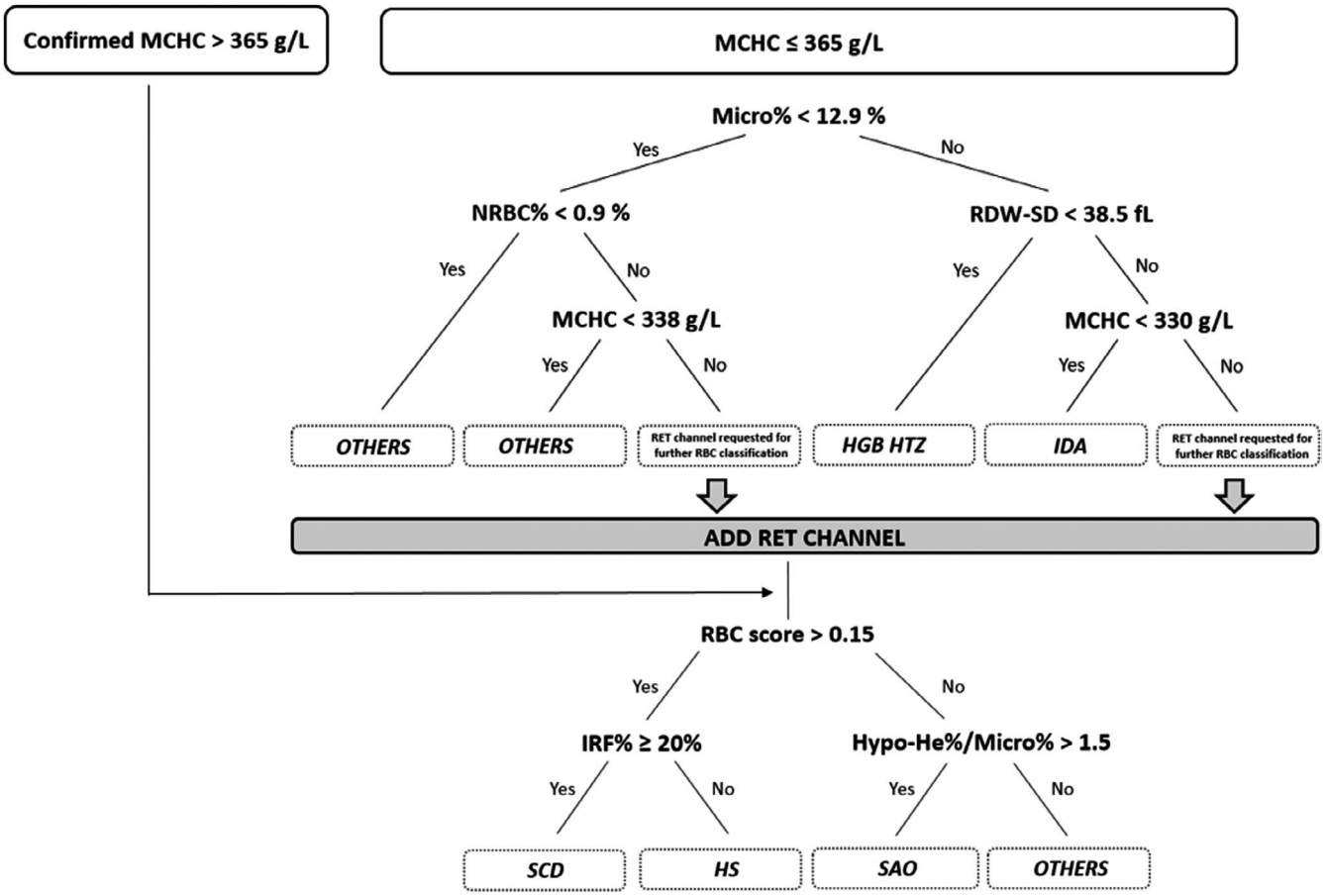

FIGURE 2 New decision tree. An additional condition has been added for patients with confirmed MCHC >365 g/L and RBC score <0.15. Group abbreviations: HGB HTZ, heterozygous haemoglobinopathy; HS, hereditary spherocytosis; IDA, iron deficiency anaemia; OTHERS, other patients who did not have a RBC disease or iron deficiency anaemia; SAO, southern Asian ovalocytosis; SCD, sickle cell disease. Parameter abbreviations; hYPO-He%, percentage of hypochromic cells; IRF%, immature reticulocyte fraction; MCHC, mean corpuscular haemoglobin concentration; Micro%, percentage of microcytes; NRBC%, nucleated RBC; RDW-SD, RBC distribution width

applicability of the ratio on a large scale and particularly in malaria-endemic regions with high prevalence.

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CONFLICT OF INTEREST

No Conflicts of Interest.

Correspondence

Vanessa Nivaggioni, Laboratoire d'hématologie biologique, Hôpital de la Timone, 264 Rue Saint Pierre, 13385 Marseille cedex 05, France.

Email: vanessa.nivaggioni@ap-hm.fr

ORCID

Vanessa Nivaggioni https://orcid.org/0000-0003-0631-7669

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