

Concordance between Reticulocyte Hemoglobin Equivalent and Reticulocyte Hemoglobin Content in CKD Patients Undergoing Hemodialysis

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ABSTRAK

Tujuan: mendapatkan korelasi dan kesesuaian antara nilai reticulocyte hemoglobin equivalent (RET-He) dan reticulocyte hemoglobin content (CHr), serta nilai cut-off RET-He sebagai target terapi suplementasi besi pada pasien dengan penyakit ginjal kronik (PGK) yang menjalani hemodialisis. **Metode:** penelitian ini menggunakan desain potong lintang terhadap 106 pasien PGK dengan hemodialisis rutin yang dilakukan pengambilan darah K₂EDTA untuk pemeriksaan RET-He dengan Sysmex XN-2000 dan CHr dengan Siemens ADVIA 2120i. **Hasil:** didapatkan korelasi sangat kuat ($r=0,91$; $p<0,0001$) dan kesesuaian yang baik antara nilai RET-He dan CHr dengan perbedaan rerata 0,5 pg. Sebanyak 96,23% data berada dalam batas kesesuaian. Nilai cut-off RET-He 29,2 pg didapatkan dari analisis kurva receiver operating characteristic (ROC) dengan CHr sebagai standar baku emas. Nilai cut-off RET-He 29,2 pg memiliki sensitivitas 95,5% dan spesifisitas 94%. **Kesimpulan:** penelitian ini menunjukkan korelasi dan kesesuaian yang baik antara RET-He dan CHr pada pasien PGK dengan hemodialisis.

Kata kunci: reticulocyte hemoglobin content (CHr), reticulocyte hemoglobin equivalent (RET-He), hemodialisis.

ABSTRACT

Aim: to evaluate the correlation and the concordance between reticulocyte hemoglobin equivalent (RET-He) and reticulocyte hemoglobin content (CHr) as well as to obtain the cut-off value of RET-He as the target of iron supplementation in chronic kidney disease (CKD) patients undergoing hemodialysis. **Methods:** a cross-sectional study was performed using K₂EDTA-anticoagulated peripheral blood samples collected from 106 CKD patients undergoing routine hemodialysis. The samples were then analyzed using both Sysmex XN-2000 and Siemens ADVIA 2120i for RET-He and CHr analysis. **Results:** a very strong correlation ($r=0.91$; $p<0.0001$) and a good concordance were found between RET-He and CHr with mean bias of 0.5 pg. The diagnostic concordance was 96.23%. The cut-off value of RET-He 29.2 pg was obtained from the receiver operating characteristic (ROC) curve with CHr as the gold standard. At this cut-off point, the sensitivity and specificity to assess the target of iron supplementation in CKD patients undergoing hemodialysis were 95.5% and 94%, respectively. **Conclusion:** the study shows a good correlation and concordance between RET-He and CHr in CKD patients undergoing hemodialysis.

Keywords: reticulocyte hemoglobin content (CHr), reticulocyte hemoglobin equivalent (RET-He), hemodialysis.

INTRODUCTION

Chronic kidney disease (CKD) is a widespread disease throughout the world, and its prevalence is increasing annually.^{1,2} Anemia is a common complication in CKD patients. The incidence of anemia is less than 10% in patients with CKD stages 1 and 2, 20-40% in CKD stage 3, 50-60% in CKD stage 4 and more than 70% in patients with CKD stage 5 or those with CKD undergoing routine hemodialysis (CKD-HD). Anemia in CKD is associated with reduced quality of life and increased risk cardiovascular disease, cognitive impairment, and mortality.^{1,3,4}

Anemia in CKD is primarily caused by inadequate renal production of erythropoietin and it is often aggravated by iron deficiency. Iron deficiency occurs in approximately 40% of CKD patients and 40-77% of CKD-HD patients. Iron deficiency causes a decrease in response to erythropoietin (EPO) therapy. Therefore, CKD-HD patients who experience iron deficiency anemia should be given adequate iron supplementation simultaneously with EPO therapy.^{1,3-7}

The gold standard to assess iron status is the iron-stained bone marrow aspiration. However, the test is invasive and difficult to assess, so it has been replaced by the conventional laboratory examination using serum ferritin and transferrin saturation. Both of these tests are affected by inflammation and therefore, these tests are replaced by alternative parameters that are more stable to assess iron status in CKD-HD patients, i.e. reticulocyte hemoglobin content (CHr) using an ADVIA analyzer or reticulocyte hemoglobin equivalent (RET-He) using a Sysmex analyzer. CHr and RET-He measure the amount of iron contained in the reticulocytes. Thus, those tests can be used to estimate the availability of iron for erythropoiesis.^{1,8-13} The 2006 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) recommended CHr of ≥ 29 pg as the target of iron supplementation in CKD-HD patients.¹⁴ Since the use of CHr has been recommended by the 2006 NKF-KDOQI, CHr can be regarded as the gold standard for assessing iron status in CKD-HD patients.

Until now, Indonesia has no data of concordance between RET-He and CHr in CKD-

HD patients as the parameters for assessing iron status. Such data is important, RET-He or CHr can be used to assess the iron status in CKD-HD patients, which make it easier for doctors to assess the target of iron supplementation if their patients examined in different laboratories which using different hematology analyzer. Similar studies were conducted in Italian dan Japanese population.^{9,15,16} RET-He and CHr values in both populations are different, so it is necessary to conduct study in Indonesian population. Therefore, we conducted a research to evaluate the correlation and the concordance between RET-He and CHr as well as to obtain the cut-off value of RET-He as the target of iron supplementation in patients with CKD-HD.

METHODS

It is a cross-sectional study. Our study was approved by Research Ethics Committee, Faculty of Medicine, Universitas Indonesia (No. 197/UN2.F1/ETIK/ 2016).

Study Subject

Subjects were CKD-HD patients in the Hemodialysis Unit of the Renal Hypertension Division of the Department of Internal Medicine of Cipto Mangunkusumo Hospital. The inclusion criteria were patients aged ≥ 18 years, willing to participate in the study and have signed the informed consent form. Being pregnant was the only exclusion criterion. Hemoglobin level, RET-He, and CHr were then analyzed at the Laboratory of Clinical Pathology, Metropolitan Medical Centre (MMC) Hospital in Jakarta. The study was conducted in April 2016 with minimum sample size of 100 patients.

Sample Analysis

Three mL of vein blood was collected from the hemodialysis access of each patient prior to hemodialysis using a sterile syringe and then it was inserted into a K₃EDTA anticoagulant tube. Each K₃EDTA-anticoagulant tube was inverted eight times to ensure homogenization between the anticoagulant and blood sample.

Hemoglobin level and RET-He were measured using Sysmex XN-2000 hematology analyzer; while CHr was measured by Siemens ADVIA 2120i hematology analyzer. Before

sample testing was performed, the instrument was calibrated. Afterwards, within-run precision and accuracy tests were carried out by measuring quality control material (XN Check and ADVIA 3•in•1 TESTpoint) of five consecutive cycles on the same day. The between-days precision test was carried out every day during the study by measuring quality control material. The within-run precision test was also carried out by measuring K₃EDTA-anticoagulated blood. The samples were stored at 20°C and were analyzed using both analyzers within three hours after blood collection. Study flowchart is shown in **Figure 1**.

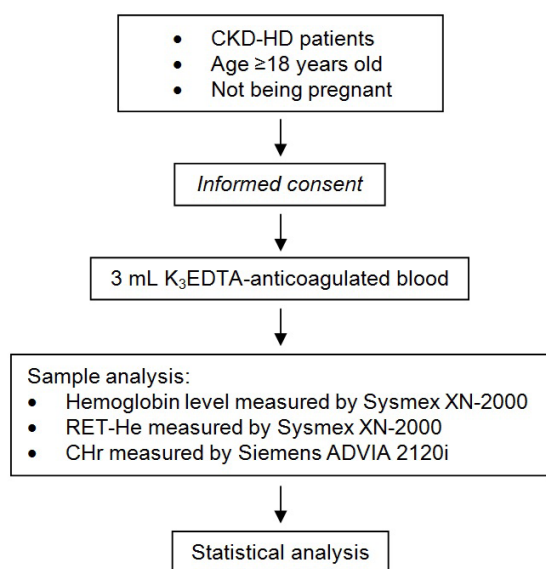


Figure 1. Study flowchart

Statistical Analysis

Statistical analysis was carried out with the use of IBM SPSS Statistics software version 20.0 for windows. Hemoglobin level, RET-He and CHr were evaluated using Kolmogorov-Smirnov normality test. The correlation between RET-He and CHr was evaluated by Pearson's correlation test for parametric data or Spearman's rank correlation test for non-parametric data. P value <0.05 was considered statistically significant.

The concordance between RET-He and CHr was evaluated by Bland Altman analysis. The difference between RET-He and CHr value was calculated and then we calculated the mean and

standard deviation of the difference. The limit of agreement was defined as mean bias (two standard deviations (SD)). The results of the concordance test were considered good if ≥95% data were within the limit of agreement. The percentage of data within the limit of agreement was called diagnostic concordance.^{17,18}

The cut-off value of RET-He as the target of iron supplementation in CKD-HD patients was evaluated by using receiver operating characteristic curve (ROC) with CHr as the gold standard. Afterwards, we obtained the sensitivity, specificity, positive and negative predictive values, as well as the area under the curve (AUC) of the cut-off value of RET-He.

RESULTS

Our study was conducted in three days. A total of 106 subjects who fulfilled the inclusion criteria were included in our study. There was no subject who fulfilled the exclusion criteria. Subject's characteristics can be seen in **Table 1**.

Table 1. Subject's characteristics

Variables	Value (N=106)
Age (years), mean (SD)	50.7 (13.1)
Sex (male), n (%)	54 (50.9)
Hemoglobin level (g/dL), mean (SD)	9.8 (1.9)
CHr (pg), mean (SD)	30.3 (2.1)
RET-He (pg), median (range)	31.3 (21.1 – 38.0)

The results of the within-run and between-days precision, and accuracy analysis for hemoglobin level, RET-He, and CHr can be seen in **Table 2**.

There was a very strong positive correlation between RET-He and CHr in CKD-HD patients as seen in **Figure 2** ($r=0.91$; $p<0.0001$). The Bland Altman analysis shows a good concordance between RET-He and CHr in CKD-HD patients with the mean bias of 0.5 pg as we can see in **Figure 3**. The limit of agreement was (-2.1)-3.1 pg and the diagnostic concordance was 96.23%.

The cut-off value of RET-He as the target of iron supplementation in CKD-HD patients was determined by the ROC curve analysis with CHr

Table 2. The results of the within-run and between-days precision, and accuracy analysis for hemoglobin level, RET-He, and CHr

	Within-run precision		Between-days precision (%)	Accuracy (%)
	Quality control material (%)	K ₃ EDTA-anticoagulated blood (%)		
Hemoglobin level	0 – 0.71	0 – 0.68	0.35 – 0.92	-0.6 – 3.28
RET-He	0.59 – 0.98	0.37 – 2.50	0.57 – 0.84	-3.50 – (-0.36)
CHr	0.25 – 0.46	0.16 – 0.31	0.63 – 0.69	-2.76 – 1.71

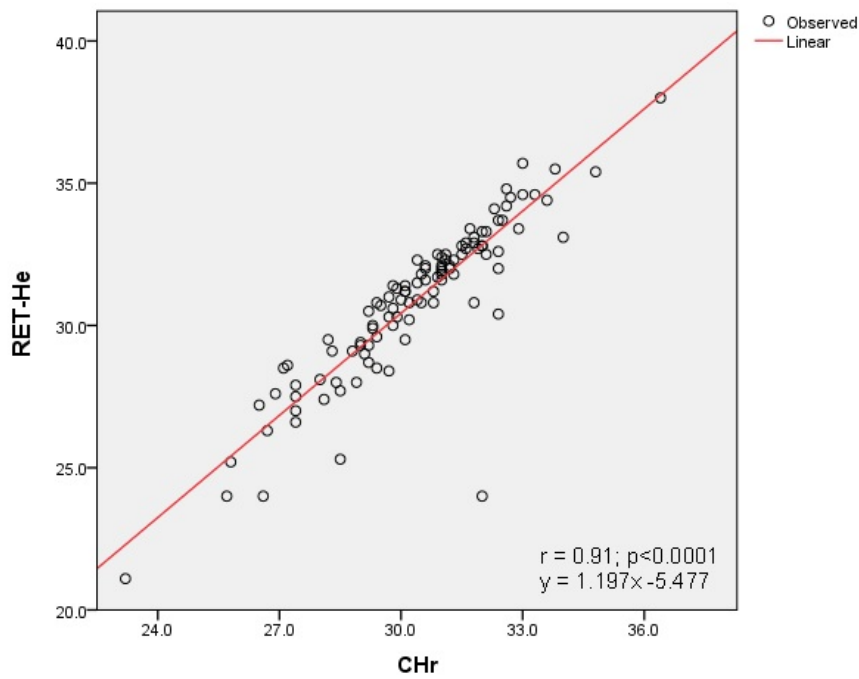


Figure 2. Spearman correlation between RET-He and CHr in CKD-HD patients

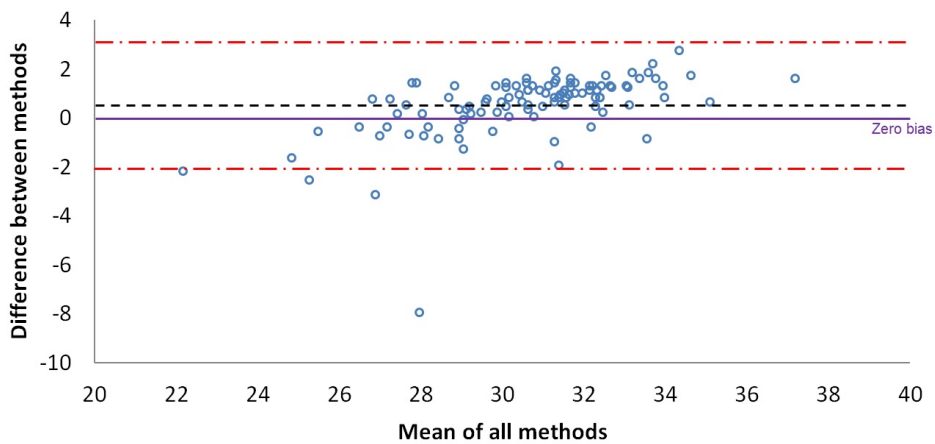


Figure 3. Bland Altman plot between RET-He and CHr in CKD-HD patients

as the gold standard, as shown in **Figure 4**. The sensitivity and specificity were optimal when the cut-off value of RET-He was at 29.2 pg. AUC was 97.9% (95% CI 95.4-100%). Based on the cut-off value, we obtained the sensitivity of 95.5%, specificity of 94%, positive predictive value of 80.8%, and negative predictive value of 98.8%.

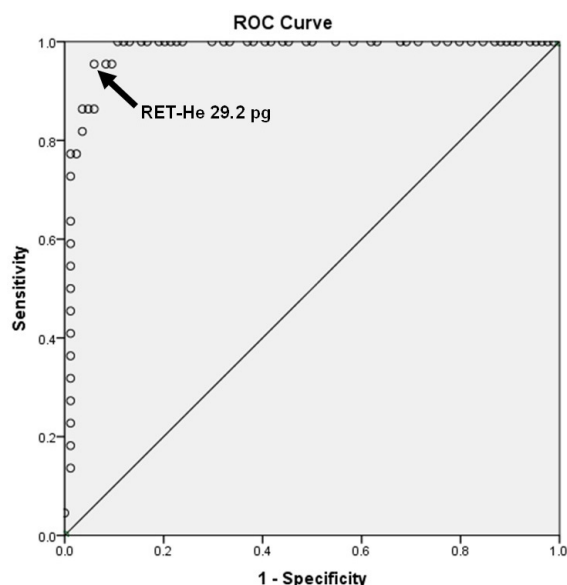


Figure 4. The ROC curve analysis of RET-He as the target of iron supplementation in CKD-HD patients

DISCUSSION

The results of the within-run and between-days precision analysis for hemoglobin level, RET-He, and CHr using quality control material and K₃EDTA-anticoagulated blood were still under the manufacturer's recommendations.^{19,20} However, the results of the within-run and between-days precision, and accuracy analysis of CHr were better than those of RET-He.

The number of subjects was 106 patients with a median age of 51.5 years (range 18-77 years) and consisting of 50.9% males and 49.1% females. Garzia et al.¹⁵ in Italy found 57 CKD-HD patients which consist of 52.6% males and 47.4% of females with a median age of 66 years (range 30-80 years). Maconi et al.¹⁶ in Italy found 200 CKD-HD patients with the median age of 63 years (range 35-78 years).

In our study, anemia occurred in 90.5% of the subjects, which is consistent with the 3rd

National Health and Nutrition Examination Survey (NHANES) stating that the incidence of anemia in CKD-HD patients is more than 70%.¹

All CKD-HD patients in our study, Maconi et al.¹⁶ and Garzia et al.¹⁵ studies have received iron supplementation therapy to maintain hemoglobin levels between 10-12 g/dL. Garzia et al.¹⁵ and Maconi et al.¹⁶ measured hemoglobin level, RET-He, and CHr every month for five months; while our study only performed the measurements once.

Comparison of hemoglobin level, RET-He, and CHr among some studies can be seen in **Table 3**. The difference of mean hemoglobin levels is probably caused by EPO dose received by our subjects, i.e. 2000-3000 IU, twice a week for hemoglobin levels of <10 g/dL and once a week for hemoglobin levels of ≥10 g/dL according to the facilities provided by the government. The EPO dose was under the dosage recommended by NKF-KDOQI 2006 and KDIGO 2012, i.e. 50-100 IU per kg body weight 3 times a week. EPO doses given to study subjects of Garzia et al.¹⁵, Maconi et al.¹⁶, and Miwa et al.⁹ were in accordance with the recommended dose of 2006 NKF-KDOQI and the 2012 KDIGO. In addition, the mean value of RET-He and CHr in our study was found to be higher than the cut-off value of iron sufficiency; thus, it supports the possibility of less EPO dose as the cause of the difference in mean hemoglobin level.

Our study showed a very strong positive correlation between RET-He and CHr in CKD-HD patients ($r=0.91$; $p<0.0001$). It also showed that the higher the value of RET-He, the higher the value of CHr. The results of our study are similar to the studies conducted by Garzia et al.¹⁵ ($r=0.93$), Maconi et al.¹⁶ ($r=0.88$; $p<0.0001$), and Miwa et al.⁹ ($r=0.86$; $p<0.01$).

Our study demonstrated that the mean bias was 0.5 pg and the diagnostic concordance was 96.23%. Moreover, Garzia et al.¹⁵ found a mean bias of 1.12 pg and a diagnostic concordance of 93.6%; while Maconi et al.¹⁶ got a mean bias of 1.04 pg and a diagnostic concordance of 97.5%. It should be noted that Garzia et al.¹⁵ and Maconi et al.¹⁶ used Sysmex XE-2100 hematology analyzer for measurement of RET-He and Siemens ADVIA 2120 hematology analyzer for

CHr measurement. In contrast, our study used Sysmex XN-2000 and Siemens ADVIA 2120i hematology analyzer. Sysmex XN-2000 is a newer series of Sysmex hematology analyzer than Sysmex XE-2100. Therefore, it is likely to cause the mean bias of our study to be better than the mean bias found in Maconi et al.¹⁶ and Garzia et al.¹⁵ The good concordance between RET-He and CHr indicates that RET-He and CHr can be used alternately for assessing iron status in CKD-HD patients. Doctors can be easier to assess the target of iron supplementation if their patients examined in different laboratories that using different hematology analyzer.

The result of ROC curve analysis our study indicated that the cut-off value of RET-He was 29.2 pg, which serves as the target of iron supplementation in CKD-HD patients and it also showed an AUC of 97.9% (95% CI 95.4-100%). The 97.9% AUC means that RET-He can assess

the achievement of target iron supplementation precisely in 97.9 of 100 cases.

The comparison of cut-off values and diagnostic values of RET-He among several studies is shown in **Table 4**. The cut-off value of RET-He in our study does not differ much with Garzia et al.¹⁵ and Maconi et al.¹⁶. The difference on cut-off value of RET-He between our study and Maconi et al.¹⁶ study as well as Garzia et al.¹⁵ study was 4.5% and 0.7%, respectively. The difference is still smaller than the manufacturer's recommendations, which is 5%.

To our knowledge, the study on the concordance between RET-He and CHr in CKD-HD patients using Sysmex XN-2000 and Siemens ADVIA 2120i hematology analyzers is the first study conducted in the world. In addition, our study has also used CHr as the gold standard for detection of functional iron deficiency as has been recommended by the 2006 NKF-KDOQI. The

Table 3. Comparison of hemoglobin level, RET-He, and CHr in CKD-HD patients

	Hematology analyzer	n	Location	Hemoglobin level (g/dL)	RET-He (pg)	CHr (pg)
Our study	XN-2000	106	Indonesia	9.8 (1.9)	31.3 (21.1 – 38.0)	30.3 (2.1)
	ADVIA 2120i					
Garzia et al. ¹⁵	XE-2100	285	Italy	10.8*	33.0 (1.2)	31.4 (1.1)
	ADVIA 2120					
Maconi et al. ¹⁶	XE-2100	200	Italy	11.6**	33.9**	32.9**
	ADVIA 2120					
Miwa et al. ⁹	XE-2100	1350	Japan	10.3 (1.1)	32.4 (4)	32.2 (2.6)
	ADVIA 120					

* mean; ** median

Table 4. Comparison of cut-off values and diagnostic values of RET-He as the target of iron supplementation in CKD-HD patients

	Hematology analyzer	Cut off value of RET-He	AUC	95% CI	Diagnostic value
Our study	XN-2000	29.2 pg	97.9%	95.4% – 100%	Sensitivity 95.5% Specificity 94% Positive predictive value 80.8% Negative predictive value 98.8%
	ADVIA 2120i				
Garzia et al. ¹⁵	XE-2100	30.5 pg	98%	96% – 99%	Sensitivity 98.4% Specificity 92.2% Positive predictive value 77.8% Negative predictive value 99.5%
	ADVIA 2120				
Maconi et al. ¹⁶	XE-2100 ADVIA 2120	29.4 pg	No data	No data	Sensitivity 100% Specificity 97.3%

amount of withdrawn blood sample is just 3 mL K₃EDTA for measuring hemoglobin levels and RET-He, provides a faster measurement result, and cheaper costs compared to conventional measurements such as hemoglobin level, serum iron, TIBC, and serum ferritin.

Given the limited time and costs, we could only perform the measurements once. Garzia et al.¹⁵ and Maconi et al.¹⁶ took measurements every month for five months; therefore, they have more data than us. More data usually will provide better concordance results.^{17,18}

CONCLUSION

We have conducted a study in 106 patients with CKD-HD and obtained a very strong positive correlation and good concordance between RET-He and CHr, which can be used by clinicians to assess the target of iron supplementation if their patients examined in different laboratories that using different hematology analyzer.

The cut-off value of RET-He as the target of iron supplementation in CKD-HD patients in our study is 29.2 pg, with CHr as the gold standard.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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