Evaluation of the new RET-He parameter versus the CHr reference method in a cohort of 57 dialysed patients in a five months follow-up

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Aim of the study

We have evaluated the concordance between the CHr parameter, used as the reference one, and the new one, the RET-He, in a cohort of dialysed patients referred to the Nephrology Unit.

Introduction

Anemia is a common feature in chronic kidney disease (CKD). In dialysed patients the development of anemia could be also complicated by associated co-morbidities; on the other hand anemia may play an important role in increasing the risk for progression to end-stage renal disease, coronary heart disease, stroke and for death too. The correction of anemia with recombinant human erythropoietin (rHuEPO) therapy and intravenous iron has produced a great improvement in CKD patient's quality of life. Patients with CKD should maintain a target Hb concentration of > 11g/dL. Iron supplementation helps to reach the targeted Hb level.
Reticulocytes are the earliest erythrocytes released into blood and circulate for 1 to 2 days: the automated availability of reticulocytes hemoglobin content (CHr) provides information on patient's erythropoiesis useful for managing therapy in real time.

Background

The CHr (ADVIA 2120 Bayer Diagnostics, Tarrytown, NY) provides a measure of iron availability to bone marrow erythropoiesis and is considered a direct marker of the iron status in hemodialysed patients receiving erythropoietin therapy as well as a strong predictor of iron deficiency (1).
The European guidelines for anemia treatment in CKD assess a value of CHr >29 pg/cell as target for iron treatment (2).
The hematology analyser Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) now provides a new parameter, the reticulocyte hemoglobin equivalent (RET-He), a measure of reticulocyte hemoglobin content, which can be used as an early indicator of iron deficiency anemia (3,4).

Methods (I)

Reference population
55 well health subjects samples, 27 males and 28 females.

Patients
57 dialysed patients 30 males, 27 females, median age 66 years (30-80).
All but three patients (affected by polycystic kidney) received rHuEPO.
Intravenously iron was administered to maintain hemoglobin level between 10 and 12 mg/dl.

Hematology parameters
K3EDTA-anticoagulated peripheral blood samples were analysed within 3 hours from withdrawal with both ADVIA 2120 and Sysmex XE-2100 hematology analyzers.
Peripheral blood of each patient was analysed monthly for five times.

Statistical Analysis
Data were evaluated using standard parametric tests and calculation was performed with the Clinical Laboratory module from Analyse-it statistical software for biomedical research.

Methods (II)

ADVIA 2120 Reticulocyte count
Reticulocytes are stained with oxazine 750 and isovolumetric sphered. The light scatter at two different angles provides the measurement of reticulocytes mean hemoglobin concentration (CHCMr) and mean volume (MCVr). CHr is a derived parameter: CHr= MCVr × CHCMr.

Sysmex XE-2100 Reticulocyte count
In the reticulocyte channel the sample, stained by a polymethine dye specific for RNA/DNA, is analyzed by flow cytometry using a semiconductor laser. A two dimensional distribution of forward-scattered light and fluorescence is presented as a scattergram indicating mature red blood cells and reticulocytes. RET-Y is the mean value of the forward-scattered light of fluorescence-labeled reticulocytes expressed in arbitrary units (AU).
The RET-He parameter is a mathematical transformation of RET-Y.

Results

At the end of the study we collected data from a total of 296 samples from each analyser.

The mean Hb value during the 5 months follow-up is 10.8 g/dL (range 7.4-13.7).

During the follow-up period two patients suffered severe gastrointestinal bleeding, one patient received antibiotic therapy for skin infection.

In Table 1 CHr and RET-He mean and median values in healthy subjects are presented.

In Figure 1, CHr and RET-He distribution curves in dialysed patients are showed.

In Figures 2 and 3, graphs of RET-He vs CHr correlation and Bland Altman plot data analysis are presented.

For the CHr value of 29 pg, the best correlation is obtained using the RET-He value of 30.5 pg.

At this cut-off point, the diagnostic concordance is 93.6%, with a sensitivity of 98.4% and a specificity of 92.2%.

Clinical predictivity is 77.8% and 99.5 for respectively positive and negative values (AUC = 0.98) (Figure 4).

	Mean	(SD)	Range (Mean + / - 2SD)	Median	Reference Interval (2.5th - 97.5th perc.)	Min value	Max value
CHr (pg)	31.41	(1.086)	29.24 – 33.58	31.60	28.66 – 33.32	28.30	33.40
RET-He (pg)	33.01	(1.229)	30.55 – 35.47	33.10	30.00 – 35.22	29.8	35.30

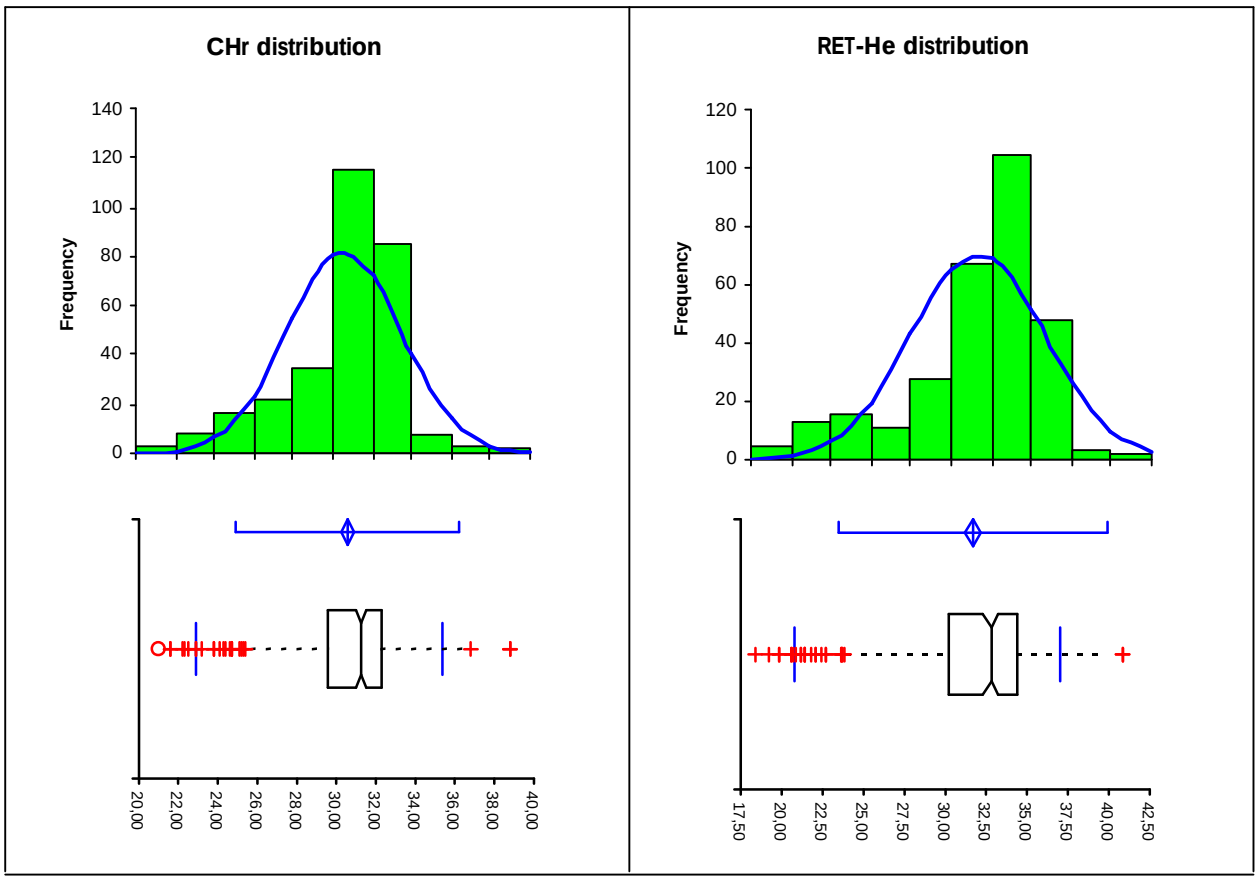

***Fig.1**. In dialysed patients the median value of CHr was 31.25 (mean 30.57 ±2.88) pg, the median RET-He was 32.84 (mean 31.69 ±4.18) pg. Both indices show a near normal distribution: the 95% central range for CHr of 22.9-35,37 pg corresponds to a range for RET-He of 20.78-37 pg.*

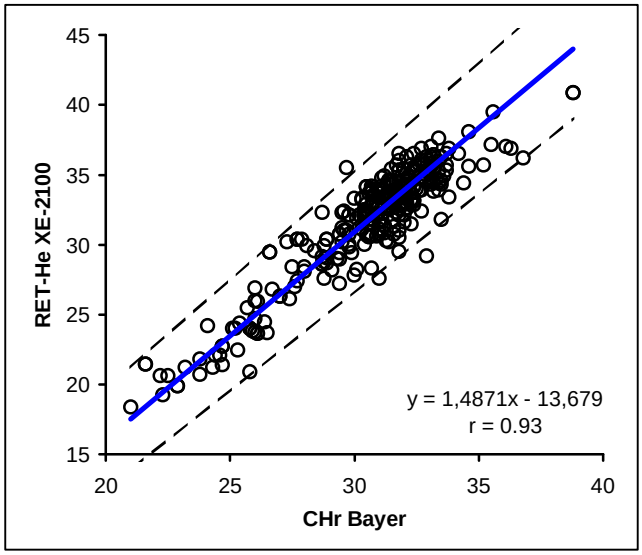

***Fig.2**. Relationship between CHr and RET-He in 296 determinations in 57 hemodialysed patients*

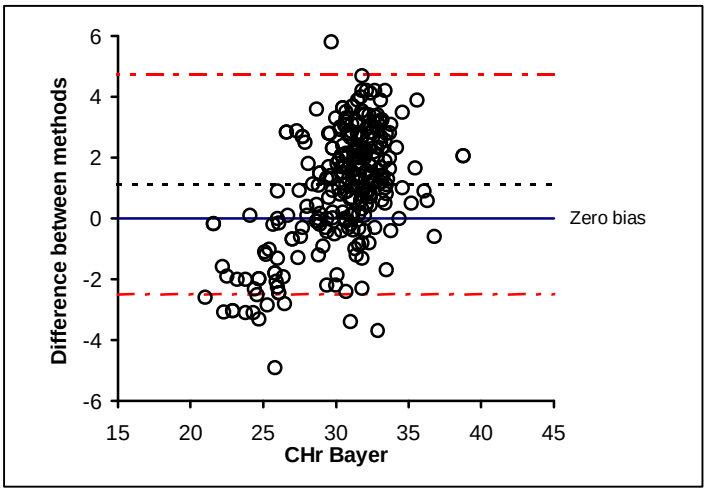

***Fig. 3.** Comparing the two parameters with the Bland & Altman plot, a mean bias of 1.12 pg emerged*

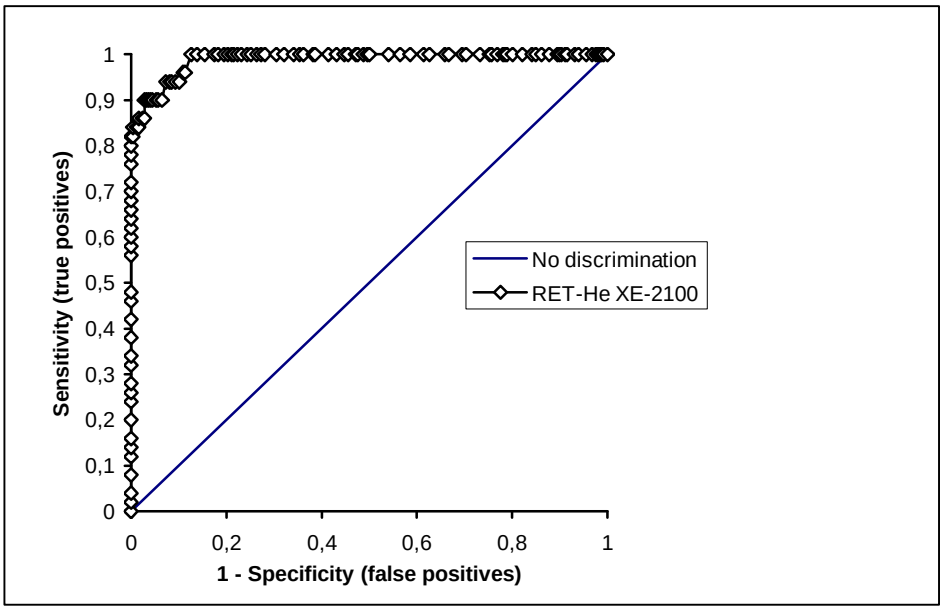

***Fig. 4**. The ROC curve analysis shows an excellent diagnostic efficiency of RET-He to evaluate patients needing iron support: the area under the curve (AUC) is 0.98 (95% C.I. 0.96-0.99)*

Conclusions

Our study demonstrates a strict correspondence between the classical CHr provided by Bayer analyzers and the new Ret-He provided by the Sysmex XE-2100, with a bias of ± 1.12 pg of Ret-He compared to the reference method (CHr). This correspondence is independent from clinical changes, as well as from therapeutic treatment of patient co-morbidities.

References

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