Title page: Increased Mean Corpuscular Haemoglobin Concentration: Artefact or true abnormality? Short running title: Increased MCHC: Artefact or pathology?

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19 pages and 5 illustrations

Title page:

Increased Mean Corpuscular Haemoglobin Concentration: Artefact or true abnormality?

Short running title: **Increased MCHC: Artefact or pathology?**

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Abstract:

Introduction: In daily practice in haematology laboratories, spurious increased MCHC induces an analytical alarm and needs prompt corrective action to ensure delivery of the right results to the clinicians. The aim of this study was to establish a "decision tree" using the new parameters Red Blood Cells (RBC-O) and Haemoglobin (HGB-O) from the Sysmex XN-10 RET obtained by flow cytometry to deliver appropriate results.

Methods: From 128 unknown patients with MCHC >365 g/L, all erythrocyte parameters including reticulocyte parameters were measured and analysed in parallel with blood smears, chemistry index and osmolarity. Differences between optical parameters (RBC-O, HGB-O) and usual parameters (RBC, HGB) obtained by impedance and photometry were reported also.

Results: Four groups were defined from observations: -RBC agglutination (n=22); -Optical interference (n=17); -RBC disease (n=18); -Others (n=71). The use of RBC-O and HGB-O permitted efficient correction of the abnormalities when RBC agglutination and/or optical interference were present in 36 of 39 patients. Reticulocyte parameters permitted to elaborate an RBC score that allowed a highly sensitive detection of RBC disease patients (17/18).

Conclusion: Based on new parameters, we propose a "decision tree" that delivers time savings and supports biological interpretation in case of elevated MCHC.

Main Body of Text:

Introduction:

Haemogram abnormality related to one or more of the measured parameters Red Blood Cells (RBC), Haemoglobin (HGB), Mean Cell Volume (MCV) or Haematocrit (HCT), leads to abnormal calculated RBC indices, especially Mean Corpuscular Haemoglobin Concentration (MCHC), which is one of the most indicative abnormalities generated by Haematology Analysers (HA), alarming the users about a spurious result. Elevated MCHC is a rare event in routine laboratory practice, but it must be managed properly [1]. In daily practice, the MCHC limit defined by the Sysmex XN-10 RET analyser (Sysmex Corporation ™, Kobe Japan) is fixed at 365 g/L. Exceeding this value leads to a suspicious "flag". This "flag" has to be considered in an accreditation context to assess the accuracy of reported parameters. Either it is just an artefact, or it refers to a true pathological sample. In fact, different aetiologies lead to spurious results of RBC, HCT or HGB measurements: 1/ the presence of abnormal RBC: as cold agglutination (CA) or RBC disease, 2/ the presence of abnormal plasma: as lipemic, icteric or haemolytic situation [1]. In case of CA, auto-antibodies agglutinate erythrocytes at a temperature below 37°C [2]. In laboratory practice, this leads to an incorrect decrease in RBC count, an untrue increase in cell volume and as a consequence to wrong erythrocyte indices [1]. In case of acquired or hereditary disorders, a modification of erythrocyte volume homeostasis is observed principally with dehydration. Water loss generates a decreased cell volume and an increased haemoglobin concentration [3]. Finally, in rare cases, increased MCHC can be found in patients with severe ionic troubles or those who have been treated with some drugs [1].

In face of a sample presenting an MCHC value exceeding 365 g/L, a suspicious "flag" appears and the sample has to be checked (plasma aspect, RBC distribution curve and the presence or not of RBC morphologic abnormalities on a blood smear). The common process for controlling these samples is time-consuming and concomitantly delays the report of true results to clinicians.

Interestingly, XN-10 RET proposes new parameters obtained by the optical method such as RBC-O or HGB-O, in parallel with the impedance method for RBC or photometry for HGB. These new parameters associated with those for reticulocytes may improve the management of MCHC > 365 g/L. Their interest was evaluated in a retrospective study that included 128 first unknown patients presenting an MCHC > 365 g/L. Using a

combination of these new parameters, we propose a “decision tree” flowchart dedicated to the fast management of high MCHC values, using a standardised validation procedure in laboratories.

Materials and Methods

Materials

The XN-10 RET uses two different technologies for achieving a full erythrocyte analysis. Erythrocytes are counted using an impedance method with a hydrodynamic focusing system in a fixed volume at room temperature. The volume for each cell is defined by the height of the peak of resistance when passing through an aperture. HCT is defined using the cumulative pulse method and MCV calculated by the ratio HCT/RBC. An additional available research parameter is the Red blood cells Most Frequent Volume (R-MFV) that defines the peak of the curve and fits the MCV in normal distribution. HGB is measured by photometry using Sodium Lauryl Sulphate Reagent. Measured parameters as HGB, RBC and HCT let one calculate MCH (HGB/RBC) and MCHC (HGB/HCT). When required, XN-10 RET can provide a second erythrocyte count (RBC-O) using fluorescence flow cytometry after stabilisation and warming at 41°C in the incubation chamber. RBC-O is a measured parameter, corresponding to total erythrocyte count, including reticulocyte counts, whereas HGB-O is a calculated parameter derived mainly from the RBC-O count and RBC Haemoglobin content (RBC-He). This parameter is based on the low angle diffraction measurement in the optical channel, and already proven to be correlated to MCH for normal patients [4]. The new parameters used and evaluated in this study provided by the optical channel are RBC-O, HGB-O, reticulocyte counts (RET), and Fragmented Red Cells (FRC). XN-10 RET is used in daily routine in the laboratory, adjustments and calibrations are checked regularly, and controls are analysed each day using both internal quality control from Sysmex^{TM} and CBC-XE, XE-Ret, XE-nRBC from Eurocell Diagnostics^{TM} (Eurocell Diagnostics, France).

Methods

All specimens in the study were adult patients from the Marseille University Hospital. The samples were collected in K3-EDTA tubes from Becton Dickinson ^{TM} (Franklin Lakes) and analysed routinely on XN-10 RET.

Comparisons and acceptable deviation between both methods

To define the capability of the HA in providing identical results for an RBC profile using impedance and optical methods, a comparison was made by including 3901 unselected adult patients over the full range of clinical values, (HGB from 31 g/L up to 201 g/L and RBC from 1.04 x 10^{12}/L up to 6.70 x 10^{12}/L) with only selective criteria that reported MCHC was lower than 366 g/L. The analysis of values between both methods for RBC and HGB led us to define: a delta RBC% = ((RBC-O – RBC)/ RBC) x 100 and a delta HGB% = ((HGB-O – HGB)/ HGB) x 100.

Increased MCHC analysis

One hundred and twenty eight unknown adult patients samples with MCHC > 365 g/L were tested in both channels: impedance and optical. The plasma aspect was optically checked to detect visible interference and samples were also analysed for chemistry indexes regarding haemolysis, lipemia and icterus with respective positive index fixed at 50, 100, 50, as defined by the Roche technology in Modular System, with the Serum Index Gen.2 (SI2) (Roche Diagnostics, Meylan, France) [5]. These chemistry indexes are calculations of absorbance measurements of diluted sample with 0.9% NaCl at three pairs of wavelengths (haemolysis: wavelengths 600/570 nm, lipemia: wavelengths 700/660 nm, icterus : wavelengths 505/480 nm).

Blood smears were reviewed by optical microscopy for RBC morphology by experimented laboratory staff. In case of RBC CA, HA underestimate the real number of RBC associated with a falsely increased MCV due to RBC clumps. So, samples were warmed at 37°C for at least one hour to obtain the true results and cold agglutinins were observed on blood smears prepared at room temperature. The possible solution with the Sysmex technology is the use of the optical channel that incubates samples at 41° C and so instantly dissociates RBC CA. To assess the validity of this technology, after incubation at 37°C of sample, results obtained by impedance method were compared with optical parameters.

Osmolarity was also calculated using the following formula: 2x[Na^+ (mmol/L)] + Urea (mmol/L) + Glycemia (mmol/L) (normal range: 280 to 300 mosmol/L) [6].

After the study of the reliability of optical parameters in comparison to impedance and photometric methods, discrepancies between both methods were analysed with regards to all available biological results. Based on these observations, all patients were first classified according to four major groups defined as:

(1) RBC agglutination (n= 22);

(2) Optical interference (n= 17);

(3) RBC disease (n= 18);

(4) Others: including unclassified and/or patients with hyposmolar plasma (n=71).

In each group described above: mean, median, and limit values for MCHC, HGB, Delta RBC%, Delta HGB%, RET, FRC were noticed and studied with regards to the confirmed abnormality of the patient.

Statistics

The reliability and agreement of measurements between the two methods were assessed using the Intraclass Correlation Coefficient (ICC) and by the Bland-Altman method. Analysis of considered RBC parameters according to different categories was performed with Mann-Whitney U-tests, and a Bonferroni correction for multiple tests. A multivariate logistic regression with stepwise selection was used to assess associations between RBC disease and blood parameters. The discriminative ability of the prediction model was measured by the area under the curve (AUC) with its confidence interval (IC) (95%). A nomogram based on the multivariate logistic regression model was developed to calculate a score called "RBC score" involving RET and FRC. All tests were two-sided at a 0.05 significance level. Analyses were carried out using R statistical software version 2.15.2 and XLSTAT 2012 from Microsoft Corporation ™.

Results

Comparisons and acceptable ranges of erythrocytes parameters between two channels

For 3901 unselected adult patients, ICC for respectively RBC vs RBC-O and HGB vs HGB-O were found at 0.990 [0.986-0.995] and 0.987 [0.982-0.992] indicating excellent concordance between impedance or photometry and optical methods. For Delta RBC%, and Delta HGB%, the 95% limits of agreement were respectively between -4.8% up to +5.5% and -5.7% up to +6.7%. Due to these results the acceptable variations were defined at ± 5 for Delta RBC% and ± 6 for Delta HGB%. Additionally, R-MFV was strictly correlated to MCV for these patients (ICC= 0.983 [0.977-0.989]). The 95% limits of agreement were between -2.1% up to 2% making both results strictly comparable.

Analysis of increased MCHC

MCHC elevated values were analysed per group for which, mean, median and limit values of erythrocytes parameters (MCHC, HGB, Delta RBC%, Delta HGB%, RET, FRC, RBC score) were noticed and studied with regards to the confirmed abnormality of patients. All results are illustrated in Table 1. The highest increase of MCHC was found for RBC agglutination group with mean value 502 g/L, whereas in group 2, 3 and 4, mean values were respectively 377, 370, 372 g/L. Concomitantly, 69 cases (54%) exceeded the defined acceptable delta% limits with either significant positive delta for RBC% (27 patients with RBC-O > RBC) or a negative one for HGB% (42 patients with HGB-O < HGB) comparing optical method to impedance and photometry. Data showed a clear difference in RBC count for patients with agglutination whereas HGB differences were marked in groups of optical interference or RBC disease. RET, FRC and RBC score appeared elevated in RBC disease compared to other groups as described in Table 1.

Table 1

Group "RBC Agglutination"

In group 1, the mean delta RBC% reached 60.2% (extreme values 13.9% to 226.1%) as described in Table 1, and the AUC of the ROC curve for the 22 patients with agglutinins with regards to the 106 patients with non-agglutinins was calculated at 0.96 with 100% sensitivity and 92% specificity (IC 95%). Leucocytes, platelets, HGB, RBC-O and R-MFV measurements at room temperature and after warming at 37°C for one hour were compared and showed no significant difference. Regression coefficients between RBC-O, R-MFV measured at room temperature and RBC, MCV after warming were higher than 0.98 for RBC-O and 0.99 for R-MFV. RBC-O was directly reported and R-MFV used to replace MCV in all 22 patients to correct the elevated MCHC whatever the initial RBC count.

Group "Optical interference"

In the 17 patients of the group optical interference with regards to chemistry indexes (8 icteric index and 9 lipemic index), the mean delta HGB% value was at -9.6% (extreme values: -0.8% to -23.5%) (Table 1). Fourteen samples out of 17 showed a delta HGB ≤ -6%. In this group, one patient showed an increased RET at 130 x 10^9/L and two others reported more than 1% FRC (1.30 and 1.14). In these three patients, the chemistry index was positive for icteric interference. HGB-0 was directly reported in all samples with delta HGB ≤ -6% in the optical interference group to correct the false MCHC.

Group "RBC disease"

The group of RBC disease included 18 patients: 15 Sickle Cell Disease (SCD) (9 S/S, 6 S/C) and 3 Hereditary Spherocytosis (HS). These patients presented a perfect correlation between both methods for RBC whereas 15 patients showed a delta HGB ≤-6%. The mean delta HGB% was -11.4% (extreme values: -1.9% to -24.5%) (Table 1). The delta HGB% was comparable to the one observed in the optical interference group but did not appear related to free plasmatic haemoglobin since plasma was checked and found clear. This observation suggested that in this group, HGB-O is underestimated due to false RBC-He measurement. Multivariate analysis of detection of RBC disease has allowed the selection of RET (Odds ratio [95%CI]: 1.04 [1.02-1.06]; *p<0.0001*) and FRC (Odds ratio [95%CI]: 4.89 [1.09-21.76]; *p=0.03*), measured by the optical channel. The established RBC score [RBC Score = 1 / (1 + exp (- (-7.6055 + 1.5873 * FRC(%) + 0.0402 * RET10^9/L))))] combining these two parameters and calculated in the nomogram (Figure 1) provided an excellent differentiation to other patients with a sensitivity at 94.4% [73%-100%] and specificity reaching 94.5% [87%-97%] at the threshold of 0.127. This score showed a better performance that the reticulocytes count at the defined threshold of 130 x 10^9/L as well as the FRC% parameter at a threshold of 0.5 %. Both thresholds were defined by ROC curve analysis (Figure 2). RBC score therefore appeared elevated in RBC disease group compared to other groups, and discriminated between group 2 and group 3. In this group, with a delta of HGB ≤- 6%, the HGB value will be conserved in absence of visible optical interference.

Figure 1

Figure 2

Group "Others"

Group "Others" included 18 patients with a hyposmolar plasma and 53 unexplained cause patients, presented a MCHC mean value of 372 g/L (extreme values 366 to 391 g/L) (Table 1). In the subgroup of 18 patients, the mean of osmolarity and hyponatremia were respectively at 248 mosmol/L and 116 mEq/L. These patients were mostly hospitalised in nephrology intensive care units. We observed that this increased MCHC was a temporary fact and disappeared when osmolarity was back to the normal range with improvement of clinical state. In the "unexplained patients", elevated MCHC was present but it could not be explained by a general approach. For few patients, various treatments were found with immunosuppressive (4 patients), chemotherapy drugs (5 patients) or antiretroviral therapy (15 patients) and uric acid increase (4 patients). So, in this group, parameters from the impedance method have to be reported to the clinicians.

To summarise, a significant increase of delta RBC > 5% is present in cases of RBC agglutination as described in box plot analysis (Figure 3a). A delta HGB ≤ -6% is significantly present in both groups optical interference and RBC disease compared to RBC agglutination and Others, respectively (Figure 3b). RBC score is significantly increased in RBC disease in comparison to the three other groups with a mean value at 0.75 (Figure 3c), suggesting that an increased RBC score would turn towards a blood smear to observe RBC morphology.

Figure 3

Discussion

The use of the optical method for RBC, HGB and reticulocytes parameters (RET, FRC), provided by the XN-10 RET analyser, associated with impedance and photometry methods, was evaluated in a retrospective cohort in the context of increased MCHC. Four groups were defined after analysis of biological and clinical results according to the increased MCHC aetiologies. In group 1 (RBC CA), spurious RBC count was obtained by impedance method. The use of optical channel with RBC-O and the use of appropriate MCV with R-MFV were reported in 100% of patients to obtain true results with a short delay, without warming sample. This manual action is not convenient because it requires time for the laboratory staff and postpones the results of patients. RBC CA could also be associated with malignant diseases or infections suggesting that the observation of leucocytes on peripheral blood smears is important for detecting malignant or reactive cells [2]. In group 2 (optical interference) and group 3 (RBC disease), delta HGB ≤-6% was principally found. In group 2 with turbidity observed in plasma, HGB measurement by photometry can be overestimated and in consequence leads to false indices: MCH and MCHC [1]. To obtain the true HGB, laboratory staff needs to replace abnormal plasma with diluent to again measure the photometric HGB. This manual method is not convenient for routine practice with a high safety risk by opening the tube as well as analytical risk with the plasma replacement. The use of the optical channel with the HGB-O instantly reported to correct the false MCHC with a short delay is therefore a good alternative in patients with latescence and/or icterus interference. It must be noticed that in case of haemolysis, it is important to be careful. In fact, it is known that some pre-analytical problems (such as delay or chaotic transport, thermal impact…) may lead to erythrocyte destruction providing an underestimation of HGB-O [5, 7]. Only in the case of “intra-vascular” haemolysis the HGB-O value will be reported with regards to clinical or biological signs. Our data analysis also let us observe that delta HGB was ≤-6% in 83% of RBC disease patients without any visible optical interference. Usually, in normal patients, HGB-O derived from RBC-

He is concordant with MCH whereas in sickle cell patients of the cohort, RBC-He is systematically underestimated compared to MCH. Optical proprieties of RBCs depend on size, shape and HGB content, so we can understand that in the presence of an important heterogeneity of RBC in volume and/or haemoglobin content observed in SCD, the HGB-O value is wrong and only photometric HGB will be reported in absence of haemolytic plasma [8]. In RBC diseases, the intrinsic RBC membrane defect in HS, the HGB-S polymerisation in SCD as well as the oxidative alterations induce modifications of permeability of cations leading to a leak of K+ and water loss of RBC [9, 10]. This intracellular dehydration results in dense erythrocytes or spherocytes observed on the peripheral blood smears and often associated to increased MCHC and abnormal erythrocyte distribution in HA [11-14]. Additionally, in SCD and HS, membrane alterations induce the release of microparticles, which result in a reduced surface-to-volume ratio of RBC and an increase in MCHC [9, 15]. In the case of proven RBC disease and in absence of optical interference, the elevated MCHC is true and will be reported to the clinicians with HGB value obtained by photometry. Reticulocyte counts and FRC were elevated in group 3 permitting to establish an RBC score, issued by a nomogram. In the cohort, this RBC score at the threshold of 0.127 discriminates patients with RBC disease from other patients with a sensitivity of 94.4% and specificity of 94.5%. This RBC score and the delta HGB are of great interest in patients with haemolytic diseases. Both are linked to Log RBC-HGB/RET-HGB studied by Maier *and al* as a marker of the erythropoietic stress due to haemolysis in sickle cell patients [16]. This RBC score needs to be studied in a large prospective study in parallel with clinical status to confirm its importance. In our cohort, the use of optical parameters did not allow the possibility of correcting the elevated MCHC in 71 patients where 18 patients had ionic trouble with deep hyposmolar plasma. Plasmatic osmolarity influences the volume and the shape of cells, usually in a hyposmolar environment where the cells are enlarged [17]. Differences exist between *in vivo* RBC and *ex vivo* RBC in diluent used by HA [18, 19]. The depth of hyposmolarity influences the cell volume measurement in the analyser [8, 20]. In the literature, elevated MCHC was found in patients treated by antiretroviral therapy, immunosuppressive drugs or chemotherapy [1]. In this group, it most frequently traduces a temporary state with no analytical interferences or RBC abnormalities identified. Finally, when the different aetiologies explaining an elevated MCHC are not found, it is recommended to verify that the pre-analytical conditions respect the requirements of instrument manufacturer. These conditions (delay of transport, delay of analysis, insufficient blood volume drawn after venepuncture in presence of salts K2-EDTA or K3…) are essential for guaranteeing accurate results.

Figure 4

To sum up, a high MCHC threshold fixed at 366 g/L was defined with regards to our results for the use of optical parameters (RBC-O, HGB-O, RET, FRC). From data analysed in this retrospective study, a "decision tree" (Figure 4) was established with the aim of providing guidance in terms of biological interpretations. The first question is to know which delta is involved:

- In the case of delta RBC > 5%, the MCHC will be recalculated with new parameters such as RBC-O and R-MFV. The use of HGB-O will depend on plasma aspect.

- In the case of delta RBC ≤ 5% and delta HGB ≤ -6%, the RBC score value will be studied. If RBC score is > 0.127, a blood smear will be performed to observe morphology RBC and MCHC won't be recalculated. If it is ≤ 0.127, MCHC will be recalculated with HGB-O in the presence of optical interference.

- In the case of delta RBC ≤ 5% and delta HGB > -6%, pre-analytical conditions and osmolarity will be checked and MCHC won't be recalculated.

To conclude, in case of elevated MCHC, our study proves the capability of XN-10 RET optical parameters to provide solutions in the majority of cases, especially concerning RBC CA and optical interference. The calculated RBC score offers a highly useful tool for managing a blood smear and specifying patients with RBC disease. This original study allows optimisation of the workflow in laboratories eliminating manual tasks, guiding biological interpretation in the case of elevated MCHC. All this will significantly reduce the delay for result delivery to the clinicians and help them in their diagnosis approach.

Table :

		MCHC(g/L)	HGB(g/L)	delta RBC%	delta HGB%	RET#(10^9/uL)	[FRC%(%)]	RBC score
RBC agglutination	Mean	502,14	106,95	**60,2%**	-2,7%	59,20	0,30	0,07
	Median	437,00	106,00	47,4%	-2,7%	50,70	0,11	0,01
	lower value	367,00	61,00	13,9%	-10,9%	7,80	0,00	0,00
	upper value	1031,00	151,00	226,1%	4,8%	207,30	2,92	0,80
Optical interference	Mean	377,17	130,61	1,2%	**-9,6%**	86,00	0,24	0,05
	Median	372,00	126,00	1,6%	-8,5%	87,70	0,00	0,02
	lower value	366,00	68,00	-4,2%	-23,5%	7,70	0,00	0,00
	upper value	445,00	189,00	4,7%	-0,8%	158,40	1,30	0,22
RBC disease	Mean	369,83	102,72	-0,4%	**-11,4%**	**211,80**	**1,39**	**0,75**
	Median	369,00	99,00	-0,6%	-11,4%	202,00	0,80	0,92
	lower value	366,00	51,00	-4,8%	-24,5%	73,30	0,00	0,01
	upper value	376,00	164,00	10,8%	-1,9%	373,60	5,89	1,00
Others	Mean	371,83	129,47	1,9%	-3,3%	69,50	0,13	0,03
	Median	370,00	136,00	1,9%	-3,2%	70,60	0,00	0,01
	lower value	366,00	52,00	-3,3%	-12,0%	4,40	0,00	0,00
	upper value	391,00	184,00	11,2%	5,6%	184,00	0,90	0,45

Table 1: Characteristics of patients' cohort sub-divided into four groups: RBC agglutination, Optical interference, RBC disease, Others. Mean, Median lower and upper values for Mean Corpuscular Haemoglobin Concentration (MCHC), Haemoglobin (HGB), Delta Red Blood Cells (Delta RBC%), Delta Haemoglobin (Delta HGB%), Reticulocyte counts (RET#), Fragmented Red Cells (FRC%) and RBC score are described in each group of patients.

Footnotes and Figure legends:

Footnotes : MCHC: Mean Corpuscular Haemoglobin Concentration, HGB: Haemoglobin, Delta RBC: Delta Red Blood Cells, Delta HGB: Delta Haemoglobin, RET: Reticulocyte counts, FRC: Fragmented Red Cells, RBC score : Red Blood Cells score.

Figure legends:

Figure 1: Nomogram based on FRC% and reticulocyte counts (RET) for predicting RBC disease. The first line corresponds to the number of points given for each parameter value: FRC (%) and RET (10^9/L) (represented in the second and third line, respectively). Total points obtained are represented in the fourth line and the fifth line allows to determine the probability of detecting an RBC disease.

For example, a patient with FRC of 2% (20 points) and with RET of 100 x 10^9/L (25 points), the nomogram would predict an RBC score of 0.40 (total points: 45).

Figure 2: ROC curves for RBC disease detection. Area under the ROC curve for RBC score (in bold line) is higher than reticulocyte count ROC curve (in dotted line) and FRC ROC curve (in dashed and dotted line).

Figure 3: Box plots comparison between four groups (RBC agglutination (red), Optical interference (green), RBC disease (dark blue) and Others (clear blue)) for three parameters: Delta RBC%, Delta HGB% and RBC score. a) Delta RBC % is significantly increased in RBC agglutination compared to the three other groups. ($P < 0.0001$), b) Negative Delta HGB% is significantly increased in both groups: optical interference and RBC disease in comparison with RBC agglutination and "Others" group (respectively $P = 0.001$, $P < 0.0001$, $P = 0.0001$, $P < 0.0001$), c) RBC score is significantly increased in RBC disease in contrast to RBC agglutination, Optical interference and "Others" groups ($P < 0.0001$).

Figure 4: "Decision Tree" defined in case of elevated MCHC > 365 g/L. Different actions will be required as a result of the findings: -Delta RBC% and/or -Delta HGB% and/or -plasma aspect and/or RBC score. RBC corresponds to RBC measured by the impedance method and HGB corresponds to HGB measured by the photometry process. RBC-O for RBC measured by optical channel, HGB-O for HGB measured by optical channel, R-MFV for Red blood cells Most Frequent Volume, defined as the peak of the RBC curve distribution.

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Conflict of interests

The authors have no competing interest.

Authors’ contribution

All authors participated substantially so as to be considered authors in this paper. All authors read and approved the final manuscript.

Y. Berda-Haddad, M. Arpin, R. Lacroix and F. Dignat-George designed the research study.

Y. Berda-Haddad, C. Faure, M. Boubaya, M. Arpin, S. Cointe and D. Frankel performed the research and analysed the data.

Y. Berda-Haddad, C. Faure, M. Boubaya wrote the paper.

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Table and Figure caption List :

Table 1: Characteristics of patients' cohort sub-divided into four groups: RBC agglutination, Optical interference, RBC disease, Others. Mean, Median lower and upper values for Mean Corpuscular Haemoglobin Concentration (MCHC), Haemoglobin (HGB), Delta Red Blood Cells (Delta RBC%), Delta Haemoglobin (Delta HGB%), Reticulocyte counts (RET#), Fragmented Red Cells (FRC%) and RBC score are described in each group of patients.

Figure 1: Nomogram based on FRC% and reticulocyte counts (RET) for predicting RBC disease. The first line corresponds to the number of points given for each parameter value: FRC (%) and RET (10^9/L) (represented in the second and third line, respectively). Total points obtained are represented in the fourth line and the fifth line allows to determine the probability of detecting an RBC disease.

For example, a patient with FRC of 2% (20 points) and with RET of 100 x 10^9/L (25 points), the nomogram would predict an RBC score of 0.40 (total points: 45).

Figure 2: ROC curves for RBC disease detection. Area under the ROC curve for RBC score (in bold line) is higher than reticulocyte count ROC curve (in dotted line) and FRC ROC curve (in dashed and dotted line).

Figure 3: Box plots comparison between four groups (RBC agglutination (red), Optical interference (green), RBC disease (dark blue) and Others (clear blue)) for three parameters: Delta RBC%, Delta HGB% and RBC score. a) Delta RBC % is significantly increased in RBC agglutination compared to the three other groups. (*P < 0.0001*), b) Negative Delta HGB% is significantly increased in both groups: optical interference and RBC disease in comparison with RBC agglutination and "Others" group (respectively *P = 0.001, P < 0.0001, P = 0.0001, P < 0.0001),* c) RBC score is significantly increased in RBC disease in contrast to RBC agglutination, Optical interference and "Others" groups (*P < 0.0001)*.

Figure 4: "Decision Tree" defined in case of elevated MCHC > 365 g/L. Different actions will be required as a result of the findings: -Delta RBC% and/or -Delta HGB% and/or -plasma aspect and/or RBC score. RBC corresponds to RBC measured by the impedance method and HGB corresponds to HGB measured by the photometry process. RBC-O for RBC measured by optical channel, HGB-O for HGB measured by optical channel, R-MFV for Red blood cells Most Frequent Volume, defined as the peak of the RBC curve distribution.