OTHERS

Original article

Preliminary assessment of the new Sysmex XN parameter Iron-Def for identifying iron deficiency

Vincenzo Roccaforte[1,2], Massimo Daves[3], Giuseppe Lippi[4], Andrea Piccin[5], Flavia Sciarini[1], Vanessa Proserpio[1], Claudio Bonato[1]

[1]*Clinical Pathology Laboratory, Hospital Alessandro Manzoni, Lecco, Italy;*
[2]*Clinical Pathology and Clinical Biochemistry, University of Milan, Milan, Italy;*
[3]*Clinical Biochemical Laboratory, Hospital of Bolzano, Bolzano, Italy;*
[4]*Section of Clinical Biochemistry, University of Verona, Verona, Italy;*
[5]*Haematology Department, Children's Health Ireland, Dublin, Ireland*

Background - Although the clinical assessment of iron status is usually based on iron stores, a rapid and accurate diagnosis of iron deficiency is challenging since ferritin is often unavailable as an urgent test and its value is frequently increased in acute phase conditions. This study was therefore aimed at evaluating the diagnostic performance of the new Sysmex XN "Iron Deficiency?" (Iron-Def) parameter for identifying patients with iron deficiency.
Materials and methods - The study population consisted of 688 consecutive patients (median age: 71 years; 341 women and 347 men), referred for routine diagnostics to the Laboratory of Clinical Pathology of Lecco Hospital, Italy. A complete clinical chemistry profile and haematological testing were performed for identifying iron deficiency anaemia.
Results - A significant negative correlation was found between Sysmex XN Iron-Def and ferritin, serum iron, mean cell haemoglobin concentration, mean cell haemoglobin, mean corpuscular volume and age, while a positive correlation was noted with transferrin, percentage of microcytic red cell, red blood cell count and red blood cell distribution width. The diagnostic accuracy of Iron-Def for identifying patients with a percentage of saturation of transferrin <15% (n=104) was 84%, with a sensitivity of 0.952 and specificity of 0.538. A sub-analysis of 71 patients with ferritin <20 ng/dL yielded an even better diagnostic performance (86%, with a sensitivity of 0.935 and specificity of 0.620).
Discussion - Although additional confirmatory investigations would be needed, the preliminary findings of our study attest that Iron-Def may be an easy, inexpensive, rapid and reliable parameter for screening iron deficiency anaemia.

Keywords: *anaemia, iron deficiency, ferritin, laboratory testing.*

INTRODUCTION

Iron deficiency is the most common nutritional deficiency around the world. This condition is currently regarded as a major global healthcare issue, given that approximately 2 billion people (i.e., over 30% of the worldwide population) have been diagnosed with iron deficiency anaemia[1]. Iron deficiency can be conventionally defined as a health-related condition, in which iron availability is insufficient to meet the body

Arrived: 23 March 2020
Revision accepted: 21 May 2020
Correspondence: Massimo Daves
e-mail: massimo.daves@sabes.com

Blood Transfus 2020; 18: 406-12 DOI 10.2450/2020.0070-20
© SIMTIPRO Srl
All rights reserved - For personal use only
No other use without premission
© SIMTIPRO Srl

needs and can manifest with or without anaemia[2], thus clearly underpinning why rapidly growing children and premenopausal women are at higher risk. Dietary insufficiency as a cause of iron deficiency is secondary to intestinal blood loss due to parasitosis in many countries around the world[3].

Early detection and prompt treatment of iron deficiency are both essential because of the crucial role played by iron in human physiology. Many studies have shown that iron deficiency is associated with longer stays in hospital, cognitive dysfunction, increased risk of falls, as well as with overall reduced life expectancy[4]. In the presence of an adequate nutritional iron supply and without concomitant inflammatory conditions, systemic homeostasis maintains plasma iron levels between 10-30 μmol/L and whole-body iron stores between 0.3-1.0 g[5]. The leading mechanism of iron homeostasis encompasses an interplay between hepcidin, a hormone produced by liver, and its receptor ferroportin, the iron exporter that allows the passage of iron from intestinal cells, macrophages and hepatocytes into the plasma[5].

The clinical assessment of iron status has long been based on body stores of this mineral, which is reflected by serum ferritin concentration[6]. Nevertheless, the accurate interpretation of ferritin levels may be challenging when iron deficiency is associated with inflammation, because ferritin is an acute phase protein and its levels may therefore be within the normal range despite the presence of true iron deficiency. The assessment of this biomarker is also rarely available as an urgent test in most laboratory medicine services, so that the diagnosis of iron deficiency may be delayed by hours or days, depending on local organisation.

Along with well-known laboratory parameters such as ferritin, transferrin, transferrin saturation and serum iron, some other tests with different diagnostic performance are available to investigate iron status. These typically include mean corpuscular volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), percentage of hypochromic red blood cells (%HRC), reticulocyte haemoglobin content (CHr), red cell zinc protoporphyrin concentration, along with soluble transferrin receptor (sTfR) and hepcidin concentrations[6]. The percentage of microcytic red blood cells (Micro%) is another emerging parameter, which can be useful in combination with other red blood cell parameters for differential diagnosis of microcytic anaemia[7]. An assessment of iron stores in bone marrow can be made using Perls' Prussian blue reaction. Although it has long been regarded as the gold standard technique, this test has some notable diagnostic drawbacks in terms of inaccuracy and imprecision, while it is also invasive and uncomfortable, carrying the risk of some clinically important complications.

Iron deficiency can be conventionally classified in two well-defined entities, absolute and functional, which have different aetiologies, as well as diverse clinical and laboratory presentations[1]. The major difference between these two types of iron deficiency is that total iron body stores may be normal or even increased in functional iron deficiency, whereas they are typically decreased in absolute iron deficiency. Inflammation is the leading cause of functional iron deficiency, though ferritin values may be may misleading for assessing iron status in this condition, as previously discussed. One important consequence of functional and total iron deficiency is the appearance of abnormalities in haemoglobin production. Since the search for new and simply laboratory parameters for diagnosing both functional and total iron deficiency is of pivotal importance, we investigated the diagnostic performance of the new parameter "Iron Deficiency?" (Iron-Def) generated by Sysmex XN 9000 haematology analysers (Sysmex©, Kobe, Japan).

MATERIALS AND METHODS

The study population consisted of 688 consecutive patients (median age: 71 years, interquartile range [IQR] 55-81 years), referred to the Laboratory of Clinical Pathology of the Hospital of Lecco (Italy), in whom the following laboratory tests were performed: serum iron, ferritin, transferrin, percentage of saturation of transferrin (TSAT) and complete blood cell count (CBC). Of these 688 patients, 341 were women (median age: 73 years, IQR 52-83 years) and 347 were men (median age: 71, IQR 58-80 years). The CBC was performed using a Sysmex XN haematology analyser, which enumerates and classifies blood cells by means of flow cytometry. More specifically, cell enumeration is accomplished with semiconductor laser counting and cells are classified by irradiation with a 633 nm laser beam and analysis of forward scattered light, side scattered light and side fluorescent light. The three signals are integrated to differentiate and count

© SIMTIPRO Srl

All rights reserved - For personal use only
No other use without premission

white blood cells, nucleated red blood cells, reticulocytes and platelets, as well as to detect abnormal and immature cells[8]. The performance of the Sysmex XN-series has been extensively evaluated by Seo *et al*., as described elsewhere[9]. Along with conventional parameters, Sysmex XN also generates an additional semi-quantitative parameter, defined "Iron-Def", whose values varies between 50 and 110. This parameter is triggered when MCHC and MCV values are decreased and the RDW-coefficient of variation is increased. The definitive underlying conditions of the flags are defined as "confidential" by Sysmex.

This combination of parameters typically characterises microcytic hypochromic anaemia due to iron deficiency. The presence of this flag should prompt additional investigations and confirmatory tests for the final diagnosis of iron deficiency anaemia. All plasma samples for assessing biochemical parameters were analysed with the automated clinical chemistry and immunochemistry analyser Dimension Vista® 1500 System (Siemens Healthcare Diagnostics, Eschborn, Germany), using proprietary reagents and according to the manufacturer's recommendations.

The correlation among the various biochemical and haematological parameters was based on Spearman's rank order test. Receiver operating characteristic curves (ROC) were constructed to explore the diagnostic performance of the Iron-Def value for detecting iron deficiency, expressed as area under the curve (AUC). Since we also aimed to identify patients with biochemical evidence of iron deficiency, we analysed the performance of Iron-Def in subjects with simultaneously low ferritin values (i.e., <20 ng/dL) and decreased value of TSAT (i.e., <15%). A serum ferritin level <30 ng/mL is the most sensitive (92%) and specific (98%) cut-off level for the identification of absolute iron deficiency, with or without anaemia, but most clinical laboratories use lower cut-off levels (i.e., between 15-25 ng/mL). The lower limit of ferritin reference range in the local laboratory is 20 ng/dL, and has been defined using internal procedures for reference range calculation, by measuring samples drawn from 120 healthy blood donors. Notably, although it is generally thought that serum ferritin values within the normal range shall efficiently rule out iron deficiency, ferritin is an acute-positive phase protein and its levels are profoundly influenced by inflammation[9]. For this reason, in patients with TSAT <15% and ferritin >20 ng/dL, C-reactive protein values were also measured to rule out the presence of an active inflammatory process. Statistical analysis was performed using the MedCalc statistical package (version 17.4.4., MedCalc Software Mariakerke Ltd, Ostend, Belgium). The value of statistical significance was set at $p<0.05$.

The investigation was based on pre-existing data extracted from the Laboratory Informatics System (LIS). All results were anonymised before evaluation, so that ethical permission and informed consent were unnecessary. The study was performed in accordance with the Declaration of Helsinki and under the terms of all relevant local legislations.

RESULTS

The values of the different parameters measured in this study are summarised in **Table I**. The Shapiro-Wilk test for normal distribution showed that all these parameters were non-normally distributed, so that they are displayed as median, 95% confidence interval (95% CI) and interquartile range (IQR).

The main results of our investigation are shown in **Table II**. A significant negative correlation was observed between Iron-Def and ferritin, serum iron, MCHC, MCH, MCV, RDW SD_fL and age, whereas a positive correlation could be found with transferrin, Micro%, red blood cell count and RDW%. No significant correlation was observed with haemoglobin and haematocrit. The diagnostic accuracy of Iron-Def with TSAT <15% was 84% (AUC, 0.837; 95% CI: 0.801-0.872), as shown in **Figure 1**. The best cut-off of Iron-Def was 90, which had a sensitivity of 0.952 and a specificity of 0.538. The sub-analysis of 71 patients with TSAT <15% and ferritin <20 ng/dL (**Table III**) is shown in **Figure 2**, highlighting that the diagnostic performance of Iron-Def was even better (AUC, 0.856; 95% CI: 0.817-0.895), and thus confirming the good diagnostic value of this index for detecting iron deficiency (**Figure 2**). The sensitivity and specificity of the Iron-Def parameter in this subset of patients were as high as 0.935 and 0.620, respectively. Interestingly, the diagnostic performance of Iron-Def in the 33 patients with TSAT <15%, ferritin >20 ng/dL and concomitantly increased C-reactive protein values (**Table IV**) was also good (AUC, 0.858).

DISCUSSION

Despite the many diagnostic advancements that have been made in laboratory haematology, the diagnosis of iron

All rights reserved - For personal use only
No other use without premission

© SIMTIPRO Srl

Table I - *Summary statistics of the parameters evaluated in the study*

Parameters	Shapiro-Wilk test for normal distribution	Median	95% CI for the median	IQR
Ferritin ng/dL	reject normality (p<0.0001)	130	107 to 149	284
Serum iron µg/dL	reject normality (p<0.0001)	60	38 to 84	45
Transferrin mg/dL	reject normality (p=0.0005)	210	205 to 216	81
TSAT %	reject normality (p<0.0001)	21	20 to 22	15
Hb g/L	reject normality (p=0.0005)	114	113 to 116	26
MCHC g/L	reject normality (p<0.0001)	322	321 to 323	17
MCH pg	reject normality (p<0.0001)	296	293 to 298	33
MCV fL	reject normality (p<0.0001)	914	910 to 921	85
MicroR%	reject normality (p<0.0001)	18	17 to 20	28
RBC (10^{12}/L)	reject normality (p<0.0001)	392	386 to 398	102
RDW-CV(%)	reject normality (p<0.0001)	146	144 to 146.19	146
RDW-SD (fL)	reject normality (p<0.0001)	485	481 to 491	485
Iron-Def	reject normality (p<0.0001)	80	80 to 80	10

95% CI: 95% confidence interval; IQR: interquartile range; TSAT: transferrin saturation; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; RBC: red blood cells; RDW: red blood cell distribution width; CV: coefficient of variation; SD; standard deviation; Iron-Def: Iron Deficiency?

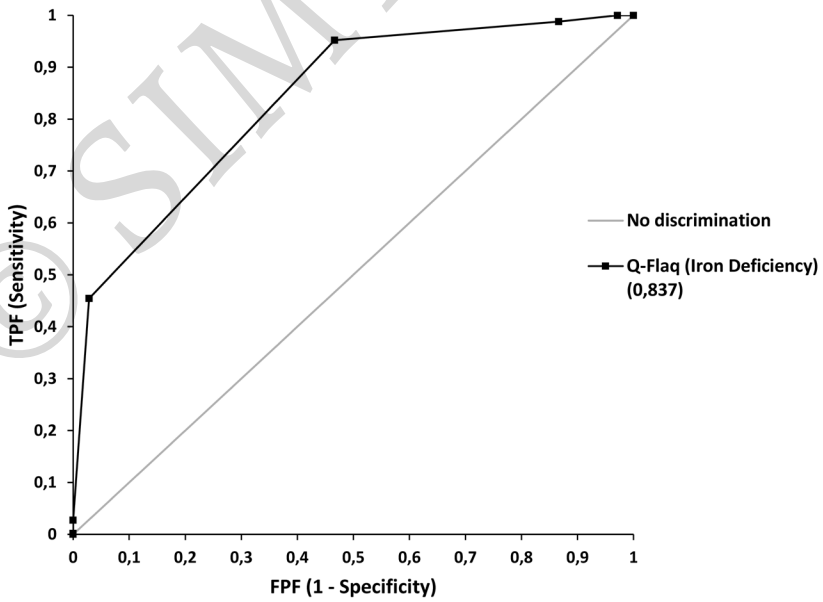


Figure 1 - Diagnostic accuracy of the Sysmex XN Iron-Def parameter in patients with transferrin saturation <15% (n=104)

©SIMTIPRO Srl

All rights reserved - For personal use only
No other use without premission

Table II - *Spearman's coefficient of rank correlation with the Iron-Def parameter*

Parameters	(Rho) 95% CI	p value
TSAT % *vs* **Iron-Def**	−0.365 −0.428 to −0.299	p<0.001
Ferritin ng/dL *vs* **Iron-Def**	−0.356 −0.421 to −0.287	p<0.001
Transferrin mg/dL *vs* **Iron-Def**	0.314 0.245 to 0.380	p<0.001
Iron µg/dL *vs* **Iron-Def**	−0.242 −0.311 to −0.171	p<0.001
Hb g/L *vs* **Iron-Def**	−0.013 −0.0877 to 0.0618	p=0.733
MCHC g/L *vs* **Iron-Def**	−0.211 −0.281 to −0.139	p<0.001
MCH pg *vs* **Iron-Def**	−0.826 −0.848 to −0.801	p<0.001
MCV fl *vs* **Iron-Def**	−0.897 −0.911 to −0.882	p<0.001
MicroR% *vs* **Iron-Def**	0.742 0.707 to 0.774	p<0.001
RBC (10^{12}/L) *vs* **Iron-Def**	0.428 0.365 to 0.487	p<0.001
HTC *vs* **Iron-Def**	0.043 −0.0312 to 0.118	p=0.253
RDW SD (fL) *vs* **Iron-Def**	−0.310 −0.376 to −0.241	p<0.001
RDW CV% *vs* **Iron-Def**	0.242 0.170 to 0.311	p<0.001
Age *vs* **Iron-Def**	−0.194 −0.265 to −0.121	p<0.001

Rho: Spearman's coefficient of rank correlation; 95% CI: 95% confidence interval; TSAT: transferrin saturation; Iron-Def: Iron Deficiency?; HB: haemoglobin; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; MicroR%: percentage of microcytic red cells; RBC: red blood cells; RDW: red blood cell distribution width; SD: standard deviation; CV: coefficient of variation.

deficiency anaemia still relies heavily on a discrete number of traditional investigations, including haemoglobin, ferritin, TSAT and serum iron[10]. Innovative diagnostic tools would therefore be welcomed for earlier and more efficient identification of subjects at risk of this condition. This study is the first that has explored the diagnostic performance of the new Sysmex XN Iron-Def parameter for identifying iron deficiency anaemia, to the best of our knowledge. Notably, this index is automatically calculated by the XN haematological analyser, so that it can be provided with no expense along with other parameters of the CBC. Taken together, the results of our investigation show that Iron-Def displays a good diagnostic performance for identifying a subset of patients with TSAT <15%, which is highly suggestive of iron deficiency. This already good performance (i.e., 84%, with a sensitivity of 0.95 and specificity of 0.54), is then magnified in patients with ferritin ≤20 ng/dL (i.e., 86%, with a sensitivity of 0.94 and specificity of 0.62), thus confirming that this parameter may be a promising index for initial screening of iron deficiency even before measuring ferritin, TSAT and serum iron. As regards the potential clinical applications of this new parameter, Munoz *et al*. recently highlighted the importance of identifying patients with iron deficiency anaemia in the Emergency Department[11]. Based on a cost-analysis, treating patients with iron deficiency by administering ferric carboxymaltose may be a cost-saving approach compared with conventional outpatient care[12]. The Iron-Def parameter is clearly meant to be used as a screening tool, for which a high sensitivity is an obvious premise. The lower specificity implies that other tests, such as hypochromic fraction of erythrocytes and reticulocyte haemoglobin equivalent[13] should be used for a definitive diagnosis, within an integrated diagnostic approach in which Iron-Def may be placed at an initial stage.

This study has some limitations which should be acknowledged. One is the relatively limited and heterogeneous sample population. Another important drawback is the lack of precise clinical information that could influence the new Iron-Def parameter. Furthermore, unfortunately, we have no data to predict the behaviour of Iron-Def in other causes of hypochromic hypochromia, such as thalassaemia and other diseases. Further studies will therefore be needed to evaluate this new parameter better in different conditions.

© SIMTIPRO Srl

Blood Transfus 2020; 18: 406-12 DOI 10.2450/2020.0070-20

All rights reserved - For personal use only
No other use without premission

Table III - *Summary statistics of the evaluated parameters in the sub-analysis of the 71 patients with transferrin saturation <15% and ferritin <20 ng/dL*

Parameters	Shapiro-Wilk test for normal distribution	Median	95% CI for the median	IQR
Ferritin ng/dL	reject normality (p<0.0001)	11	107 to 149	8
Serum iron µg/dL	reject normality (p<0.0001)	28	38 to 84	20
Transferrin mg/dL	reject normality (p=0.0005)	288	205 to 216	96
TSAT %	reject normality (p<0.0001)	7,7	20 to 22	5,2
Hb g/L	reject normality (p=0.0005)	113	113 to 116	37
MCHC g/L	reject normality (p<0.0001)	309	321 to 323	24
MCH pg	reject normality (p<0.0001)	252	293 to 298	43
MCV fL	reject normality (p<0.0001)	824	910 to 921	105
MicroR%	reject normality (p<0.0001)	83	17 to 20	109
RBC (10^{12}/L)	reject normality (p<0.0001)	447	386 to 398	91
RDW-CV(%)	reject normality (p<0.0001)	163	144 to 146.19	39
RDW-SD (fL)	reject normality (p<0.0001)	466	481 to 491	100
Iron-Def	reject normality (p<0.0001)	90	80 to 80	10

95% CI: 95% confidence interval; IQR: interquartile range; TSAT: transferrin saturation; Hb: haemoglobin; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; MicroR%: percentage of microcytic red cells; RBC: red blood cells; RDW: red blood cell distribution width; CV: coefficient of variation; SD: standard deviation; Iron-Def: Iron Deficiency?

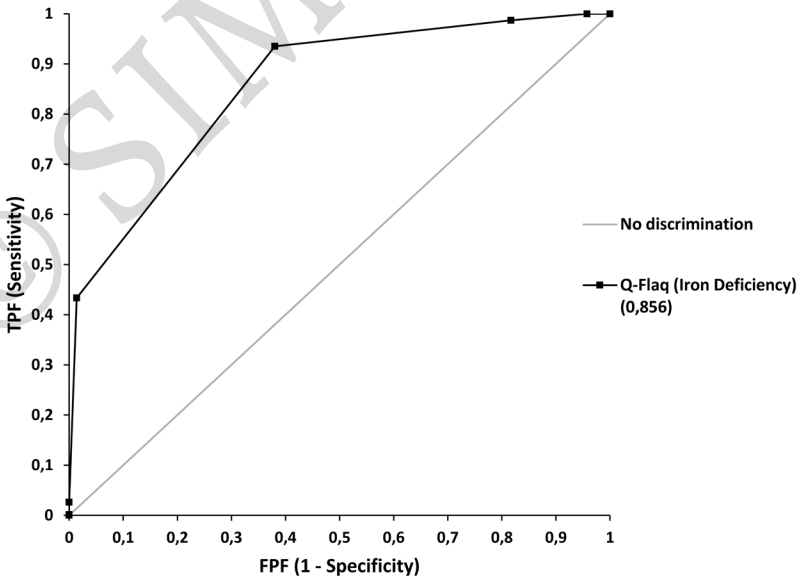


Figure 2 - Diagnostic accuracy of the Sysmex XN Iron-Def parameter in patients with transferrin saturation <15% and ferritin ≤ 20 ng/dL (n=71)

©SIMTIPRO Srl

All rights reserved - For personal use only
No other use without premission

Table IV - *Summary statistics of the evaluated parameters in the sub-analysis of the 33 patients with transferrin saturation <15%, ferritin >20 ng/dL and concomitantly increased C-reactive protein values*

Parameters	Shapiro-Wilk test for normal distribution	Median	95% CI for the median	IQR
Ferritin ng/dL	reject Normality (p<0.0001)	142	71 to 217	255
Serum iron µg/dL	reject normality (p<0.0001)	26	19 to 28	14
Transferrin mg/dL	reject normality (p=0.0005)	192	166 to 233	86
TSAT %	reject normality (p<0.0001)	8,5	7,8 to 9,9	4
Hb g/L	reject normality (p=0.0005)	102	95 to 107	21
MCHC g/L	reject normality (p<0.0001)	318	310 to 322	18
MCH pg	reject normality (p<0.0001)	274	266 to 282	34
MCV fL	reject normality (p<0.0001)	873	824 to 892	81
MicroR%	reject normality (p<0.0001)	47	36 to 65	48
RBC (10^{12}/L)	reject normality (p<0.0001)	382	361 to 400	66
RDW-CV(%)	reject normality (p<0.0001)	156	150 to 172	34
RDW-SD (fL)	reject normality (P<0.0001)	491	479 to 513	64
Iron-Def	reject normality (p<0.0001)	90	80 to 90	10

95% CI: 95% confidence interval; IQR: interquartile range; TSAT: transferrin saturation; Hb: haemoglobin; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; MicroR%: percentage of microcytic red cells; RBC: red blood cells; RDW: red blood cell distribution width; CV: coefficient of variation; SD: standard deviation; Iron-Def: Iron Deficiency?

CONCLUSIONS

In conclusion, the results of our study suggest that Iron-Def may be used as easy, inexpensive and rapid parameter for initial screening for iron deficiency anaemia. Confirmatory investigations are needed to confirm our preliminary findings.

AUTHORSHIP CONTRIBUTIONS

RV and DM contributed equally to this work.
Conception and design: RV, DM and BC. Collection and assembly of data: RV, PA, PV, DM. Provision of study materials or patients: RV, DM, SF. Data analysis and interpretation: all Authors. Manuscript writing: LG, DM and RV. Final approval of manuscript: all Authors.

The Authors declare no conflicts of interest.

REFERENCES

1. Carcoub P, Vandewalle C, Peoc'h K. Using transferrin saturation as a diagnostic criterion for iron deficiency: a systematic review. Crit Rev Clin Lab Sci 2019; **56**: 526-32.
2. Cappellini MD, Comin-Colet J, de Francisco A et al. Iron deficiency across chronic inflammatory conditions: international expert opinion on definition, diagnosis, and management. Am J Hematol 2017; 92: 1068-78.
3. Andrews NC. Disorders of iron metabolism. N Engl J Med 1999; **341**: 1986-94.
4. Keshav S, Stevens R. New concepts in iron deficiency anaemia. Br J Gen Pract 2017; **67**: 10-1.
5. Ganz T. Anemia of inflammation. N Engl J Med 2019; **381**: 1148-57.
6. Thomas DW, Hincliffe RF, Briggs C, et al. Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol 2013; **161**: 639-48.
7. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how?. Int Jnl Lab Hem 2016; **38** (Suppl.1): 123-32.
8. Daves M, Zagler EM, Cemin R, et al. Sample stability for complete blood cell count using the Sysmex XN haematological analyser. Blood Transfus 2015; **13**: 576-82.
9. Seo YJ, S.-T. Lee, S.H. Kim. Performance evaluation of the new hematology analyzer Sysmex Xn-series. Int Jnl Lab Hem 2015; **37**: 155-64.
10. Lippi G, Plebani M. Recent developments and innovations in red blood cells diagnostics. J Lab Precis Med 2018; **3**: 68.
11. Muñoz M, Gómez-Ramírez S, Besser M, et al. Current misconceptions in diagnosis and management of iron deficiency. Blood Transfus 2017; **15**: 422-37.
12. Quintana-Díaz M, Muñoz-Romo R, Gómez-Ramírez S, et al. A fast-track anaemia clinic in the Emergency Department: cost-analysis of intravenous iron administration for treating iron-deficiency anaemia. Blood Transfus 2017; **15**: 438-46.
13. Urrechaga E, Boveda O, Aguayo FJ, et al. Percentage of hypochromic erythrocytes and reticulocyte hemoglobin equivalent predictors of response to intravenous iron in hemodialysis patients. Int J Lab Hematol. 2016; **38**: 360-5.

All rights reserved - For personal use only
No other use without premission

© SIMTIPRO Srl