Diagnosis of Iron Deficiency in Patients Undergoing Hemodialysis

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Abstract

To diagnose iron deficiency in patients undergoing hemodialysis, the percentage of hypochromic RBCs (with cellular hemoglobin concentration <280 g/L [HYPO%]) and mean reticulocyte hemoglobin content (CHret) provided by the Siemens ADVIA 120 and 2120 analyzers (Siemens Diagnostic Solutions, Tarrytown, NY) were proposed as alternatives to biochemical tests. Sysmex, with its XE-5000 analyzer (Sysmex, Kobe, Japan), also proposed the percentage of erythrocytes with cellular hemoglobin content lower than 17 pg (%Hypo-He) and equivalent of the mean reticulocyte hemoglobin content (Ret-He) with similar clinical applications.

Our aim was to verify the clinical usefulness of the biochemical and cellular parameters as predictors of iron deficiency in patients undergoing long-term hemodialysis. We studied 69 patients undergoing hemodialysis 3 times weekly. The baseline values of serum ferritin and percentage of transferrin saturation were poor predictors of iron responsiveness. Better ability was demonstrated by reticulocyte indices (area under the curve [AUC], 0.74 for CHret and 0.72 for Ret-He; best cutoff values, 31.2 and 30.6 pg, respectively) and erythrocyte parameters (AUC, 0.72 for HYPO% and 0.68 for %Hypo-He; best cutoff values, 5.8 and 2.7, respectively).

The newly proposed Ret-He and %Hypo-He can provide clinicians with information equivalent to CHret and HYPO%.

Anemia is a common complication in people affected by chronic kidney disease, especially patients undergoing hemodialysis. Correction of the anemia yields numerous benefits: a higher tolerance for physical activity,[1,2] an improvement of cognitive and cardiovascular functions,[3,4] a better quality of life,[5,6] reduced hospitalization, and lower mortality.[7] Anemia is corrected with the administration of erythropoiesis-stimulating agents (ESAs). The therapeutic goal is to reach a hemoglobin concentration between 11.0 and 12.0 g/dL (110-120 g/L).[8-10]

In patients undergoing hemodialysis and treated with ESAs, iron-deficient erythropoiesis frequently develops. The iron deficiency can be absolute (eg, malnutrition, gastrointestinal bleeding, chronic blood retention in the dialysis circuit, and frequent blood collections) or functional (ie, limitation of bone marrow erythropoietic activity by inability to mobilize sufficient iron from body storage sites); in this situation the body's total iron stores may be normal.

The iron deficit limits the effectiveness of the therapy with ESAs, and, to optimize the treatment, patients must receive an intravenous (IV) iron supplement.[11-13] Because parenteral iron administration has potential risks that are immediate (eg, toxic effects and anaphylactic reactions) and long-term (eg, decreased polymorphonuclear leukocyte function, increased risk of infections, organ damage), it is essential to select patients who need iron supplementation.

Best-practice guidelines have been developed to provide indications for the use of potentially useful parameters for identifying and managing iron deficiency in patients undergoing hemodialysis: the European Best Practice Guideline and the US Kidney Disease Outcomes Quality Initiative (KDOQI) **Table 1**.[8-10] The main differences concern the

Table 1
Summary of Renal Anemia Guidelines for Patients Undergoing Hemodialysis

	European Best Practice Guidelines	US Kidney Disease Outcome Quality Initiative
Target hemoglobin level, g/dL (g/L)	>11.0 (110)	11.0-12.0 (110-120)
Ferritin, ng/mL (pmol/L)	>100 (225)	>200 (449)
Transferrin saturation (%)	>20	≥20
HYPO%	<10	—
CHret (pg)	>29	>29

CHret, mean reticulocyte hemoglobin content; HYPO%, percentage of erythrocytes with cellular hemoglobin concentration lower than 280 g/L.

threshold value of the serum ferritin concentration (100 vs 200 ng/mL [225 vs 449 pmol/L]) and, overall, the use of the percentage of erythrocytes with cellular hemoglobin concentration lower than 280 g/L (HYPO%), which represents the percentage of hypochromic RBCs. Even though HYPO% has been demonstrated as one of the best predictors of iron deficiency, it is not included in the KDOQI guidelines because the measured value depends on the time elapsed between collection and analysis. In fact, the erythrocytes in samples stored at room temperature tend to swell progressively, with consequent reduction of the cellular hemoglobin concentration and an increase of HYPO%. In contrast, the mean reticulocyte hemoglobin content (CHret) parameter that indicates the average hemoglobin content of the reticulocytes remains stable over time. When HYPO% is used, the suggested threshold is 10%, but more recent studies indicate better diagnostic efficiency by using values at a lower threshold, around 6% to 7%.[14,15]

The erythrocyte and reticulocyte parameters proposed by the guidelines are available only with ADVIA 120 and 2120 analyzers (Siemens Diagnostic Solutions, Tarrytown, NY) and have, therefore, limited availability. Recently, the Sysmex XE-5000 analyzer (Sysmex, Kobe, Japan) was marketed. It provides a reticulocyte parameter, the equivalent of the mean reticulocyte hemoglobin content (Ret-He), similar to CHret, and the percentage of erythrocytes with cellular hemoglobin content lower than 17 pg (%Hypo-He), based on the cell-by-cell determination of the hemoglobin content. The percentage of hypochromic RBCs obtained with the 2 different analyzers, while based on different cellular indices (hemoglobin content vs concentration), should theoretically provide parallel indications in iron-deficiency states.

The main goal of this work is the study of the %Hypo-He and Ret-He parameters provided by the XE-5000 in comparison with the more familiar HYPO% and CHret obtained with the ADVIA, in order to identify the cutoff values for %Hypo-He and Ret-He that correspond to the threshold values suggested by the guidelines and scientific literature for HYPO% and CHret and to evaluate the effectiveness of hypochromic erythrocytes and reticulocyte parameters in predicting the response to iron administration in patients undergoing hemodialysis in comparison with biochemical indices such as the percentage of transferrin saturation (TSAT%) and serum ferritin levels.

Materials and Methods

Analytic Methods

The CBC, including HYPO%, and the reticulocyte indices, including CHret, were obtained with the ADVIA 120. HYPO% is, by the manufacturer's definition, the percentage of erythrocytes with a corpuscular hemoglobin concentration of less than 280 g/L. The instrument provides the concentration of hemoglobin on a cell-by-cell basis by using a "high angle scatter" determination on the previously "sphered" erythrocytes. CHret represents the average of the values of the hemoglobin content of individual reticulocytes. The hemoglobin content of each reticulocyte is the product of hemoglobin concentration times the cell volume, both obtained by measures based on the "light scattering" method.[16]

The percentage of erythrocytes with a low hemoglobin content (%Hypo-He) and the Ret-He were obtained with the Sysmex XE-5000. These parameters were obtained by using a "forward light scatter" measure that, because of the particular characteristics of the system, correlates to the cellular hemoglobin content.[17,18] Because it is not a direct measurement of the cell hemoglobin content, the term *equivalent* was proposed by the manufacturer for this parameter. The instrument defines cells with a low hemoglobin (equivalent) content as those erythrocytes with a value lower than an arbitrary preset threshold equivalent to 17 pg. Ret-He represents the average of the distribution of the values of the hemoglobin content of individual reticulocytes.

The serum iron level was determined by a colorimetric method and the serum ferritin level with an immunometric method (Roche Diagnostics, Milan, Italy); transferrin and C-reactive protein (CRP) values were obtained with an immunonephelometric method (Siemens Diagnostic Solutions, Milan, Italy), and the TSAT% was obtained by calculation.

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Study Design

To analyze the predictive efficiency of the suggested parameters in guiding the treatment of patients with anemia who were undergoing hemodialysis, we studied 69 patients undergoing long-term hemodialysis 3 times per week and receiving ESA maintenance therapy. Patients with hemoglobin concentrations of more than 12.0 g/dL (120 g/L), recent bleeding, clinically evident inflammatory or infectious disease, malignancy, hemoglobinopathy, and a requirement for blood transfusion were excluded. The trial lasted a total of 21 weeks.

Because before enrollment the majority of patients received IV iron, the administration was interrupted in the 3 weeks before the study to obtain a reasonable wash-out. From the total of enrolled patients, 10 were lost because of complications or death. Therefore only 59 completed the trial.

In this study, "responders" (ie, probably iron-deficient) were defined as patients who had an increase in hemoglobin of at least 1.0 g/dL (10 g/L) compared with baseline at any time after the third week from the administration of IV iron. The administration of IV iron gluconate (Ferlixit, Aventis Pharma, Milan, Italy) and α-darbepoetin (Aranesp, Amgen, Milan, Italy) was based on parameters suggested by the guidelines with the objective of reaching and maintaining a hemoglobin concentration between 11.0 and 12.0 g/dL (110-120 g/L).

Blood samples for CBC and reticulocyte indices were obtained before the first-of-the-week hemodialysis session, every week for the first 12 weeks and monthly for another 2 months (total, 21 weeks). All other parameters (serum ferritin, TSAT%, and CRP) were measured every 4 weeks. The samples for CBC and reticulocytes were analyzed within 3 hours from sampling in parallel with the ADVIA 120 and XE-5000 analyzers. Informed consent was obtained from participants before enrollment.

Statistical Analysis

Regression analysis was used to compare HYPO% and %Hypo-He and to calculate the best fit between the 2 variables. The differences between responders and nonresponders were evaluated by using the Student *t* test method, and a *P* value less than .05 was considered significant.

To identify the efficiency of the test and the optimal cutoff values for predicting the response to iron administration, a receiver operating characteristic analysis was performed, and the sensitivity and specificity were calculated at various cutoff levels.

Results

Comparison of Methods

When HYPO% and %Hypo-He were compared, the numeric difference between the parameters increased progressively with the increase of the value of HYPO%. The best fit is a nonlinear relationship (polynomial equation of second order: $y = 0.2016 + 0.3981x + 0.00356x^2$). By using this equation, it is possible to calculate the values of %Hypo-He corresponding to the threshold suggested by international guidelines for HYPO%; these values are 2.7% and 4.5% vs 6% and 10%, respectively.

Predictive Value for a Positive Response in HD Patients

Table 2 summarizes the data (mean and SD) at baseline for all patients subdivided into responders and nonresponders. At baseline, responders had lower values of hemoglobin, TSAT%, and CHret, whereas the HYPO% was higher, and the serum ferritin level showed no substantial difference. Statistical differences between groups were found only for hemoglobin, CHret, and Ret-He. The prevalence of CRP of more than 6 mg/L (57.1 nmol/L; used as marker of inflammation) was approximately the same in both groups (58% vs 51%).

Figure 1 shows the receiver operating characteristic curve concerning the parameters considered in this study: HYPO% (and %Hypo-He), CHret (and Ret-He), serum ferritin, and TSAT%. **Table 3** gives the areas under the curve (AUCs) with the confidence intervals, the significance of the difference from .5 (equivalent to no predictive ability), the best cutoff, and the corresponding sensitivity and specificity. Statistically significant differences from .5 were observed for erythrocyte (AUCs, 0.72 and 0.68) and reticulocyte (AUCs, 0.74 and 0.72) parameters, but not for TSAT% and serum ferritin. No statistical differences were found between HYPO% and %Hypo-He or between CHret and Ret-He.

The best threshold values for predicting response to iron administration were as follows: 5.8 for HYPO% (sensitivity, 45%; specificity, 87%), a result better than the

Table 2
Baseline Characteristics of Patients Classified as Responders and Nonresponders to Intravenous Iron Administration*

	Responders	Nonresponders	*P*
Hemoglobin (g/L)	107.1 ± 9.1	113.3 ± 5.4	.0063
HYPO%	6.4 ± 4.9	3.8 ± 5	.0580
%Hypo-He	2.3 ± 2.2	1.6 ± 2.3	.2809
CHret (pg)	31.3 ± 1.9	33.0 ± 2.9	.0077
Ret-He (pg)	30.6 ± 2.9	32.9 ± 4.1	.0129
Ferritin (ng/mL)	255 ± 296	220 ± 181	.6247
Transferrin saturation (%)	16 ± 9	19 ± 9	.3328
CRP (mg/L)	13.6 ± 18.3	7.3 ± 6.3	.1334

CHret, mean reticulocyte hemoglobin content; CRP, C-reactive protein; HYPO%, percentage of erythrocytes with cellular hemoglobin concentration lower than 280 g/L; %Hypo-He, percentage of erythrocytes with cellular hemoglobin content lower than 17 pg; Ret-He, equivalent of the mean reticulocyte hemoglobin content.

* Data are given as mean ± SD. The values for hemoglobin are given in Système International (SI) units; to convert to conventional units, divide by 10. The values for ferritin and CRP are given in conventional units; conversions to SI units are as follows: ferritin (pmol/L), multiply by 2.247; CRP (nmol/L), multiply by 9.524.

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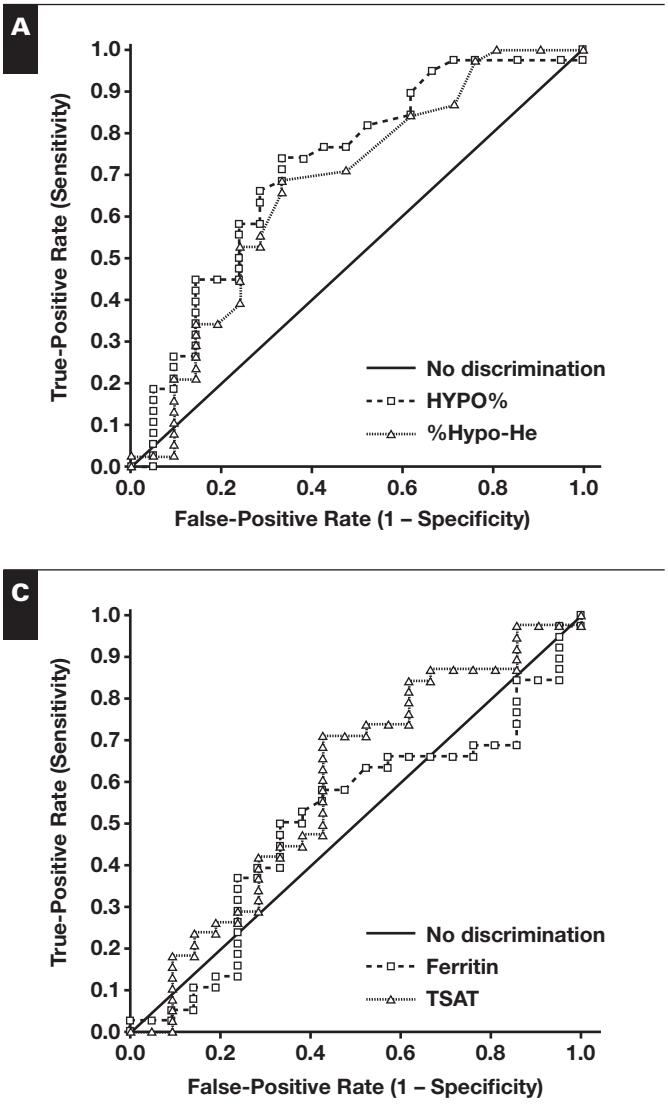

Figure 1 Results of the receiver operating characteristic curves. **A**, Hypochromic RBCs. **B**, Reticulocyte hemoglobin content. **C**, TSAT percentage and serum ferritin level. CHret, mean reticulocyte hemoglobin content; HYPO%, percentage of erythrocytes with cellular hemoglobin concentration lower than 280 g/L; %Hypo-He, percentage of erythrocytes with cellular hemoglobin content lower than 17 pg; RET-He, equivalent of the mean reticulocyte hemoglobin content; TSAT, transferrin saturation.

one obtained using the threshold of 10% (sensitivity, 26%; specificity, 91%); 2.7 for %Hypo-He (sensitivity, 34%; specificity, 87%); 31.2 for CHret (sensitivity, 47%; specificity, 83%); 30.6 for Ret-He (sensitivity, 45%; specificity, 83%). The reticulocyte parameters are the best predictors, immediately followed by hypochromic RBCs, whereas the biochemical indices of iron metabolism as TSAT% and serum ferritin are useless.

It is interesting to observe that the best threshold for Hypo-He obtained through this approach (2.7%) can be

Table 3
Diagnostic Characteristics of Tests for Predicting Iron Deficiency

Test	AUC	95% CI	*P**	Cutoff	Sensitivity (%)	Specificity (%)
CHret (pg)	0.74	0.60-0.89	<.001	31.2	47	83
Ret-He (pg)	0.72	0.58-0.86	<.003	30.6	45	83
HYPO%	0.72	0.58-0.86	<.001	5.8	45	87
%Hypo-He	0.68	0.54-0.82	<.014	2.7	34	87
Ferritin (ng/mL)	0.53	0.38-0.69	.470	—	—	—
Transferrin saturation (%)	0.56	0.40-0.72	.130	—	—	—

AUC, area under the curve; CHret, mean reticulocyte hemoglobin content; CI, confidence interval; HYPO%, percentage of erythrocytes with cellular hemoglobin concentration lower than 280 g/L; %Hypo-He, percentage of erythrocytes with cellular hemoglobin content lower than 17 pg; Ret-He, equivalent of the mean reticulocyte hemoglobin content.
* Significance of the difference from .5 (equivalent to no predictive ability).

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superimposed on the values obtained with the polynomial regression when 6% of HYPO was used as the reference.

Discussion

Functional or absolute iron deficiency is a limiting factor for the efficacy of ESAs in patients undergoing hemodialysis. The administration of IV iron can cause unwanted effects, immediate and long-term, so it becomes important to select patients who need iron supplementation and possibly have the parameters useful in predicting an effective response. Several national and international guidelines suggest using some widely available biochemical markers for recognizing iron deficiency: TSAT% and serum ferritin. However TSAT% is strongly influenced by the daily fluctuation of serum iron levels, and the serum ferritin value is an acute phase protein and, thus, can be increased in chronic inflammatory diseases such as uremia. The guidelines for treating anemia in patients undergoing hemodialysis and receiving ESAs and IV iron agree on the lower values of TSAT (<20%) at which therapy has to be started but disagree on the upper value of ferritin, which should not be exceeded to avoid the risk of acute and chronic toxicity.[14,19-21]

Erythrocyte and reticulocyte indices such as the HYPO% and the reticulocyte hemoglobin content provide direct information on bone marrow iron availability and on its use for hemoglobin synthesis. So, they can be used as alternatives. However, the HYPO% is strongly influenced by the time between sampling and analysis, and it is not recommended by the KDOQI guidelines. The same time limits when samples were stored at room temperature were found for %Hypo-He, but with a decrease of the value over time as opposed to the HYPO% behavior (M.B., personal observations).

The major obstacle to the use of these parameters is their reduced availability. In fact, they are provided by only one manufacturer (Siemens) with the ADVIA 120 and 2120 analyzers. The recently marketed XE-5000 analyzer offers some RBC and reticulocyte parameters (%Hypo-He and Ret-He) with the same clinical applications, extending the adoption of the use of these parameters.

In exploring the efficiency of biochemical and cellular parameters of iron status in predicting response to IV iron in patients undergoing hemodialysis, we observed that baseline TSAT% and iron ferritin levels were not predictive of iron responsiveness. These results agree with the conclusions of several other studies.[22-24] Instead, the cellular parameters showed better ability to discriminate iron deficiency from iron repletion with an acceptable compromise between specificity and sensitivity.

The best threshold for CHret was 31.2 pg (30.6 pg for Ret-He), a value higher than the target of 29 pg proposed by the guidelines but closer to the results obtained in other recent works.[25-27] This finding shows that 29 pg as a cutoff value is exceedingly low as a response predictor. The best combination of sensitivity and specificity for HYPO% was at a threshold of 5.8%, very close to the 6% suggested by more recent studies and guidelines.[14,15,28]

The results of this study are in agreement with those of others concerning the hierarchy of the parameters as predictors of response to anemia treatment[14,15,29]; however, the sensitivity, specificity, and AUCs were lower. These differences are probably due to the clinical status of the enrolled patients. On one hand, the hemoglobin concentration and serum ferritin values at baseline were higher in our study, on average, than in the studies by Tessitore and others[14] and Chuang and others[29]; on the other hand, the amount of administered therapy was lower in our patients (with a mean patient dose per week of 98 mg of iron gluconate and 39 μg of α-darbepoetin) than that in the study by Bovy and others.[15]

The fact that with the same administered ESA therapy (mean patient dose per week of 36.5 vs 40.5 μg; $P = .63$) the nonresponders received less iron compared with responders (mean patient dose per week of 80 vs 108 mg; $P = .06$) can be explained by the higher concentration of hemoglobin, a higher CHret, a lower value of HYPO%, and a slightly higher TSAT%; by contrast, the ferritin concentrations were similar. The lack of response could, therefore, depend on insufficient administration of iron.

Therefore, the CHret and Ret-He parameters showed the best predictive power with the threshold values being very near each other: 31.2 vs 30.6 pg. The HYPO% parameter, even with the limits derived from the storage time of the sample, proved to be useful in predicting (with a best threshold value of 5.8%) and influencing therapy choices. Likewise, the new parameter %Hypo-He can be used with different values (with a threshold of 2.7%) as an instrumental alternative for the same clinical applications.

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References

1. Braumann KM, Nonnast-Daniel B, Boning D, et al. Improved physical performance after treatment of renal anemia with recombinant human erythropoietin. *Nephron*. 1991;58:129-134.

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2. Robertson HT, Haley NR, Guthrie M, et al. Recombinant erythropoietin improves exercise capacity in anemic hemodialysis patients. *Am J Kidney Dis*. 1990;15:325-332.
3. Wolcott DL, Marsh JT, La Rue A, et al. Recombinant human erythropoietin treatment may improve quality of life and cognitive function in chronic hemodialysis patients. *Am J Kidney Dis*. 1989;14:478-485.
4. Foley RN, Parfrey PS, Harnett JD, et al. The impact of anemia in cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Am J Kidney Dis*. 1996;28:53-61.
5. Evans RW, Rader B, Manninen DL, and the Cooperative Multicenter EPO Clinical Trial Group. The quality of life of hemodialysis recipients treated with recombinant human erythropoietin. *JAMA*. 1990;263:825-830.
6. Delano BG. Improvement in quality of life following treatment with r-HuEPO in anemic hemodialysis patients. *Am J Kidney Dis*. 1989;14:14-18.
7. Locatelli F, Conte F, Marcelli D. The impact of haematocrit levels and erythropoietin treatment on overall and cardiovascular mortality and morbidity: the experience of the Lombardy Dialysis Registry. *Nephrol Dial Transplant*. 1998;13:1642-1644.
8. Locatelli F, Aljama P, Barany P, et al. Revised European Best Practice Guidelines for the management of anaemia in patients with chronic renal failure. *Nephrol Dial Transplant*. 2004;19(suppl 2):ii1-ii47.
9. KDOQI; National Kidney Foundation. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease [published correction appears in *Am J Kidney Dis*. 2006;48:518]. *Am J Kidney Dis*. 2006;47(suppl 3):S11-S145.
10. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease: 2007 update of hemoglobin target. *Am J Kidney Dis*. 2007;50:471-530.
11. Fishbane S, Maesaka JK. Iron management in end-stage renal disease. *Am J Kidney Dis*. 1997;29:319-333.
12. Taylor JE, Peat N, Porter C, et al. Regular low-dose intravenous iron therapy improves response to erythropoietin in haemodialysis patient. *Nephrol Dial Transplant*. 1996;14:1079-1083.
13. Coyne D. Challenging the boundaries of anemia management: a balanced approach to i.v. iron and EPO therapy. *Kidney Int Suppl*. 2006;69:S1-S3.
14. Tessitore N, Solero GP, Lippi G, et al. The role of iron status markers in predicting response to intravenous iron in haemodialysis patients on maintenance erythropoietin. *Nephrol Dial Transplant*. 2001;16:1416-1423.
15. Bovy C, Gothot A, Delanaye P, et al. Mature erythrocyte parameters as new markers of functional iron deficiency in haemodialysis: sensitivity and specificity. *Nephrol Dial Transplant*. 2007;22:1156-1162.
16. Tycko DH, Metz MH, Epstein EA, et al. Flow-cytometric light scattering measurement of red blood cell volume and hemoglobin concentration. *Appl Opt*. 1985;24:1355-1365.
17. Buttarello M, Temporin V, Ceravolo R, et al. The new reticulocyte parameter (RET-Y) of the Sysmex XE-2100: its use in the diagnosis and monitoring of posttreatment sideropenic anemia. *Am J Clin Pathol*. 2004;121:489-495.
18. Franck S, Linssen J, Messinger M, et al. Potential utility of Ret-Y in the diagnosis of iron-restricted erythropoiesis. *Clin Chem*. 2004;50:1240-1242.
19. Fishbane S, Shapiro W, Dutka P, et al. A randomized trial of iron deficiency testing strategies in hemodialysis patients. *Kidney Int*. 2001;60:2406-2411.
20. Chang CH, Chang CC, Chiang SS. Reduction in erythropoietin doses by the use of chronic intravenous iron supplementation in iron-replete hemodialysis patients. *Clin Nephrol*. 2002;57:136-141.
21. Singh AK, Coyne DW, Shapiro W, et al. Predictors of the response to treatment in anemic hemodialysis patients with high serum ferritin and low transferrin saturation. *Kidney Int*. 2007;71:1163-1171.
22. Mittman N, Sreedhara R, Mushnick R, et al. Reticulocyte haemoglobin content predicts functional iron deficiency in hemodialysis patient receiving rHuEPO. *Am J Kidney Dis*. 1997;30:912-922.
23. Fishbane S, Kowalsky EA, Imbriano LJ, et al. The evaluation of iron status in hemodialysis patients. *J Am Soc Nephrol*. 1996;7:2654-2657.
24. Lin JL, Chang MY, Tan DT, et al. Short-term small-dose intravenous iron trial to detect functional iron deficiency in dialysis patients. *Am J Nephrol*. 2001;21:91-97.
25. Kaneko J, Miyazaki S, Hirasawa Y, et al. Transferrin saturation versus reticulocyte haemoglobin content for iron deficiency in Japanese hemodialysis patients. *Kidney Int*. 2003;63:1086-1093.
26. Mitsuiki K, Harada A, Miyata Y. Assessment of iron deficiency in chronic hemodialysis patients: investigation of cutoff values for reticulocyte haemoglobin content. *Clin Exp Nephrol*. 2003;7:52-57.
27. Kim JM, Ihm CH, Kim HJ. Evaluation of reticulocyte haemoglobin content as marker of iron deficiency and predictor of response to intravenous iron in haemodialysis patients. *Int J Lab Hematol*. 2008;30:46-52.
28. The Renal Association. Clinical Practice Guidelines Module 2: complications. 2007. http://www.renal.org/Clinical/GuidelinesSection/ComplicationsofCKD.aspx#full_S3. Accessed December 14, 2008.
29. Chuang CL, Liu RS, Wei YH, et al. Early prediction of response to intravenous iron supplementation by reticulocyte haemoglobin content and high-fluorescence reticulocyte count in haemodialysis patients. *Nephrol Dial Transplant*. 2003;18:370-377.

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