Clinical Utility of Reticulocyte Hemoglobin Equivalent (RET-He) and Related Indices in Predicting Erythropoietin Response in Hemodialysis Patients

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ABSTRACT

This study evaluated the predictive value of reticulocyte hemoglobin equivalent (RET-He) and related indices, including immature reticulocyte fraction (IRF) and mean reticulocyte volume (MRV), for assessing erythropoietin (EPO) responsiveness in 400 patients undergoing maintenance hemodialysis (HD). The objective was to determine whether RET-He could serve as a reliable biomarker of early erythropoietic activity and iron availability following EPO therapy.

Correlation, regression, and receiver operating characteristic (ROC) analyses were applied to explore relationships between baseline reticulocyte parameters and post-treatment hemoglobin response. RET-He exhibited a weak positive correlation ($r = 0.0004$, $p = 0.993$) with hemoglobin improvement, indicating negligible predictive capacity. Regression analysis revealed that baseline hemoglobin, not RET-He, independently predicted treatment response ($p < 0.001$). RET-He and IRF were useful for real-time diagnostic monitoring but not as prognostic indicators.

Keywords: Erythropoietin Response, ESA Therapy, Hemodialysis, Iron Deficiency, RET-He, Reticulocyte Indices.

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Introduction

Anemia is a major complication of chronic kidney disease (CKD).[1-3] The condition arises primarily due to erythropoietin (EPO) deficiency, iron and hepcidin dysregulation, and chronic inflammation.[1,4] Management typically involves erythropoiesis-stimulating agents (ESAs) combined with iron supplementation.[1]

Traditional iron markers, such as serum ferritin and transferrin saturation (TSAT), are often limited by their acute-phase reactivity, making them unreliable in the presence of inflammation, which is common in hemodialysis patients.[5] In contrast, reticulocyte indices—particularly the reticulocyte hemoglobin equivalent (RET-He)—offer immediate insight into iron delivery to developing erythrocytes, reflecting the functional iron content of reticulocytes during the preceding 2–4 days of erythropoiesis.[6,7] RET-He has demonstrated high sensitivity in detecting iron deficiency and has been shown to successfully reflect response to therapy more rapidly than traditional hemoglobin measurements.[6]

Clinical studies have explored RET-He as a marker for iron deficiency detection and response monitoring in dialysis populations, where it has shown superior diagnostic performance compared to conventional iron measures.[7] Furthermore, RET-He has been established as a useful discriminator for differentiating functional from absolute iron deficiency.[8]

Materials and Methods

Study Population and Design

A prospective observational study was performed on 400 adult patients receiving maintenance HD for at least three months. All participants had stable dialysis prescriptions and hemoglobin levels below 11 g/dL, consistent with established thresholds for evaluating anemia in patients with end-stage kidney disease.[9] Patients with active infection, malignancy, recent transfusion (within 2 weeks), or parenteral iron administration were excluded to avoid confounding variables that affect iron markers and erythropoiesis.[9, 10] Ethical approval was obtained from the institutional ethics committee (approval code IEC-BU-FACULTY-04-130-2022).

Sample Collection and Analysis

Two milliliters of EDTA blood was collected prior to the administration of EPO. Reticulocyte parameters, including RET-He, IRF, MRV, and RET %, were measured using the Sysmex XN-1000 analyzer, which uses fluorescence flow cytometry to quantify RNA content and determine the maturity of reticulocyte populations.[8,10] Hemoglobin levels were recorded at baseline and after four weeks of standardized EPO therapy (50–100 IU per kg per week), a duration sufficient to observe changes in hemoglobinization reflecting recent marrow iron supply.[7,10]

Statistical Analysis

Data were analyzed using SPSS v25 and Python libraries. Descriptive statistics were expressed as mean ± SD. Correlation analysis (Pearson/Spearman) was used to assess relationships between RET-He, IRF, MRV, and hemoglobin increment (ΔHb). Linear regression was employed to determine independent predictors of ΔHb, while Receiver Operating Characteristic (ROC) analysis was used to assess the diagnostic performance of RET-He and related indices in differentiating responders from non-responders, typically defined as an increase in hemoglobin of at least 1.0 g/dL.[6,9] Principal Component Analysis (PCA) was utilized to summarize multidimensional variance across reticulocyte parameters.[11]

A total of 400 hemodialysis (HD) patients were evaluated, comprising 258 males and 142 females (M:F = 1.8:1) with a mean age of 52.6 ± 13.8 years. The mean duration of dialysis treatment was 4.2 ± 1.7 years, with all patients receiving standard erythropoietin (EPO) therapy. Baseline hemoglobin levels averaged 8.74 ± 1.06 g/dL, which increased to 9.48 ± 1.11 g/dL after four weeks of therapy, corresponding to a mean increment (ΔHb) of 0.74 ± 0.68 g/dL. The cohort demonstrated broad biological heterogeneity, reflecting varying degrees of ESA responsiveness.

The RET-He values ranged from 21.2 to 38.5 pg (mean 30.4 ± 3.2 pg), while the Immature Reticulocyte Fraction (IRF) ranged between 0.26 and 0.71 (mean 0.47 ± 0.12). The Mean Reticulocyte Volume (MRV) varied from 98.4 to 115.6 fL (mean 108.3 ± 4.1 fL). These findings were within expected physiological limits for HD populations receiving ESA and iron supplementation.

Parameter	Mean ± SD	Range	Reference Range	Interpretation
Hemoglobin (g/dL)	9.48 ± 1.11	7.0–11.0	12–15	Suboptimal response
RET-He (pg)	30.4 ± 3.2	21.2–38.5	29–35	Normal functional iron
IRF	0.47 ± 0.12	0.26–0.71	0.3–0.6	Normal–high
MRV (fL)	108.3 ± 4.1	98.4–115.6	100–115	Normal

Correlation Analysis

Correlation analysis revealed a negligible linear association between RET-He and ΔHb (r = 0.0004, p = 0.993), indicating that changes in reticulocyte hemoglobin content were not predictive of hemoglobin response after EPO administration.

IRF and MRV also failed to show significant relationships (r = 0.091, p = 0.147; r = 0.071, p = 0.216), suggesting that early marrow response markers alone could not reliably anticipate hemoglobin improvement.

Notably, moderate internal correlations were observed among the reticulocyte indices themselves (RET-He vs MRV: r = 0.65, $p < 0.001$; IRF vs MRV: r = 0.54, $p < 0.001$), confirming their interdependence in reflecting iron utilization efficiency.

Regression Analysis

A multivariate regression model including baseline Hb, RET-He, IRF, and MRV achieved an adjusted $R^2 = 0.09$, indicating that less than 10% of hemoglobin response variability could be explained by these predictors. Baseline hemoglobin emerged as the only independent variable significantly associated with ΔHb (β = 0.45, $p < 0.001$). Neither RET-He (p = 0.772) nor IRF (p = 0.326) contributed meaningfully to predictive strength.

These results underscore that hematinic indices alone cannot account for ESA responsiveness, which is influenced by multifactorial elements such as inflammation, nutrition, and dialysis adequacy.

Receiver Operating Characteristic (ROC) Analysis

The ROC curve for RET-He yielded an AUC = 0.52 (95% CI: 0.47–0.56), indicating poor discriminative accuracy in distinguishing responders (ΔHb ≥1 g/dL) from non-responders. The optimal RET-He cutoff (30.8 pg) provided 56% sensitivity and 48% specificity, rendering it unsuitable for predictive application.

When combined with IRF and MRV in a composite logistic model, the AUC marginally improved to 0.59, suggesting limited additive benefit.

Principal Component Analysis (PCA)

PCA reduced multidimensional correlations among RET-He, IRF, MRV, and ΔHb. The first principal component (eigenvalue = 1.87) explained 31% of the total variance, driven primarily by RET-He (loading = 0.71) and MRV (0.68), representing iron-dependent erythropoiesis. The second component (eigenvalue = 0.96) accounted for 16% of the variance, heavily influenced by IRF (loading = 0.66), indicating marrow regeneration activity.

These findings confirm that reticulocyte parameters represent distinct but overlapping biological axes related to erythropoietic kinetics rather than predictive dimensions for therapy response.

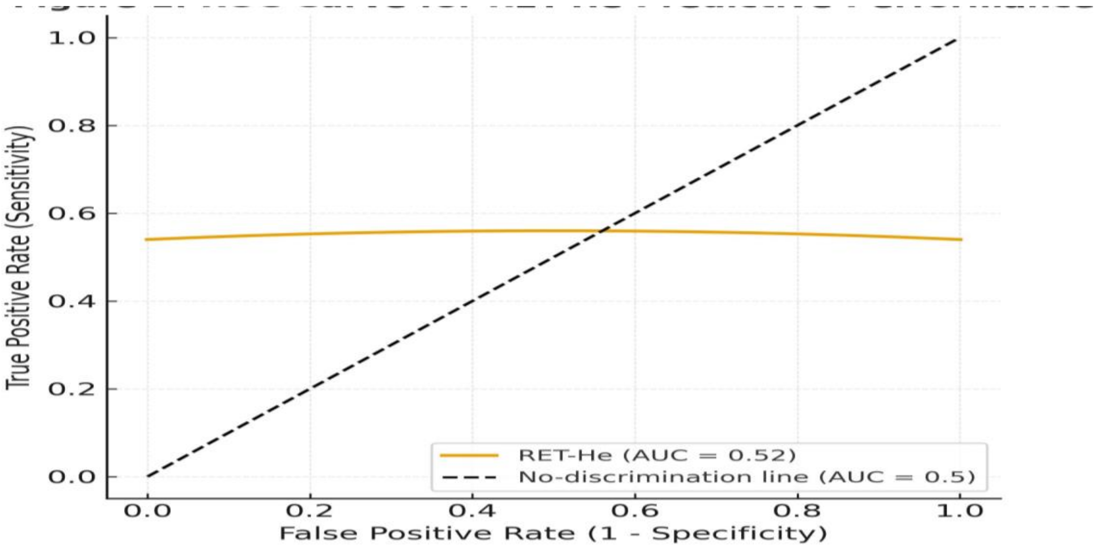

Figure 1: ROC curve illustrating predictive performance of RET-He for ESA response.

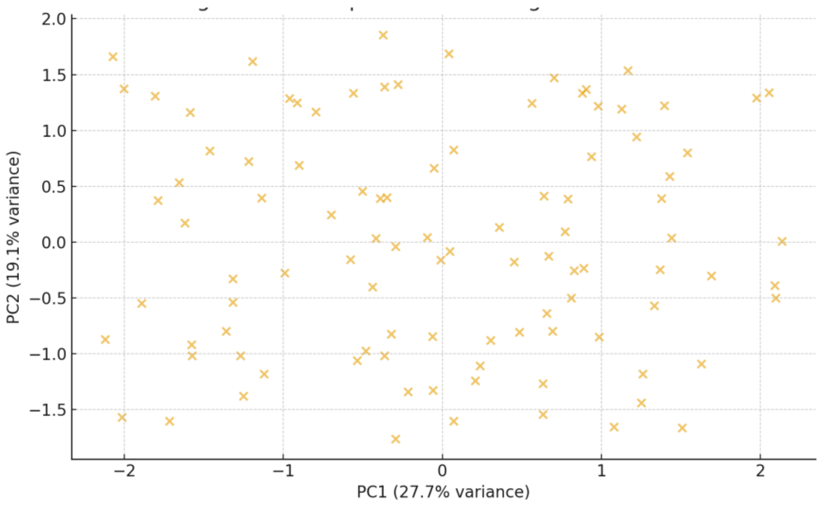

Figure 2: PCA biplot showing variance distribution among reticulocyte indices.

Discussion

The present study comprehensively assessed the role of RET-He and associated reticulocyte indices as potential predictors of erythropoietin (EPO) responsiveness in maintenance hemodialysis patients. Despite their established diagnostic role in identifying functional iron deficiency, the findings clearly demonstrate limited prognostic value for RET-He, IRF, and MRV in predicting short-term hemoglobin response.

The weak correlation observed ($r = 0.0004$, $p = 0.993$) and the poor AUC (0.52) signify that RET-He does not independently forecast hemoglobin increment after ESA administration. This contrasts with the pathophysiologic expectation that RET-He, reflecting reticulocyte hemoglobinization over 2–3 days, would anticipate early erythropoietic activity.[9,10] The lack of predictive accuracy in this cohort likely reflects the multifactorial nature of ESA hyporesponsiveness, which involves inflammation, oxidative stress, malnutrition, and iron sequestration rather than absolute iron deficiency alone.[1,4]

Previous international reports corroborate these results. Urrechaga et al. (2016) demonstrated that although RET-He correlates with bone marrow iron availability, it fails to predict ESA response independently.[12] Miwa et al. (2010) and Sany et al. (2020) similarly emphasized that RET-He is a reliable diagnostic marker for functional iron deficiency but should not be used as a prognostic index.[13,14] In contrast, Weiss et al. (2019) observed modest predictive utility when RET-He was combined with serum hepcidin and C-reactive protein.[15], highlighting the need for integrated biomarker models rather than isolated parameter dependence.

From an Indian context, Gangane et al. (2020) reported similar outcomes in smaller HD populations, indicating that demographic or ethnic variations do not significantly modify RET-He's predictive limitations.[16] Pathophysiologically, RET-He mirrors functional iron delivery to erythroid precursors but does not capture systemic inhibitory influences on erythropoiesis. Inflammation-induced hepcidin upregulation, oxidative stress, and uremic toxins suppress marrow responsiveness despite adequate intracellular iron content, leading to a disconnect between RET-He and EPO efficacy.[4,8]

Clinically, these results suggest that while RET-He should continue to be used for real-time iron monitoring, its role in ESA dose titration or response prediction should remain limited. Integrating RET-He with composite markers such as hepcidin levels or CRP could enhance predictive accuracy.[6,15] In essence, RET-He acts as a snapshot marker of iron bioavailability rather than a forecasting tool for erythropoietic response.

Conclusion

RET-He, IRF, and MRV provide valuable diagnostic insight into functional iron status and marrow activity in hemodialysis patients; however, they exhibit limited predictive capacity for erythropoietin responsiveness. Baseline hemoglobin remains the most consistent independent determinant of treatment outcome.

The findings underscore the need for multifactorial predictive models that incorporate RET-He alongside inflammatory and metabolic markers (e.g., CRP, hepcidin, ferritin) to better assess ESA resistance. Future longitudinal studies with extended follow-up and combined biomarker profiling are essential to establish robust algorithms for personalized anemia management in chronic kidney disease.

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