Red Cell Disorders

Clinical utility of the new Sysmex XE 2100 parameter - reticulocyte hemoglobin equivalent - in the diagnosis of anemia

Our aim was to establish the reference values of the new parameter reticulocyte hemoglobin equivalent (RET-He) and to investigate its role in differentiating between iron deficiency anemia and anemia of chronic diseases. We found that RET-He was useful for diagnosing iron deficiency anemia. A cut-off point of 25 pg provided a specificity of 0.81 and a sensitivity of 0.76.

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The reticulocyte channel of the Sysmex XE-2100 counter (Sysmex, Japan) provides a parameter called RET-Y by measuring the forward light scatter histogram, expressed in arbitrary units (channel number). This parameter is dependent on the hemoglobin content of the reticulocytes (CHr). A high correlation between RET-Y and the CHr parameter (ADVIA-120) has been shown.[1-2] Both are suitable parameters for monitoring iron deficiency.[3-6] RET-Y can be transformed into RET-He, expressed as picograms, by applying a recently published regression plot (RET-He=5.5569$e^{0.001RET\text{-}Y}$).[1]

In clinical practice, differentiating between iron deficiency anemia (IDA) and anemia of chronic disorders (ACD) can be difficult in some patients.[7] Despite their limitations, serum transferrin receptor (sTfR) and the ratio of sTfR/log serum ferritin (sFt) are useful variables for this purpose.[8-9] The aim of our study was to establish the reference values of RET-He and to investigate its role in the study of IDA versus ACD.

A total of 504 peripheral blood samples were analyzed. There were 196 samples from healthy individuals (reference group), 53 from patients with ACD and 127 from patients with iron-deficiency, including 45 cases of IDA, 36 cases of mild-IDA and 46 cases of storage iron deficiency (Table 1). RET-He was also investigated in 95 patients with mixed analytical patterns (mixed group) and in 33 with thalassemia trait. Iron parameters, including sFt and sTfR, were evaluated by automated methods (Tinaquant, Roche Diagnostics). A diagnostic study of IDA and ACD calculated the cut-off points by logistic regressions and receiver-operating characteristic (ROC)

Table 1. Characteristics of the groups.

	n	*Hb (g/L)*	*sFt (µg/L)*	*sTfR (mg/L)*	*RET-He (pg)*
Reference group[1]	196	M: 148±8.8 (135-172) F: 134±7.3 (121-153)	99±93 (14-432)	3.07±0.7 (1.9-4.9)	33.4±1.5 (29.8-37.7)
ACD	53	M: 98±13.9 (70-120) F: 91±9.4 (76-109)	810±877 (101-4657)	3.1±1.1 (1.1-6.5)	29.2±3.6 (21.4-35.1)
IDA	45	M: 89±19.4 (48-119) F: 82±18.7 (39-108)	8±5 (2-19)	12.2±6.4 (4.9-33.1)	21.8±3.9 (15.2-30.6)
Mild-IDA	36	M: 124±3.2 (121-128) F: 117±2.9 (111-120)	10±4 (3-19)	5.2±1.5 (3-8.7)	28.9±1.8 (23.7-31.5)
SID	46	M: – F: 128±5.9 (121-144)	9±2 (4-12)	4.4±1.2 (2.7-7.5)	30.9±2.5 (22.8-36.3)
Mixed group	95	M: 97±14.6 (54-120) F: 95±14.4 (60-110)	186±201 (21-1101)	5.6±2.9 (1.5-16.8)	28.8±(18.8-38.7)
Thalassemia trait	33	M: 125±15.8 (104-162) F: 110±9.8 (92-133)	224±304 (22-1524)	–	21.8±(18-26.7)
Diagnostic study[2]	ACD n=53 IDA n=37	Hb (g/L)	sFt (µg/L)	sTfR (mg/L)	RET-He (pg)
IDA	37	91±13 (72-119)	8±5 (2-19)	11±5.6 (4.9-28.7)	22.6±3.8 (16.2-30.6)

Mean ± 1 standard deviation. Maximum and minimal values in brackets. M: males; F: females; ACD: anemia of chronic disorders; IDA: iron deficiency anemia; SID: storage iron deficiency; Hb: hemoglobin; sFt: serum ferritin; sTfR: serum transferrin receptor; RET-He: reticulocyte Hb equivalent. Inclusion criteria: Reference group: healthy individuals with blood cell counts and biochemical iron variables within the reference range (sFt >13 µg/ and. sedimentation rate (SR) <20mm/h). ACD: Hb <120g/L for M and <110g/L for F, MCV 80-98 fl; RDW<16.5%; serum iron <9 µmol/L; TIBC <40Imol/L; sFt >100 µg/L and SR >50 mm/h. IDA: Hb <120g/L for M <110 g/L for F; MCV <98 fL; low serum iron + high TIBC + Tf saturation <15% + sFt <20 µg/L. Mild-IDA: IDA with Hb 120-130g/L for M and 110-120g/L for F. SID: Normal Hb levels with sFt <13 µg/L. Mixed Group: Hb <120g/L for M and <110g/L for F, with a mixed iron biochemical pattern. Based on an iron exam in the bone marrow or on the response to iron treatment, ID was diagnosed in 53 and ACD in 42. Thalassemia trait: iron sufficient adults with beta or δ-β thalassemia trait.
[1]Reference values in our laboratory were obtained from 121 normal individuals (M: 55, F: 66). [2]There was no statistical difference between ACD and IDA in Hb. RET-He was significantly decreased in IDA (p<0.001).

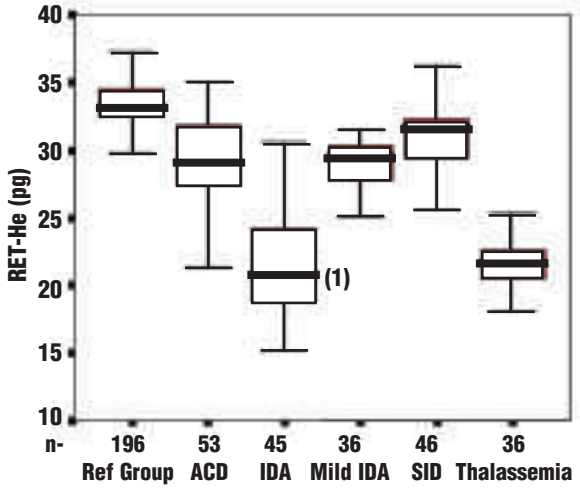

Figure 1. Box-and-whisker plot showing the distribution of RET-He in the different groups. Ref. Group: reference group; ACD: anemia of chronic disease; IDA: iron deficiency anemia; SID: storage iron deficiency. (1) t-test: IDA vs Ref. group $p<0.001$ and IDA vs ACD $p<0.001$.

curves. The κ index and the positive and negative agreement were assessed in order to determine the concordance between the different parameters.

The values of RET-He were normally distributed. The reference range for RET-He was 30.2-36.7 pg (RET-Y values 169-189). RET-He was slightly lower in the ACD group than in the reference group, but markedly reduced in IDA (Figure 1). In cases with iron deficiency, a correlation between hemoglobin and RET-He was observed ($r=0.79$, $p<0.001$). The diagnostic study between IDA and ACD was performed in a subgroup of 90 cases with Hb>70g/L. The best parameters for differentiating IDA from ACD were sTfR (cut-off point 5.6 mg/L) and sTfR/log sFt (cut-off point 3.1), with area under the curve (AUCROC) of 0.99 and 1.0, respectively. The optimal cut-off point for RET-He was 25 pg (RET-Y 150), which provided an AUC^{ROC} of 0.90, with a sensitivity of 0.76 and a specificity of 0.81. An overall agreement of 79% was observed between the results of RET-He and sTfR, and of 80% for sTfr/log sFt (κ index 0.53 and 0.55).

When the diagnostic parameters were applied to the mixed group, RET-He showed a high specificity for IDA (0.95), with an AUCROC of 0.76. As the concordance with the sTfR in this group was low (κ index < 0.4), we suggest that RET-He can play a complementary role to sTfR in the diagnosis of IDA. RET-He increased the sensitivity of sTfR in this group, from 0.72 to 0.81. Moreover, as RET-He provided the diagnosis of IDA in 32% of the patients with a mixed pattern, sTfR quantification would only be required in the remaining cases. Finally, very low values of RET-He were observed in thalassemia trait, despite these patients having Hb levels practically in the reference range.

There has been much interest in the potential use of new reticulocyte parameters in the diagnosis of anemias and in monitoring erythropoiesis. CHr has been used as a diagnostic tool, together with biochemical markers, to distinguish IDA from ACD and from the combined state ID/ACD.[10] This is the first study to define the normal values of RET-He. As expected, RET-He was significantly lower in cases with IDA (Figure 1). In the diagnosis study, RET-He values lower than 25 pg discriminated IDA from ACD, with an overall agreement with sTfR and sTfR/log sFt of 80%. A higher cut-off point of RET-Y (channel 162) was recently established to discriminate IDA from the normal status.[2] Our study shows that RET-He is useful in differentiating IDA from ACD, as is CHr.[3-4] As expected, the diagnostic efficiency of RET-He was lower in the mixed group, but it could play a complementary role with sTfR in the discrimination of IDA from ACD. A direct diagnosis of IDA can be made with RET-He in a one-third of patients with mixed pattern anemia.

In conclusion, our study shows that the new RET-He parameter is useful for diagnosing IDA. RET-He, in conjunction with standard blood cell counts and iron parameters, enables a diagnosis to be made rapidly. We also show that RET-He is helpful in identifying thalassemia carriers.

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