Appendix A: Literature Search Strategy

Database(s): Ovid MEDLINE(R) 1948 to July Week 2 2011, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations July 21, 2011, EBM Reviews - Cochrane Central Register of Controlled Trials 3rd Quarter 2011

1) percentage of hypochromic erythrocytes.mp. [mp=ps, rs, ti, ot, ab, nm, hw, ui, sh, kw] (15)
2) (percentage adj2 hypochromic erythrocytes).tw. (15)
3) *Reticulocytes/pa [Pathology] (77)
4) (%HYPO and CHr).mp. [mp=ps, rs, ti, ot, ab, nm, hw, ui, sh, kw] (11)
5) *Erythrocyte Indices/ (783)
6) Erythrocyte Count.mp. [mp=ps, rs, ti, ot, ab, nm, hw, ui, sh, kw] (10128)
7) *Erythrocytes/an, du, me [Analysis, Diagnostic Use, Metabolism] (18919)
8) *Erythrocytes/pa (994)
9) Erythropoiesis/ph (1271)
10) *Reticulocytes/ch (65)
11) *Reticulocyte Count/ (156)
12) Ferritins/bl (7434)
13) *Hemoglobins/an (5118)
14) *Erythrocyte Indices/ (783)
15) Reticulocytes/me (4605)
16) Transferrin/an (3892)
17) TSAT.tw. (221)
18) exp Anemia, Hypochromic/ or exp Anemia, Iron-Deficiency/ (15078)
19) hypochromic an?emia.mp. (802)
20) hypochromic erythrocytes.mp. (40)
21) Anemia, Iron-Deficiency/dt (1439)
22) (transferrin adj saturation).af. (2614)
23) exp transferrin/ (14770)
24) hepcidin.af. (1413)
25) ((soluble or serum) adj transferrin).af. (3648)
26) ((soluble or serum) adj transferrin adj receptor).af. (822)
27) (zinc adj protoporhyrin).af. (5)
28) erythrocyte zinc protoporhyrin.mp. (1)
29) superconducting quantum interference device.mp. (345)
30) *Biological Markers/an, bl, me [Analysis, Blood, Metabolism] (11098)
31) *ferritins/ or *apoferritins/ (6927)
32) acute-phase proteins/ or exp transferrin/ (19169)
33) transferrin.mp. (28365)
34) Transferrin/ad, an, bl, du, de, me, pk, tu [Administration & Dosage, Analysis, Blood, Diagnostic Use, Drug Effects, Metabolism, Pharmacokinetics, Therapeutic Use] (10637)
35) or/1-34 (104459)
36) exp "sensitivity and specificity"/ (347657)

37) exp Predictive Value of Tests/ (116486)
38) exp ROC CURVE/ (20708)
39) exp Mass Screening/ (88916)
40) exp diagnosis/ (5609927)
41) exp REPRODUCIBILITY OF RESULTS/ (224874)
42) exp false negative reactions/ or false positive reactions/ (31445)
43) predictive value.tw. (47133)
44) (sensitivity or specificity).tw. (616885)
45) accuracy.tw. (179753)
46) screen$.tw. (378934)
47) diagno$.tw. (1399885)
48) roc.tw. (15058)
49) reproducib$.tw. (95276)
50) (false positive or false negative).tw. (41652)
51) likelihood ratio.tw. (4915)
52) accuracy.tw. (179753)
53) di.fs. (1755561)
54) biological variability.mp. (700)
55) reference values.tw. (8341)
56) reference standard$.tw. (6786)
57) or/36-56 (7398453)
58) (NeoRecormon or Aranesp or Methoxy Polyethylene Glycol Epoetin Beta or MIRCERA or Epoetin or Dynepo or PDpoetin).af. or (NeoRecormon or Aranesp or Methoxy Polyethylene Glycol Epoetin Beta or MIRCERA or Epoetin or Dynepo or PDpoetin).tw. (2810)
59) (epogen or epotin or betapoietin or relpoietin or epokine or procrit or eprex or darbopoietin).af. or (epogen or epotin or betapoietin or relpoietin or epokine or procrit or eprex or darbopoietin).tw. (252)
60) (Ferumoxytol or Feraheme or Iron Dextran or DexFerrum or INFeD or Ferrous fumarate or ferrous gluconate or ferrous sulfate or ferrous sulphate or carbonyl iron or polysaccharide iron complex or Icar or Feosol or Ircon or Hemocyte or Nephro-Fer or Feostat or Ferro-DSS or Ferro-Sequels or Fergon or Fer-Gen-Sol or Fer-In-Sol or Mol-Iron or Feratab or Ferrex or Niferex or Hytinic or Fe-Tinic or Iron sucrose or Venofer or Sodium ferric gluconate or Ferrlecit).mp. or exp Iron/ or exp Ferric Compounds/ or exp Ferrous Compounds/ or exp Anemia, Iron-Deficiency/ or ferrous.mp. (105161)
61) exp recombinant erythropoietin/ or recombinant erythropoietin.mp. (6065)
62) or/58-61 (111284)
63) exp Renal Replacement Therapy/ or exp Renal Dialysis/ or exp Kidney Transplantation/ or exp Kidney Function Tests/ or renal.mp. or nephro$.mp. or kidney.mp. or ur?emia.tw. or h?emodialysis.tw. (827443)
64) hemodialysis.af. (45683)
65) peritoneal dialysis.mp. or exp Peritoneal Dialysis/ (24886)
66) exp Kidney Diseases/ or exp Kidney Failure, Chronic/ or chronic kidney disease.mp. or exp Chronic Disease/ or exp Kidney Glomerulus/ (584160)
67) or/63-66 (1060229)
68) 35 and 57 and 67 (5403)

69) remove duplicates from 68 (5089)
70) 35 and 62 and 67 (2619)
71) remove duplicates from 70 (2409)
72) 69 and 71 (1287)
73) 69 not 72 (3802)
74) 73 or 71 (6211)
75) limit 74 to (addresses or bibliography or biography or case reports or comment or congresses or consensus development conference or dictionary or directory or festschrift or in vitro or interactive tutorial or interview or lectures or legal cases or legislation or news or newspaper article or overall or patient education handout or periodical index or portraits or "scientific integrity review" or twin study) [Limit not valid in CCTR; records were retained] (458)
76) 74 not 75 (5753)

Appendix B: Excluded Studies

Of the 661 articles obtained for full-text screening, 30 were included and 631 were excluded on double, independent full-text screening because they did not meet one or more of the PICO criteria for a particular key questions. The two most common reasons for rejection were: a) no diagnostic outcomes reported (studies reported only correlations between markers or the measurements of levels of markers before and after treatment); b) no comparative data for the outcomes of management strategies where treatment decisions were guided by test results (newer versus classical markers). The 631 excluded references are listed below, in alphabetic order of first author's surname, along with the reason for exclusion for each.

1) [No authors listed]. Serum ferritin concentrations after intravenous iron-dextran. Lancet. 1(8279):1017-8, 1982 May 1. PMID: 6122828
Not biomarkers of interest - older markers only (KQ2)

2) Abbas EE,Afioni N,Al WJ,Bakr MA,Dham R,Donia A,Droubi N,Khidir E,Mathew CM,Mitwali AH,Naga S,Pingle A,Rashed A,Roshdy A,Shaheen F,Shaibani B,Shaibani FM,Shaker DS,Sheiban A,Solieman M. The new rHuEPO alpha (epotin) in the management of anemia of end-stage renal disease in patients on maintenance hemodialysis. Transplant Proc. 36(6):1805-11, 2004 Jul-Aug. PMID: 15350482
Single arm Tx cohort (KQ3)

3) Acharya VN,Sinha DK,Almeida AF,Pathare AV. Effect of low dose recombinant human omega erythropoietin (rHuEPO) on anaemia in patients on hemodialysis. J Assoc Physicians India. 43(8):539-42, 1995 Aug. PMID: 8772973
Single arm treatment cohort and analytic validity data for older markers only

4) Adachi Y,Nishio A. N-terminal pro-brain natriuretic peptide in prevalent peritoneal dialysis patients. Adv Perit Dial. 24:75-8, 2008. PMID: 18986006
Not a biomarker of interest (N-terminal BNP)

5) Agarwal AK,Silver MR,Reed JE,Dhingra RK,Liu W,Varma N,Stehman-Breen C. An open-label study of darbepoetin alfa administered once monthly for the maintenance of haemoglobin concentrations in patients with chronic kidney disease not receiving dialysis. J Intern Med. 260(6):577-85, 2006 Dec. PMID: 17116009
Single arm Tx cohort (KQ3)

6) Agarwal R,Davis JL,Hamburger RJ. A trial of two iron-dextran infusion regimens in chronic hemodialysis patients. Clin Nephrol. 54(2):105-11, 2000 Aug. PMID: 10968685
Only older markers studied in terms of analytic validity data

7) Agarwal R,Leehey DJ,Olsen SM,Dahl NV. Proteinuria induced by parenteral iron in chronic kidney disease--a comparative randomized controlled trial. Clin J Am Soc Nephrol. 6(1):114-21, 2011 Jan. PMID: 20876669
Not test-directed Tx

8) Agarwal R,Rizkala AR,Bastani B,Kaskas MO,Leehey DJ,Besarab A. A randomized controlled trial of oral versus intravenous iron in chronic kidney disease. Am J Nephrol. 26(5):445-54, 2006. PMID: 17035697
Analytic validity data only (KQ2)

9) Agarwal R,Vasavada N,Sachs NG,Chase S. Oxidative stress and renal injury with

intravenous iron in patients with chronic kidney disease. Kidney Int. 65(6):2279-89, 2004 Jun. PMID: 15149341
Not test-directed Tx

10) Aggarwal HK,Nand N,Singh S,Singh M,Hemant,Kaushik G. Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India. 51:170-4, 2003 Feb. PMID: 12725261
Treatment not based on markers and anlaytic validity data for only older markers

11) Aggarwal HK,Tziviskou E,Bellizzi V,Khandelwal M,Moupas L,Bargman JM,Jassal SV,Oreopoulos DG. Prolonged administration over six hours of large doses of intravenous iron saccharate (500 mg) prevents severe adverse reactions in peritoneal dialysis patients. Perit Dial Int. 22(5):636-7, 2002 Sep-Oct. PMID: 12455582
Treatment no based on biomarker

12) Ahluwalia N,Lammi-Keefe CJ,Bendel RB,Morse EE,Beard JL,Haley NR. Iron deficiency and anemia of chronic disease in elderly women: a discriminant-analysis approach for differentiation. Am J Clin Nutr. 61(3):590-6, 1995 Mar. PMID: 7872225
Population group not of interest.

13) Ahsan N,Groff JA,Waybill MA. Efficacy of bolus intravenous iron dextran treatment in peritoneal dialysis patients receiving recombinant human erythropoietin. Adv Perit Dial. 12:161-6, 1996. PMID: 8865893
N<10, Single arm treatment cohort, analytic validity data for older markers only

14) Ahsan N. Infusion of total dose iron versus oral iron supplementation in ambulatory peritoneal dialysis patients: a prospective, cross-over trial. Adv Perit Dial. 16:80-4, 2000. PMID: 11045266
Analytic validity data for only older markers studied

15) Ahsan N. Intravenous infusion of total dose iron is superior to oral iron in treatment of anemia in peritoneal dialysis patients: a single center comparative study. J Am Soc Nephrol. 9(4):664-8, 1998 Apr. PMID: 9555669
Comparison of Different cutoffs

16) Ahsan N. ITDI is the preferred treatment for iron supplementation in rHuEpo-treated anemic PD patients. Adv Perit Dial. 14:228-31, 1998. PMID: 10649730
Treatment based only on single marker and analytic validity data for only older markers studied

17) Akcicek F,Ozkahya M,Cirit M,Ok E,Unsal A,Toz H,Celik A,Atabay G,Basci A. The efficiency of fractionated parenteral iron treatment in CAPD patients. Adv Perit Dial. 13:109-12, 1997. PMID: 9360661
not test-directed Tx (KQ3)

18) Akhmedkhanov SS,Shamov RI. [Characteristics of the outcomes of different clinical forms of iron deficiency anemia and various aspects of its ambulatory treatment]. [Russian]. Sov Med. (9):73-5, 1991. PMID: 1798932
Article in russian; appears to be analytical validity data only; the google translate was not clear;

19) Albertazzi A,Di LL,Daniele F,Battistel V,Colombi L. Efficacy and tolerability of recombinant human erythropoietin treatment in pre-dialysis patients: results of a multicenter study. Int J Artif Organs. 21(1):12-8, 1998 Jan. PMID: 9554820
Analytic validity data for only older markers studied

20) Albitar S,Meulders Q,Hammoud H,Soutif C,Bouvier P,Pollini J. Subcutaneous versus intravenous administration of erythropoietin improves its efficiency for the treatment of anaemia in haemodialysis patients. Nephrol Dial

Transplant. 10 Suppl 6:40-3, 1995. PMID: 8524493
Analytic validity data only for older markers

21) Ali M,Rigolosi R,Fayemi AO,Braun EV,Frascino J,Singer R. Failure of serum ferritin levels to predict bone-marrow iron content after intravenous iron-dextran therapy. Lancet. 1(8273):652-5, 1982 Mar 20. PMID: 6121967
Analytic validity data only for older markers

22) Aljama P,Ward MK,Pierides AM,Eastham EJ,Ellis HA,Feest TG,Conceicao S,Kerr DN. Serum ferritin concentration: a reliable guide to iron overload in uremic and hemodialyzed patients. Clin Nephrol. 10(3):101-4, 1978 Sep. PMID: 699405
Analytic validity data for older markers only

23) Allegra V,Mengozzi G,Martimbianco L,Vasile A. Long-term monitoring of iron stores in renal transplant recipients. Nephron. 55(4):440-1, 1990. PMID: 2392202
Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

24) Allegra V,Mengozzi G,Spulzaro P,Tenti M,Amendolagine F,Vasile A. [Serum ferritin in subjects with chronic renal insufficiency undergoing hemodialytic treatment]. [Italian]. Minerva Nefrol. 30(1):33-42, 1983 Jan-Mar. PMID: 6843884
Article in Italian; Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

25) Allegra V,Mengozzi G,Vasile A. Iron deficiency in maintenance hemodialysis patients: assessment of diagnosis criteria and of three different iron treatments. Nephron. 57(2):175-82, 1991. PMID: 1902285
Treatment fixed and not based on biomarker and analytic validity data for only older markers studied in terms of response or non response to treatment

26) Al-Shohaib S,Shaker DS,Ghaedi BB,Alyarim M,Emara S,Behairy M. The hematopoietic effect of Epotin (recombinant human erythropoietin-alpha) on maintenance hemodialysis end-stage kidney disease patients. Transplant Proc. 42(3):753-9, 2010 Apr. PMID: 20430164
Single arm Tx cohort (KQ3)

27) Altintepe L,Kurtoglu E,Tonbul Z,Yeksan M,Yildiz A,Turk S. Lower erythropoietin and iron supplementation are required in hemodialysis patients with hepatitis C virus infection. Clin Nephrol. 61(5):347-51, 2004 May. PMID: 15182130
Analytic validity data for only older markers studied

28) Alvo M,Elgueta L,Aragon H,Cotera A. [Correction of anemia in hemodialysis, effect of intravenous iron without erythropoietin]. [Spanish]. Rev Med Chil. 130(8):865-8, 2002 Aug. PMID: 12360794
Single arm treatment cohort and only one biomarker studied with no comparison

29) Ammirati AL,Watanabe R,Aoqui C,Draibe SA,Carvalho AB,Abensur H,Drumond SS,Moreira J,Bevilacqua JL,Silva AC,Tatsch F,Canziani ME. [Hemoglobin levels in hemodialysis patients treated with epoetin: a Brazilian experience]. [Portuguese]. Rev Assoc Med Bras. 56(2):209-13, 2010 Mar-Apr. PMID: 20498997
Single arm Tx cohort (KQ3); Portugese article

30) Amon O,Altrogge H,Kemper M,Strehlau J,Muller-Wiefel DE. Increased need of erythropoietin during peritonitis in children on continuous peritoneal dialysis. Adv Perit Dial. 10:318-20, 1994. PMID: 7999857
Only one biomarker of interest studied in

terms of change in mean value after treatment

31) Anastassiades EG,Howarth D,Howarth J,Shanks D,Waters HM,Hyde K,Geary CG,Yin JA,Gokal R. Monitoring of iron requirements in renal patients on erythropoietin. Nephrol Dial Transplant. 8(9):846-53, 1993. PMID: 8255518
Analytic validity data for older markers only

32) Andre JL,Deschenes G,Boudailliez B,Broux F,Fischbach M,Gagnadoux MF,Horen B,Lahoche-Manucci A,Macher MA,Roussel B,Tsimaratos M,Loirat C. Darbepoetin, effective treatment of anaemia in paediatric patients with chronic renal failure. Pediatr Nephrol. 22(5):708-14, 2007 May. PMID: 17216497
Single arm Tx cohorts (KQ3)

33) Anirban G,Kohli HS,Jha V,Gupta KL,Sakhuja V. The comparative safety of various intravenous iron preparations in chronic kidney disease patients. Ren Fail. 30(6):629-38, 2008. PMID: 18661414
Not test-directed treatment, Tx was fixed

34) Anker SD,Colet JC,Filippatos G,Willenheimer R,Dickstein K,Drexler H,Luscher TF,Mori C,von Eisenhart RB,Pocock S,Poole-Wilson PA,Ponikowski P,FAIR-HF committees and investigators. Rationale and design of Ferinject assessment in patients with IRon deficiency and chronic Heart Failure (FAIR-HF) study: a randomized, placebo-controlled study of intravenous iron supplementation in patients with and without anaemia. Eur J Heart Fail. 11(11):1084-91, 2009 Nov. PMID: 19875408
Not CKD patients

35) Anraku M,Kitamura K,Shintomo R,Takeuchi K,Ikeda H,Nagano J,Ko T,Mera K,Tomita K,Otagiri M. Effect of intravenous iron administration frequency on AOPP and inflammatory biomarkers in chronic hemodialysis patients: a pilot study. Clin Biochem. 41(14-15):1168-74, 2008 Oct. PMID: 18692036
Not test-directed treatment, Tx was fixed

36) Antonov S,T'rkolev N,Chal'kova G,Kuleva V. [Study of iron depot in patients on periodic hemodialysis by determination of serum ferritin]. [Bulgarian]. Vutr Boles. 21(5):34-42, 1982. PMID: 7164404
Article in Bulgarian; Not biomarkers of interest - older markers only (KQ2)

37) Anuradha S,Singh NP,Agarwal SK. Total dose infusion iron dextran therapy in predialysis chronic renal failure patients. Ren Fail. 24(3):307-13, 2002 May. PMID: 12166697
Single arm Tx cohort (KQ3); no Dx information

38) Arabul M,Gullulu M,Yilmaz Y,Eren MA,Baran B,Gul CB,Kocamaz G,Dilek K. Influence of erythropoietin therapy on serum prohepcidin levels in dialysis patients. Med Sci Monit. 15(11):CR583-7, 2009 Nov. PMID: 19865058
Analytic validity data only

39) Arndt U,Kaltwasser JP,Gottschalk R,Hoelzer D,Moller B. Correction of iron-deficient erythropoiesis in the treatment of anemia of chronic disease with recombinant human erythropoietin. Ann Hematol. 84(3):159-66, 2005 Mar. PMID: 15565327
Non CKD-patients

40) Asanuma M,Taguchi C,Uesaka H,Kumagai T,Seino K,Hosokawa H,Yamauchi F,Fujishima M,Murai K,Ishida Y. Discrepancy between the percentage of hypochromic erythrocytes and the reticulocyte hemoglobin content in hemodialysis patients with recombinant human erythropoietin therapy. Lab Hematol. 11(2):124-30, 2005. PMID: 16024336
Sample size <10

41) Ashby DR,Gale DP,Busbridge M,Murphy KG,Duncan ND,Cairns TD,Taube DH,Bloom SR,Tam FW,Chapman RS,Maxwell PH,Choi P. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int. 75(9):976-81, 2009 May. PMID: 19212416
Analytic validity data only (for KQ2)

42) Aucella F,Vigilante M,Scalzulli P,Musto P,Prencipe M,Valente GL,Carotenuto M,Stallone C. Synergistic effect of desferrioxamine and recombinant erythropoietin on erythroid precursor proliferation in chronic renal failure. Nephrol Dial Transplant. 14(5):1171-5, 1999 May. PMID: 10344357
Single arm Tx cohort (KQ3)

43) Auerbach M,Winchester J,Wahab A,Richards K,McGinley M,Hall F,Anderson J,Briefel G. A randomized trial of three iron dextran infusion methods for anemia in EPO-treated dialysis patients. Am J Kidney Dis. 31(1):81-6, 1998 Jan. PMID: 9428456
No comparison between biomarkers and treatment is fixed.

44) Aviles B,Coronel F,Perez-Garcia R,Marcelli D,Orlandini G,Ayala JA,Rentero R. [Anemia management in haemodialysis. EuCliD database in Spain]. [Spanish]. Nefrologia. 22(6):555-63, 2002. PMID: 12516289
Treatment not based on markers and only one biomarker studied with no comparison

45) Baiardi P,Piazza V,Montagna G,Mazzoleni MC. Use of statistical classifiers as support tools for the diagnosis of iron-deficiency anemia in patients on chronic hemodialysis. Stud Health Technol Inform. 43 Pt B:666-70, 1997. PMID: 10179750
sensitivity and specificity comparison only between older markers

46) Bailie GR,Frankenfield DL,Prowant BF,McClellan W,Rocco MV. Erythropoietin and iron use in peritoneal dialysis patients. Report from the 1997 HCFA end-stage renal disease core indicators project. Am J Kidney Dis. 33(6):1187-9, 1999 Jun. PMID: 10352214
Single arm Tx cohort (KQ3)

47) Bailie GR,Plitnick R,Eisele G,Clement C,Rasmussen R. Experience with subcutaneous erythropoietin in CAPD patients. Adv Perit Dial. 7:292-5, 1991. PMID: 1680449
Single arm treatment cohort and analytic validity data for older markers only

48) Baillie FJ,Morrison AE,Fergus I. Soluble transferrin receptor: a discriminating assay for iron deficiency. Clin Lab Haematol. 25(6):353-7, 2003 Dec. PMID: 14641138
<10 pts with CKD in ACD group

49) Baker LR,Cattell WR,Child JA,Savdie E. Iron therapy in maintenance haemodialysis. Clin Sci Mol Med Suppl. 48(6):529-32, 1975 Jun. PMID: 1056283
Single marker studied and treatment is not based on marker

50) Bakkaloglu SA,Ekim M,Tumer N,Akar N,Uysal Z. Soluble transferrin receptor is not a reliable marker of iron deficiency in pediatric CAPD patients. Perit Dial Int. 22(5):621-5, 2002 Sep-Oct. PMID: 12455576
Only Analytic validity data (KQ2)

51) Baldus M,Salopek S,Moller M,Schliesser J,Klooker P,Reddig J,Gansert U,Brass H. Experience with zinc protoporphyrin as a marker of endogenous iron availability in chronic haemodialysis patients. Nephrol Dial Transplant. 11(3):486-91, 1996 Mar. PMID: 8710158
Analytic validity data only (KQ2)

52) Barosi G,Merlo C,Palestra P,Liberato NL,Guarnone R,Di DF,Piazza V,Salvadeo A. Variations in

erythropoiesis and serum ferritin during erythropoietin therapy for anaemia of end-stage renal disease. Acta Haematol. 90(1):13-8, 1993. PMID: 8237268
Analytic validity data only (KQ2)

53) Barraclough KA,Noble E,Leary D,Brown F,Hawley CM,Campbell SB,Isbel NM,Mudge DW,van Eps CL,Sturtevant JM,Johnson DW. Rationale and design of the oral HEMe iron polypeptide Against Treatment with Oral Controlled Release Iron Tablets trial for the correction of anaemia in peritoneal dialysis patients (HEMATOCRIT trial). BMC Nephrol. 10:20, 2009. PMID: 19635169
Not test-directed treatment, Tx was fixed

54) Barrios Y,Espinoza M,Baron MA. [Pro-hepcidin, its relation with indicators of iron metabolism and of inflammation in patients hemodialyzed treated or not with recombinant erythropoietin]. [Spanish]. Nutr Hosp. 25(4):555-60, 2010 Jul-Aug. PMID: 20694291
Analytic validity data only (for KQ2)

55) Barton JC,Barton EH,Bertoli LF,Gothard CH,Sherrer JS. Intravenous iron dextran therapy in patients with iron deficiency and normal renal function who failed to respond to or did not tolerate oral iron supplementation. Am J Med. 109(1):27-32, 2000 Jul. PMID: 10936475
Non CKD patients.

56) Bastani B,Jain A,Pandurangan G. Incidence of side-effects associated with high-dose ferric gluconate in patients with severe chronic renal failure. Nephrology. 8(1):8-10, 2003 Feb. PMID: 15012743
Single arm Tx cohort (KQ3)

57) Beallo R,Dallman PR,Schoenfeld PY,Humphreys MH. Serum ferritin and iron deficiency in patients on chronic hemodialysis. Trans Am Soc Artif Intern Organs. 22:73-9, 1976. PMID: 951893
Only older markers studied

58) Bedani PL,Cecchetti E,Gilli P. Effectiveness of subcutaneous low-dose erythropoietin in patients with chronic renal failure despite functional iron deficiency. Nephron. 71(4):459-60, 1995. PMID: 8587628
Population <10 and analytic validity data for older markers only

59) Beerenhout C,Bekers O,Kooman JP,Van der Sande FM,Leunissen KM. A comparison between the soluble transferrin receptor, transferrin saturation and serum ferritin as markers of iron state in hemodialysis patients. Nephron. 92(1):32-5, 2002 Sep. PMID: 12187081
Analytic validity data (KQ2)

60) Beguin Y,Loo M,R'Zik S,Sautois B,Lejeune F,Rorive G,Fillet G. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood. 82(7):2010-6, 1993 Oct 1. PMID: 8400253
Analytic validity data only (KQ2)

61) Beguin Y,Loo M,R'Zik S,Sautois B,Lejeune F,Rorive G,Fillet G. Quantitative assessment of erythropoiesis in haemodialysis patients demonstrates gradual expansion of erythroblasts during constant treatment with recombinant human erythropoietin. Br J Haematol. 89(1):17-23, 1995 Jan. PMID: 7833259
Analytic validity data only (KQ2)

62) Bell JD,Kincaid WR,Morgan RG,Bunce H,Alperin JB,Sarles HE,Remmers AR. Serum ferritin assay and bone-marrow iron stores in patients on maintenance hemodialysis. Kidney Int. 17(2):237-41, 1980 Feb. PMID: 7382271
Not a biomarker of inoterest (KQ2) - only older biomarkers evaluated

63) Bergmann M,Grutzmacher P,Heuser J,Kaltwasser JP. Iron metabolism under rEPO therapy in patients on maintenance hemodialysis. Int J Artif Organs.

13(2):109-12, 1990 Feb. PMID: 2347653
Analytic validity data only for older markers

64) Besarab A,Amin N,Ahsan M,Vogel SE,Zazuwa G,Frinak S,Zazra JJ,Anandan JV,Gupta A. Optimization of epoetin therapy with intravenous iron therapy in hemodialysis patients. J Am Soc Nephrol. 11(3):530-8, 2000 Mar. PMID: 10703677
Comparison of Different cutoffs

65) Besarab A,Kaiser JW,Frinak S. A study of parenteral iron regimens in hemodialysis patients. Am J Kidney Dis. 34(1):21-8, 1999 Jul. PMID: 10401011
Analytic validity data only (for KQ2); No test-directed Tx (KQ3)

66) Beshara S,Barany P,Gutierrez A,Wikstrom B,Swedish Investigators Group. Varying intervals of subcutaneous epoetin alfa in hemodialysis patients. JN, J. nephrol.. 17(4):525-30, 2004 Jul-Aug. PMID: 15372414
Analytic validty data only for older markers

67) Bezwoda WR,Derman DP,Bothwell TH,MacPhail AP,Torrance JD,Milne FJ,Meyers AM,Levin J. Iron absorption in patients on regular dialysis therapy. Nephron. 28(6):289-93, 1981. PMID: 7312083
Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

68) Bhandari S,Brownjohn A,Turney J. Effective utilization of erythropoietin with intravenous iron therapy. J Clin Pharm Ther. 23(1):73-8, 1998 Feb. PMID: 9756115
Single arm Tx cohort (KQ3)

69) Bhandari S,Brownjohn AM,Turney JH. Response of mean reticulocyte haemoglobin content to intravenous iron therapy in haemodialysis patients. Nephrol Dial Transplant. 13(12):3276-7, 1998 Dec. PMID: 9870516
Single arm study with same treatment provision. Analytic validity data only (KQ2)

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Single arm study

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Single arm Tx cohorts

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Single arm Tx cohort (KQ3); no Dx information

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single arm, fixed treatment

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Non-CKD patienots

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Dx information for older markers only; Single arm Tx cohort (KQ3)

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TfR-ferritin Index is a combination of a new and old marker

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non CKD-patienots

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Population non CKD

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No biomarker studied

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Single arm treatment cohort and only one marker studied

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n Dx information; Single arm Tx cohort (KQ3)

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Treatment fixed and analytic validity data only for one newer marker

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Narrative review

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Only one biomarker present

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N<10, single arm treatment cohort and analytic validity data for older markers only

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Single arm Tx cohort (KQ3)

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n Dx information; Single arm Tx cohort (KQ3)

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Tretament is fixed and analytic validity data only (KQ2)

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Treatment is fixed and analytic validity data (KQ2)

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Treatment not based on biomarkers. Only older markers studied in terms of analytic validity data

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Treatment is not based on biomarker and no biomarker comparison present

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Only one biomarker of interest studied

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Not a biomarker of inoterest (KQ2) - only older biomarkers evaluated

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Analytic validity data for only older markers studied

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N<10 in one group and only older markers studied in terms of mean values in different groups

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No comparison of biomarkers and treatment not based on markers

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Article in Spanish

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Single arm treatment cohort and no comparison between older markers

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Narrative review

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Changes in mean values of older markers only studied over the course of therapy and treatment not based on markers
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Not biomarkers of interest - older biomarkers only
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Single arm treatment cohort and no comparison between markers
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Comparison of mean values of older markers only pre and post treatment
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Analytical validity data only (KQ2)
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Language other than english

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Not a biomarker of interest

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Not biomarkers of interest - older biomarkers only

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Single arm Tx cohort (KQ3); No Dx information

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Single arm treatment cohort. Change in mean values of only older markers studied

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No comparison between biomarkers. Just the data gathered at baseline

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Analytical validity data only (KQ2)

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Not test-directed Tx (KQ3)

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Cost effectiveness analysis done

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Treatment not based on biomarker

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Only one biomarker studied and treatment is not based on biomarker

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Single arm treatment cohort and only one biomarker studied

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No comparison between biomarkers and treatment is not based on any biomarker

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No Dx information, no test-directed Tx

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Not test-directed Tx (KQ3)

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Narrative review

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Single arm Tx cohorts (KQ3)

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Not CKD patients

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Analytical validity data only (KQ2)

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Case study(n<10)

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Single arm treatment cohort and no comparison between biomarkers
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Single arm Tx cohort (KQ3); No Dx information
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Change in mean values of older markers studied and treatment is not based on biomarker
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Mean values of only older biomarkers studied in different groups
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Non CKD-patients

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Non CKD-patients

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Not a biomarker of interest; only older markers used

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No Dx information; Not test-directed Tx

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Single arm Tx cohort

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N<10; Single arm Tx cohort (KQ3); No Dx information

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Treatment fixed. Only older markers studied with no comparison with newer ones.
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Changes in mean values of older markers studied pre and post treatment
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Single arm Tx cohort, no Dx information (French)

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Not test-directed Tx

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Single arm Tx cohort (KQ3)

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Not biomarkers of interest - older markers

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Treatment not based on biomarker

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Analytic validity data (KQ2)

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Comparison of Different cutoffs

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Not test-directed Tx (KQ3)

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Not biomarkers of interest - older markers only (KQ2)

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Single arm Tx cohort (KQ3); No Dx information

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Not biomarkers of interest - older biomarkers only

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Non-CKD patients

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Experimental lab study

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Analytical validity data only (KQ2); Logistic regression only looks at r-HuEPO independence, not iron deficiency

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Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only (KQ2)

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Not test-directed Tx

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Single arm treatment cohort. Change in mean values of only older markers studied pre and post treatment

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Single arm Tx cohort (KQ3); No Dx information; CKD patients - N<10

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Single arm Tx cohort (KQ3); No Dx information

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N<10; Single arm Tx cohort (KQ3); Not biomarkers of interest - older biomarkers only & Analytical validity data only (KQ2)

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Comparison of Different cutoffs

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Not test-directed Tx

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Treatment fixed and not based on biomarkers. No biomarker comparison

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Only newer biomarker discussed. In vitro study?

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Not test-directed Tx (KQ3)

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Analytic validity data only for older markers

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Changes in mean values of older markers studied pre and post treatment

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Treatment not based on biomarker

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Change in mean values of only older markers after the treatment is studied

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Sensitivity and specificity comparison between older markers only

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Changes in mean values of older markers studied pre and post treatment

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Single arm Tx cohort (KQ3); No Dx information

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Non CKD patients and analytic validity data only between older markers

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Not biomarkers of interest - older markers;

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Treatment not based on biomarker.

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Treatment not based on biomarker and population group not of interest

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Non CKD patients and single arm treatment cohort

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Analytic validity data only (KQ2)

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Sensitivity and specificty comparison between older markers only

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Only one biomarker studied

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Not test-directed Tx (KQ3)

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Analytic validity data only for older markers

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Only one biomarker studied

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Analytic validity data only (for KQ2)

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Single arm Tx cohort (KQ3); No Dx information

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case series; N<10

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Not test-directed Tx (KQ3); Not biomarkers of interest - older markers only

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Narrative review

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Only newer markers compared

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Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only

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Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only

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Analytical validity data only (KQ2); Single arm Tx cohort (KQ3)

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Not biomarkers of interest - ferrokinetic parameters (KQ2)

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Comparison of mean values of older markers in different groups

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Comparison of Different cutoffs

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Treatment fixed and not dependent on markers

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Single arm Tx cohort (KQ3); Analytical validity data only (KQ2)

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Treatment is fixed and n<10 in one treatment arm. Analytic vaidity data for older markers only

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Only one biomarker studied

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Single arm Tx cohort (KQ3)

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Only one biomarker studied and no EPO or iron treatment given

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Treatment fixed and analytic validity data for older markers only

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Single arm Tx cohort (KQ3)

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Analytic validity data only (KQ2)

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non CKD-patienots

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Analytic validity data for older markers only

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Comparison of mean values of older markers in different groups

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Comparison of Different cutoffs

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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narrative review

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Analytic validity data only

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Only older markers studied in terms of analytic validity

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Single arm Tx cohort (KQ3)

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Single arm treatment cohort and analytic validity data only

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No CKD population

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No comparison between markers and treatment is not dependent on markers. Newer marker ferrum

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No marker used and treatment is not dependent on marker

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No comparison between older and newer markers but only older markers mean levels studied with respect to two different treatments

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Not test-directed Tx

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Sensitivity, specificity and predictive values comparison only between older markers

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Analytic validity data only (KQ2)

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not test-directed Tx

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Only analytic validity data. ISAT newer marker used

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No PDF

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Single arm treatment cohort and analytic validity data for older markers only

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Analytic validity data for older markers only

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Treatment fixed and not based on biomarker

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Analytical validity data only (KQ2)

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Analytic validity data only (for KQ2)

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Analytic validity data for older markers only

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Treatment no dependent on markers and analytic validity data for older markers

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New marker erythrocyte ferritin and analytic validity data for markers

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Treatment not dependent on markers and analytic validity data for older markers only

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not test-directed Tx

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Treatment no dependent on markers and only one marker studied

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Single arm Tx cohort (KQ3)

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Single arm treatment cohort and only older markers studied

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Analytical validity data only (KQ2)

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Not biomarkers of interest (KQ2)

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non-CKD population

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Sensitivity comparison only between two newer biomarkers, one of which is not listed in the protocol

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Analytic validity data for older markers only

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Anlytic validity data only (for KQ2)

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Single arm cohort study and analytic validity data only

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Single arm Tx cohort (KQ3); No Dx information

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Single arm Tx cohorts (KQ3)

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Non-CKD population

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Not test-directed Tx (KQ3); no Dx information

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Single arm Tx cohort (KQ3); No Dx information

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Not test-directed Tx

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No biomarker comparison and treatment fixed

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Single arm Tx cohort (KQ3)

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Not test-directed Tx

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Analytic validity data only (KQ2)

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non-CKD population; Not biomarkers of interest (KQ2);

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While ROC information is available the it looks like most of the population is non-CKD

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Single arm Tx cohort (KQ3); Not

biomarkers of interest - older markers only (KQ2)

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N<10; Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Single arm tx cohort, outcome is mortality - if outcome were a diagnostic one, e.g. response to Tx then we would include for diagnostic odds ratios)

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The CDT biomarker discussed is not of interest

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No comparison between biomarkers influencing treatments

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Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Analytic validity data only (for KQ2)

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Single arm cohort and no comparison between newer and older markers

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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Single arm Tx cohort (KQ3); No Dx information

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Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

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not test-directed Tx

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Analytic validity data only (for KQ2)

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Analytic validity data (KQ2)

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Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

628) Zelichowski G,Lubas A,Wankowicz Z. [Effectiveness of subcutaneous and intravenous epoetin alpha in haemodialysed patients]. [Polish]. Pol Merkuriusz Lek. 17(98):143-7, 2004 Aug. PMID: 15603323
Single arm Tx cohort (KQ3); Not biomarkers of interest - older markers only & Analytical validity data only (KQ2)

629) Zhang Y,Thamer M,Cotter D,Kaufman J,Hernan MA. Estimated effect of epoetin dosage on survival among elderly hemodialysis patients in the United States. Clin J Am Soc Nephrol. 4(3):638-44, 2009 Mar. PMID: 19261818
Single arm Tx cohort - registry data (KQ3); No Dx information

630) Zheng S,Coyne DW,Joist H,Schuessler R,Godboldo-Brooks A,Ercole P,Brennan DC. Iron deficiency anemia and iron losses after renal transplantation. Transpl Int. 22(4):434-40, 2009 Apr. PMID: 19076330
Single arm study

631) Zupan IP,Varl J,Kovac D,Cernelc P,Mlakar U,Andoljsek D,Pretnar J,Zver S,Modic M. Indices of iron status in patients treated by chronic haemodialysis. Pflugers Arch. 442(6 Suppl 1):R202-3, 2001. PMID: 11678340
Analytical validity data only (KQ2)

Appendix C: Blank Extraction Form

A. Source and Extractor

Author, Year			**Site, Country**		
Extractor			**Funding source**		
RefID		**PMID**		Is there >1 form for this RefID (Y/N)?	
Key Question					

KQ1: Management of Iron deficiency anemia with biomarkers
KQ2: Diagnostic accuracy; 2a:Reference standards; 2b:Diagnostic utility as an add-on test; 2c:Adverse events of testing;
KQ3:Effect on Treatment - Intermediate outcomes & Adverse events;
KQ4: Influencing factors

B. Study description

Study design [1]	**Recruitment method** [2]	**Sampling population (can be more than one category)** [3]		**Age Group** [4]
Inclusion criteria	**Exclusion criteria**	**Multi-center (Y/N)?** **If yes, how many centers (if reported)?**		**Setting** [6]
		Enrollment period: **Dates:** **Follow up duration:**[5]		
Comments (e.g./o selection or other bias):				

[1] Cross Sectional; Case Control; Retrospective Cohort; Prospective Cohort; Non-Randomized comparative study; RCT; Other (mention)
[2] Consecutive patients; Random sampling; Convenience sample; Selected sample; Other (mention)
[3] CKD patients with Stage (specify) whether on HD or PD (specify); ESRD patients; Kidney transplant patients; Other (mention)
HD=Hemodialysis, PD=Peritoneal dialysis, ESRD=End Stage Renal Disease (not related to CKD staging)
[4] Pediatric (incl. Adolescent); Adolescent (11-18 yrs); Adult (>18 yrs); Adult & Adolescent; Adult & Pediatric (incl Adoloscent); Adult
[5] Mean only; If estimated, please add “estimated” in brackets. If median, SE, range, IQR, or other, specify these.
[6] hospitals; Dialysis centers;Outpatient (health care setting);Emergency Dept;Community (non-health care setting);Other (mention)

C. Participant characteristics *

N enrolled	N analyzed	Cause of CKD (N, %)	Male, (n/N, %)	Age, y **	Race (n/N, %)	Blood Urea Nitrogen (mg/dL) **	Creatinine (mg/dL) **	Creatinine clearance (mL/min) **
eGFR (mL/min) **	Method to calculate eGFR***	Hb (g/dL)	Ht %	S Ferritin ng/ml	TSAT %	ESA dose IU/wk		
HD (n/N, %)	PD (n/N, %)	Hypertension %	CVD %	Diabetes, %	Is this a Special Population? **** (which?)			
Comments								

*Add headings for additional subgroups if presented.
** Mean only; If estimated, please add "estimated" in brackets. If median, SE, range, IQR, or other, specify these.
*** MDRD, Cockcroft-Gault, CKD-EPI, Mayo, Schwartz (children), Cystatin C
****Only if it is NOT a typical CKD, HD or PD or kidney transplant patient; if any of these four leave it blank. If not any of these 4, add description

D. Diagnostic performances [assumes gold standard]

Analysis #	Index Test [1]	Outcome of interest [2]	Markers used for Diagnosis (List Reference test (/s)) [3]	Definition of Response to Tx – variable [4]	Dx trial treatment (iron/EPO, dose, duration etc.)	# Participants recruited who did not receive the index test	# Participants recruited who did not receive the reference test

[1] **Index Test:** CHr (Hb content of reticulocytes); %HYPO (% of hypochromic RBC); ZPP (erythrocyte zinc protoporphyrin); Soluble transferrin receptor; Hepcidin; Superconducting quantum interference device (SQUID); Other (mention)
[2] Markers for Diagnosis; Response to Tx
[3] **Reference Test:** Serum Ferritin; TSAT; Iron binding capacity; Serum iron; Bone marrow iron; Other (mention). The markers used can be a single reference test; a combination of reference tests; a combination of reference and index tests; Other (mention)
[4] Hb g/dL, Ht %, Other (mention)

Analysis #	Index test Cut-off	Outcome of interest	Sn data		Sp data		Sens (95% CI)	Spec (95% CI)	ROC (AUC, ± SD/SE,	Other / Comments
			TP	FN	TN	FP				

			(I+R+)	(I-R+)	(I-R-)	(I+R-)			p value)	

E. Concordance [agreement between measurements – assume no gold standard]

Comparison		N Enrolled	N Analyzed	Concordance Metric*	Value (95% CI or LOA)**	Other Text Description (eg, of bias)
Index	Ref					

* Bland Altman plot; LOA, Limits of Agreement (±2SD); NOT correlation coefficients and OLS regression
** Delete or correct the incorrect value/item. If change, highlight yellow.

F. Predictive performances (KQ2)

Comparison / Risk factor (Enter unit in text field if applicable) [1]	Predictor Unit	Predictor Type (continuous/binary)	Predictor Cut-off (if binary)	Outcome of interest [2]	Markers used for Diagnosis (List Reference test (/s)) [3]	Definition of Response to Tx – variable [4]	Statistical test used/estimate (HR/RR/OR/AdjOR)	Value (HR/RR/OR/AdjOR)	(95% CI)	p-value
Multivariate (Y/N)										
If multivariate model, list covariates in model										

[1] **Tests:** CHr (Hb content of reticulocytes); %HYPO (% of hypochromic RBC); ZPP (erythrocyte zinc protoporphyrin); Soluble transferrin receptor; Hepcidin; Superconducting quantum interference device (SQUID); Serum Ferritin; TSAT; Iron binding capacity; Serum iron; Bone marrow iron; Other (mention)
[2] Markers for Diagnosis; Response to Tx
[3] The markers used can be a single reference test; a combination of reference tests; a combination of reference and index tests; Other (mention)
[4] Hb g/dL, Ht %, Other (mention)

G. Subgroup Analysis (KQ4)

Use the tables in section D, E and F (as appropriate) and add a column in the beginning titled "subgroup" to indicate the subgroups for which results are being reported.

H. Adverse Effects or Harms Associated with Testing (KQ2b)

I. Methodological Quality

Indicator	Comments
Prospective or Retrospective?	
Was patient selection consecutive? (Y/N/Not mentioned)	
Are subjects representative of the patients who will receive this test? (Y/N/Unclear) [a]	
Were inclusion and exclusion criteria defined? (Y/N/Unclear)	
Clear description of population studied (Y/N)	
Was recruitment period defined? (Y/N)	
Adequate description of tests? (Y/N)	
Is the reference standard likely to correctly classify the target condition? (Y/N/Unclear)[b]	
Is verification bias unlikely? (Y/N/Unclear) (if no/unclear, describe)[c]	
Index Test Readers BLINDED to Reference Test Results? (Y/N/No data)	
Was time interval between index and reference test reported? (Y/N/Unclear)	
Analytic problem? (Y/N) (if yes describe) [d]	
Were statistical tests to quantify uncertainty included eg 95%CI? (Y/N)	
Were uninterpretable/ intermediate test results reported? (Y/N/Unclear) [e]	
Were withdrawals from the study explained? (Y/N/Unclear)	
Data loss / not analyzed (%)	
Overall Quality (A/B/C) [f]	
Briefly mention why you decided on the grade	

(a) Reported estimates of diagnostic accuracy may have limited clinical applicability (generalisability) if the spectrum of tested patients is not similar to the patients in whom the test will be used in practice. The spectrum of patients refers severity of the underlying target condition, demographic features and differential diagnosis and/or co-morbidity. The judgement should be based on both the method of recruitment and the characteristics of those recruited; **(b)** If the reference standard used is likely to correctly classify the target condition OR is the best method available ; **(c)** Verification bias occurs when not all of the study group receive confirmation of the diagnosis by the reference standard; generally only occurs when patients are tested by the index test prior to the reference standard; **(d)** e.g., improper accounting for multiple measurements in same patient (should do clustered analysis); **(e)** Uninterpretable / indeterminate / intermediate results typically go unreported & are removed from the analysis, leading to biased assessments. Whether bias will arise depends on the possible correlation between uninterpretable test results and the true disease status; **(f)** Quality: Grade A (good) studies fulfill most commonly held concepts of high quality, including the following: blinding of assessors to results of the other test, blinding to clinical information, enrollment of consecutive patients, random order of measurements or simultaneous measurements with the compared methods, clear description of the evaluated population, setting, and measurement methods; appropriate measurement of outcomes; appropriate statistical and analytic methods and reporting; no reporting errors; not excessive data loss (<20%); and no obvious bias. Grade B (moderate) studies may be susceptible to some bias, but not sufficient to invalidate the results. Such studies do not meet the criteria described in category A. They have some deficiencies but none likely to cause major bias. Study may be missing information making assessment of the limitations and potential problems difficult. Grade C (poor) studies are subject to significant bias that may invalidate the results. Such studies may have serious errors in design, analysis or reporting. These studies may have large amounts of missing information or discrepancies in reporting.

Appendix D. Test Performance of Newer Markers for Assessing Iron Status as Defined by Classical Laboratory Markers

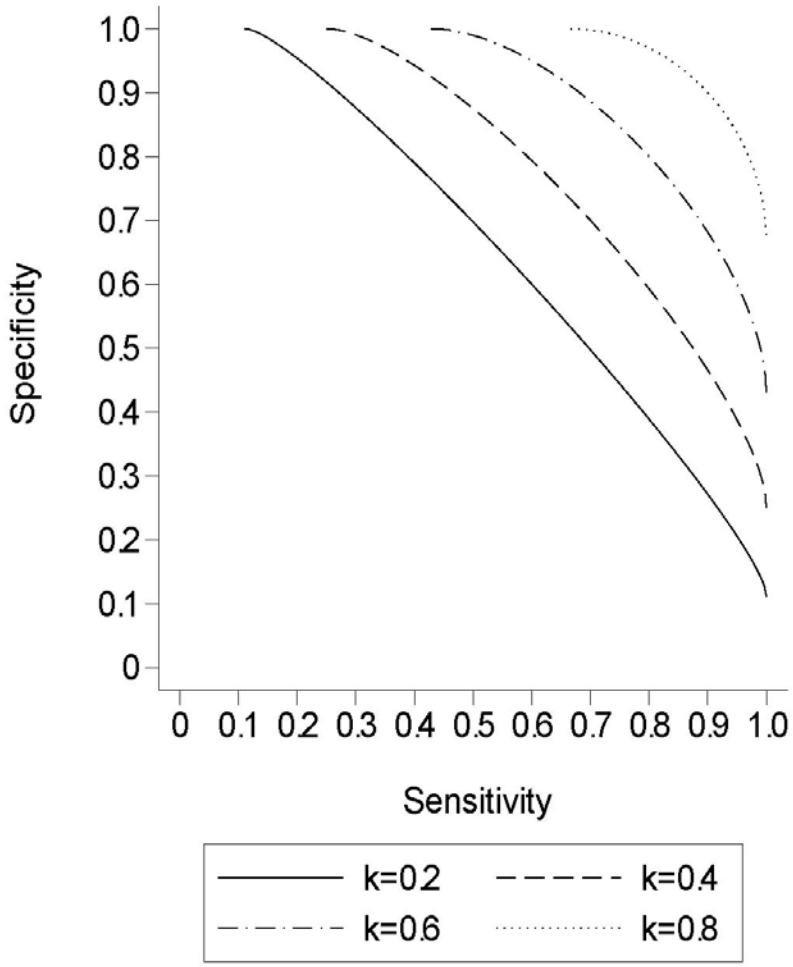
Methods for Statistical Analyses – Relationships between Analytic Sensitivity and Specificity, and Test Agreement)

There is a lack of a generally-accepted reference standard test for determining iron deficiency in the setting of CKD.[1] Thus, we can expect that both newer and the classical laboratory biomarkers of iron status can err, i.e., the results of either test could be different than the "true" iron status. In such a case, assessing concordance may be the only meaningful option if none of the compared tests is an obvious choice for a reference standard, e.g., when both tests are alternative methodologies to measure the same quantity.

When the original studies used classical markers of iron status to define iron deficiency and used it as the reference standard in calculating the test accuracy (sensitivity and specificity) of newer markers of iron status, we considered these data are analytic sensitivity and specificity (or technical test performance) of newer markers.[2] To facilitate the interpretation of analytic sensitivity and specificity, whenever possible, we calculated Cohen's kappa statistic. The Cohen's kappa statistic can be calculated based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity comparing a newer marker of iron status with a "reference standard," as defined by the classical markers of iron status in the original studies. We used the Landis and Koch[3] interpretation of values of kappa to determine the level of agreement: <0, poor agreement; 0 to 0.20, slight agreement; 0.21 to 0.4, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; 0.81 to 1.0, almost perfect agreement.

When Cohen's kappa statistic could not be calculated due to insufficient data in the original studies, we used a graph that visualized the relationships between sensitivity, specificity, and kappa to aid our interpretations of the test agreements, based on the reported sensitivities and specificities (**Figure D1**).[4] This graph was produced based on the formulas describing the analytic relationship between sensitivity, specificity, kappa, and raw agreement for the 2x2 contingency tables. Four curves representing the ceiling of the four kappa coefficients were plotted on a squared space of sensitivity and specificity values. The curves provide all of the sensitivities and specificities pairs below which kappa cannot be achieved. For example, for k = 0.8 (which is considered to indicate substantial agreement), the line, sensitivity equal to specificity, intersects the elliptical curve for k = 0.8 exactly at (0.9, 0.9). Thus, these sensitivities and specificities, both of which are below 0.9, can never produce a kappa of 0.8 or greater.

Figure D1. Relationships between statistical measures of test performance: analytic sensitivity and specificity, and kappa (k) statistic



Results –Concordance between newer markers and classical markers of iron status

In this section, we summarize the findings from 15 cross-sectional studies evaluating the test performance of newer markers for assessing iron status as defined by classical laboratory markers.[5-19] Thirteen studies enrolled adult hemodialysis (HD CKD) patients. One study enrolled adult peritoneal dialysis (PD CKD) and nondialysis (ND CKD) patients,[11] and another study enrolled pediatric HD and PD CKD patients.[10] Many of these studies evaluated more than one newer marker, including content of hemoglobin in reticulocytes (CHr), percent hypochromic red blood cells (%HYPO), reticulocyte hemoglobin content (RetHe), soluble transferrin receptor (sTfR), and erythrocyte zinc protoporphyrin (ZPP). Studies used a variety of definitions for iron deficiency using classical laboratory markers, such as transferrin saturation (TSAT) or ferritin.

As described in the Methods, "reference standard" tests for determining iron deficiency in the context of CKD are lacking, and both newer and classical laboratory markers are subject to measurement errors. Thus, whenever it is possible, we either calculated Cohen's kappa statistic based on the 2x2 contingency tables or estimated kappa based on a graph depicting the

relationships between sensitivity, specificity, and kappa to aid our interpretation of test agreements (**Figure D1**).[4]

Summary of key points

- Among adult HD CKD patients:
  - Content of hemoglobin in reticulocytes (CHr) and classical laboratory markers (TSAT or ferritin, alone or in combination) have poor to moderate test agreements.
  - Percent hypochromic red blood cells (%HYPO) and classical laboratory markers (TSAT or ferritin, alone or in combination) have poor to fair agreements.
  - Soluble transferring receptor (sTfR) and classical laboratory markers (TSAT or ferritin, alone or in combination) have poor to fair agreements.
  - Erythrocyte zinc protoporphyrin (ZPP) and classical laboratory marker (TSAT<20%) have poor to fair agreements for assessing iron status in HD CKD patients with normal iron store as indicated by serum ferritin concentrations (>100 ng/mL). Higher ZPP cutoffs were associated with better agreements with TSAT <20%.

Content of Hemoglobin in Reticulocytes

Key Points

Seven cross-sectional studies, enrolling a total of 911 adult HD CKD patients (individual counts ranged from 22 to 217 patients in each study),[6,7,9,15,16,18,19] and one cross-sectional study with 19 PD CKD patients,[11] evaluated the test performance of CHr for assessing iron status as defined by classical laboratory markers. Studies enrolled primarily older patients who received maintenance ESA treatment; however, maintenance ESA doses varied across studies. Baseline iron status (based on mean serum ferritin and TSTA concentrations) also varied across studies.

CHr and classical laboratory markers (TSAT or ferritin) have poor to moderate agreements for assessing iron status in HD CKD patients.

Detailed Synthesis (Table D1 to D3)

Seven cross-sectional studies, enrolling a total of 833 adult HD CKD patients (individual counts from 22 to 217 patients in each study),[6,7,9,15,16,18,19] and one cross-sectional study with 19 PD CKD patients,[11] evaluated the test performance of CHr for assessing iron status as defined by classical laboratory markers. Four studies were conducted in Japan, two in the U.K., one in Germany, and one in South Korea. Studies enrolled primarily older men and women (mean age ranged from 49 to 62 years old). Baseline mean Hb concentrations ranged from 9.4 to 10.3 g/dL (reported in 5 studies). Four of the eight studies reported a mean ferritin concentration less than 100 ng/mL (indicating an insufficient iron store status),[6,7,18,19] while the other four reported a mean ferritin concentration greater than 100 ng/mL at baseline (ranging from 198 to 427 ng/mL).[9,11,15,16] Baseline mean TSAT ranged from 20 to 32 percent. Most patients received maintenance ESA treatment (one study enrolled 29 patients who had not received ESA treatment), but the maintenance ESA doses varied across studies (**Table D1**).

The test performance of CHr was assessed using four different reference standards of iron status as defined by classical laboratory markers: 1) TSAT ≤15%;[9] 2) TSAT ≤20%;[6,7,15,19] 3) ferritin ≤100 ng/mL;[7] and 4) TSAT ≤20% or ferritin ≤100 ng/mL.[11,15,16,18] Seven studies used

variable CHr cutoffs to define iron deficiency (ranging from <26 to <35 pg),[6,7,9,15,16,18] of which only two both used cutoffs of CHr <31 and <32 pg.[16,18] One study analyzed the test performance of CHr as a continuous measure.[19]

The reported sensitivity and specificity pairs at different CHr cutoffs, as well as the estimated agreements between CHr and classical markers of iron status, are summarized in **Tables D2 and D3**. Seven studies (6 studies in HD CKD patients and 1 study in PD CKD patients) showed poor to moderate test agreements between CHr and classical markers of iron status.[6,7,9,11,15,16,18] Most studies were small in sample size (N<100) and thus cannot provide precise test agreement estimates. Moreover, the heterogeneity in the test comparisons, iron status of the study populations, and background treatment may explain the inconsistencies in the test agreements across studies.

Another study of 149 HD CKD patients performed multivariate analyses, and showed that Chr was the most significant predictor among the markers examined (including hematocrit, %HYPO, log ferritin, sTfR, and epoetin user status). The multivariate analyses also showed that each 1 pg decrease in CHr and one standard deviation decrease (equivalent to 2.25 pg) in CHr were associated with a 1.88 (95 percent CI, 1.39 to 2.53) and 4.11 (95 percent CI, 2.10 to 8.05) fold increase, respectively, in the odds of iron deficiency (defined as TSAT < 20 percent).[19]

Table D1. Characteristics of cross-sectional studies evaluating the test performance of reticulocyte hemoglobin content (CHr) for assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Adult HD CKD						
Bhandari, 1997[6] [9398126] UK	HD CKD	22 / 22	Male (%): 73 Age (yr): 62 Race (%): NR	NR	Hb (g/dL): NR Hct (%): NR ferritin (ng/mL): 33.4 µg/L [range, 4 to 56 µg/L] TSAT (%): 25.1	ESA dose: 6055 IU/wk Iron washout: No washout
Bhandari, 1998[7] [9589378] UK	HD CKD	72 / 72	Male (%): 72 Age (yr): 62 Race (%): NR	NR	Hb (g/dL): NR Hct (%): NR ferritin (ng/mL): NR (≤100 in 64%) TSAT (%): NR (≤20 in 51%)	ESA dose: 2000 IU/wk Iron washout: No washout
Cullen, 1999[9] [10193816] Germany	HD CKD	36 / 25	Male (%): 47 Age (yr): 64 Race (%): 100% Caucasian	NR	Hb (g/dL): 9.4 Hct (%): NR ferritin (ng/mL): 427.3 TSAT (%): 19.5	ESA dose: NR (All patients received ESA treatment intermittently before the start of the study) Iron washout: No washout
Kaneko, 2003[15] [12631092] Japan	HD CKD	197 / 197	Male (%): 61 Age (yr): 59 Race (%): NR	NR	Hb (g/dL): NR Hct (%): 31.3 ferritin (ng/mL): 247.4	ESA dose: 4116 IU/wk Iron washout:

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
					TSAT (%): 25.8	No washout
Kim, 2008[16] [18190467] South Korea	HD CKD	140 / 140	Male (%): 49 Age (yr): 56 Race (%):NR	NR	Hb (g/dL): 10 Hct (%): 32.6 ferritin (ng/mL): 224.5 TSAT (%): 27.6	ESA dose: NR (all patients received rHuEpo alpha intravenously) Iron washout: 3 wks
Miwa, 2010[18] [19624802] Japan	HD CKD	217 / 752 samples (multiple samples from single subject)	Male (%): 59 Age (yr): 58 Race (%):NR	NR	Hb (g/dL): 10.3 Hct (%): 33 ferritin (ng/mL): 74.4 TSAT (%): 20.8	ESA dose: 5069 IU/wk Iron washout: No washout and no change allowed in dose
Tsuchiya, 2003[19] [12608554] Japan	HD CKD	149 / 149	Male (%): 38 Age (yr): 55 Race (%): NR	NR	Hb (g/dL): 10.2 Hct (%): 32.4 ferritin (ng/mL): 98.1 TSAT (%): 23	ESA dose: 93.1 IU/kg/wk (n=120, patients received EPO treatment) Iron washout: No washout and no change allowed in dose
Adult PD CKD						
Eguchi, 2010[11] [20415234] Japan	PD CKD	19 / 85 samples (multiple samples from single subject)	Male (%): 26 Age (yr): 48.6 Race (%):NR	BUN (mg/dL): 56 Creatinine (mg/dL): 11.6	Hb (g/dL): 9.8 Hct (%): 29.9 ferritin (ng/mL): 197.8 TSAT (%): 32.6	ESA dose: 17,833 IU/ month (Epoetin-beta); 88.2 µg/ month (darbepoetin-alpha) Iron washout: NR

BUN=blood urea nitrogen; CHr=content of hemoglobin in reticulocytes; CKD=chronic kidney disease; Dx=diagnosis; ESA=erythropoiesis stimulating agents; ESRD=end stage renal disease; Hb=hemoglobin; HD=hemodialysis; Hct=hematocrit; IV=intravenous; IU=international units; NR=not reported; PD=peritoneal dialysis; TSAT=transferrin saturation; wk=week; yr=year

Table D2. Summary of results for the test agreements between reticulocyte hemoglobin content (CHr) and classical markers of iron status

			TSAT ≤15%	TSAT ≤20%	Ferritin ≤100 ng/mL	TSAT ≤20% or Ferritin ≤100 ng/mL
Study	**$N_{analyzed}$**	**CHr Cutoff (pg)**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**
Adult HD CKD						

			TSAT ≤15%	TSAT ≤20%	Ferritin ≤100 ng/mL	TSAT ≤20% or Ferritin ≤100 ng/mL
Study	**$N_{analyzed}$**	**CHr Cutoff (pg)**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**
Cullen, 1999[9]	25	<26	0.74 (0.29, 0.74)			
Bhandari, 1997[6]	22	<26.5		0.47 (-0.03, 0.67)[c]		
Kaneko, 2003[15]	127	<29.0		<0.20[a]		
Miwa, 2010[18]	752 samples	≤30				0.20-0.40[a]
Kim, 2008[16]	140	<31				0.76 (0.64, 0.76)
Miwa, 2010[18]	752 samples					0.40[a]
Kaneko, 2003[15]	127	<31.5		0.20[a]		
Kaneko, 2003[15]	127	<32.0		0.20-0.40[a]		
Miwa, 2010[18]	752 samples					0.40[a]
Miwa, 2010[18]	752 samples	<32.2				0.20-0.40[a]
Kim, 2008[16]	140	<32.4				0.77 (0.64, 0.82)
Kaneko, 2003[15]	127	<32.5		0.20-0.40[a]		
Kaneko, 2003[15]	127	<33.0		0.20-0.40[a]		0.62 (0.51, 0.62)
Miwa, 2010[18]	752 samples	<33				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<34				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<35				0.20-0.40[a]
Tsuchiya, 2003[19]	149	Per 1 pg decrease		Adj. OR=1.88 (CI 1.39, 2.53)		
Tsuchiya, 2003[19]	149	Per 1 SD (2.25 pg) decrease		Adj. OR=4.11 (CI 2.10, 8.05)		
Bhandari, 1998[7]	72	"Reduced CHr (normal range: 25.9-30.6)"		0.21 (-0.01, 0.35)	0.06 (-0.14, 0.20)	
Adult PD CKD						
Eguchi, 2010[11]	85 samples	<33.8				0.20-0.40[a]

Adj OR=adjusted odds ratio; CHr=content of hemoglobin in reticulocytes; CI=95% confidence interval; CKD=chronic kidney disease; HD=hemodialysis; PD=peritoneal dialysis; SD=standard deviation

[a] Kappa was estimated based on the location of the reported sensitivity and specificity pair in Figure 3 (Chpater 2) depicting the relationship between statistical measures of test performance.
[b] Calculated Cohen kappa statistics based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity.
[c] Estimated values: CHr was used as the reference standard and TSAT as the index test in the original study that a reported sensitivity of 85% and a specificity of 76%.

Table D3. Study results for reticulocyte hemoglobin content (CHr) – assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Lab Analysis or Assay	Index Test Cut-Off (pg)	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sensitivity, %	Specificity, %	Other Results	Risk of Bias[a]
Adult HD CKD									
Kaneko, 2003[15] [12631092] Japan	Flow Cytometry (ADVIA)	<29.0	TSAT ≤20%	34[d]	197[d]	9.9	87.5	NR	Medium
		<31.5	TSAT ≤20%			40.8	74.4		
		<32.0	TSAT ≤20%			52.1	75.6		
		<32.5	TSAT ≤20%			59.2	62.7		
		<33.0	TSAT ≤20%			69	57		
Kim, 2008[16] [18190467] South Korea	ADVIA 120 haematologic analyser (Bayer Diagnostics, Tarrytown, NY, USA)	<31	TSAT <20% or ferritin <100 µg/L	38	140	72	100	NR	Medium
		<32.4	TSAT <20% or ferritin <100 µg/L			96	84		
		<33	TSAT <20% or ferritin <100 µg/L			100	68		
Tsuchiya, 2003[19] [12608554] Japan	Advia 120 Autoanalyser, Bayer Diagnostics	Per 1 pg decrease	TSAT <20%	NR	149	NR	NR	OR: 2.26 (1.72,2.98), P<0.001 AdjOR[b]: 1.88 (1.39,2.53), P<0.001	Medium
		Per 1 SD (2.25 pg) decrease	TSAT <20%		149			OR: 6.27 (3.37,11.68) AdjOR[b]: 4.11 (2.10,8.05)	
Bhandari, 1997[6] [9398126] UK	Bayer automated Tehnicon H3 analyser	<26.5	TSAT < 20%	36	22	47[c]	95[c]	NR	High
Bhandari, 1998[7] [9589378] UK	Bayer automated Tehnicon H3 analyser	Reduced CHr (normal range-25.9-30.6)	TSAT ≤20%	64	72	33	89	From ROC analysis, CHr "failed to show as a strong performance"	High

Study, Year [Pubmed ID] Country	Lab Analysis or Assay	Index Test Cut-Off (pg)	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sensitivity, %	Specificity, %	Other Results	Risk of Bias[a]
		Reduced CHr (normal range-25.9-30.6)	ferritin ≤100 µg/L			74	27	From ROC analysis, CHr "failed to show as a strong performance"	
Cullen, 1999[9] [10193816] Germany	Technicon H*3 hematology analyzer, Bayer Diagnostic	<26	TSAT ≤15%	46	24	73	100	NR	High
Miwa, 2010[18] [19624802] Japan	Advia 120, Siemens	<30	TSAT ≤20% or ferritin ≤100 ng/mL	45	752 samples (from 153 patients)	42.8	91.8	NR	High
		<31	TSAT ≤20% or ferritin ≤100 ng/mL			51.9	85.7		
		<32	TSAT ≤20% or ferritin ≤100 ng/mL			60.8	76.8		
		<32.2	TSAT ≤20% or ferritin ≤100 ng/mL			74.3	68.3		
		<33	TSAT ≤20% or ferritin ≤100 ng/mL			74.3	64.9		
		<34	TSAT ≤20% or ferritin ≤100 ng/mL			83.5	53		
		<35	TSAT ≤20% or ferritin ≤100 ng/mL			89.7	37.5		
Adult PD CKD									
Eguchi, 2010[11] [20415234] Japan	NR	<33.8	TSAT ≤20% or ferritin ≤100 ng/mL	NR	NR	60	64	NR	High

Adj OR=adjusted odds ratio; CHr=content of hemoglobin in reticulocytes; CI=confidence interval; Dx=diagnosis; HD=hemodialysis; NR=not reported; OR=odds ratio; SD=Standard deviation; Sens=sensitivity; Spec=specificity; PD=peritoneal dialysis; TSAT=transferrin saturation

[a] Based on QUADAS.
[b] Adjusted for age, gender, duration of hemodialysis, hematocrit, CHr, %HYPO, $\log_{10}$ ferritin, sTfR, and epoetin user status.
[c] Estimated values: CHr was used as the reference standard and TSAT as the index test in the original study that reported sensitivity of 85% and a specificity of 76%.
[d] Total number of patients analyzed and prevalence of iron deficiency were based on the data reported in Table 1 of the original article. However, the estimated specificity based on the reported 2x2 table does not match the reported specificity in the text; therefore, we cannot use the 2x2 table to estimate kappa.

Percent Hypochromic Red Blood Cells

Key Points

Four cross-sectional studies, with a total of 495 adult HD CKD patients (per study enrollment ranged from 72 to 202 patients) evaluated the test performance of CHr for assessing iron status as defined by classical laboratory markers.[7,9,17,19] Studies enrolled primarily older patients who received maintenance ESA treatment, although the maintenance ESA doses varied from study to study. Baseline iron status, based on mean serum ferritin and TSAT concentrations, also varied across studies.

Overall, %HYPO and classical laboratory markers (TSAT or ferritin) have poor to fair agreements for assessing iron status in HD CKD patients.

Detailed Synthesis (Table D4 to D6)

Four cross-sectional studies, with a total of 495 adult HD CKD patitents, evaluated the test performance of %HYPO for assessing iron status as defined by classical laboratory markers.[7,9,17,19] Studies were conducted in several different countries: the U.K., Germany, Poland, and Japan. All studies enrolled primarily older men and women (mean age ranged from 55 to 64 years old). Baseline mean Hb concentrations ranged from 8.8 to 10.2 g/dl (reported in 3 studies). Of the four included studies, two reported a mean ferritin concentration less than 100 ng/mL (indicating an insufficient iron store status),[7,19] and the other two reported a mean ferritin concentration of 274 and 427 ng/mL at baseline.[9,17] Baseline mean TSAT ranged from 20 to 38 percent across studies. Most patients received maintenance ESA treatment (a total of 74 patients did not received ESA treatment), although the maintenance ESA doses varied across studies (**Table D4**).

The test performance of %HYPO was assessed using three different reference standards of iron status (as defined by classical laboratory markers): 1) TSAT ≤ 15%;[9] 2) TSAT ≤ 20%;[7,17,19] and 3) ferritin ≤100 ng/mL.[7] Three studies used variable %HYPO cutoffs to define iron deficiency ranging from >1.5% to >10%,[9,17] with two studies using the same cutoffs of >5% and >10%. One study analyzed the test performance of %HYPO as a continuous measure.[7]

The reported sensitivity and specificity pairs at different %HYPO cutoffs, as well as the estimated agreements between %HYPO and classical markers of iron status, are summarized in **Tables D5 and D6**. Three studies showed poor to fair test agreements between %HYPO and classical markers of iron status in HD CKD patients,[7,9,17] one of which also reported that %HYPO "failed to show as a strong performance as measure of iron deficiency" based on ROC analyses.[7] The heterogeneity in the test comparisons, iron status of the study populations, and background treatment may explain the inconsistencies in the test agreements across studies.

Another study, enrolling149 HD CKD patients, showed that, when compared to a %HYPO ≤1.5 percent, a %HYPO ≥4.1% and a %HYPO between 1.6 and 4.0% were associated with a respective 8.53 (95 percent CI, 3.42 to 21.26) and 2.20 (95 percent CI, 0.88 to 5.48) fold increase in the odds of iron deficiency (defined as a TSAT < 20 percent).[19] However, %HYPO was not a significant predictor of iron deficiency in a multivariate model including other markers (including hematocrit, CHr, log ferritin, sTfR, and epoetin user status).

Table D4. Characteristics of cross-sectional studies evaluating the test performance of percent hypochromic red blood cell (%HYPO) for assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Adult HD CKD						
Bhandari, 1998[7] [9589378] UK	HD CKD	72 / 72	Male (%): 72 Age (yr): 62 Race (%): NR	NR	Hb (g/dL): NR Hct (%): NR ferritin (ng/mL): NR (≤100 in 64%) TSAT (%): NR (≤20 in 51%)	ESA dose: 2000 Iron washout: NR
Cullen, 1999[9] [10193816] Germany	HD CKD	36 / 25	Male (%): 47 Age (yr): 64 Race (%): 100% Caucasian	NR	Hb (g/dL): 9.4 Hct (%): NR ferritin (ng/mL): 427.3 TSAT (%): 19.5	ESA dose: NR (All patients received ESA treatment intermittently before the start of the study) Iron washout: No washout
Matuszkiewicz-Rowinska 2003[17] [14682204] Poland	HD CKD	186 / 186	Male (%): 55 Age (yr): 18 to 75 Race (%): NR	NR	Hb (g/dL): 8.8 Hct (%): 26.7 ferritin (ng/mL): 274 TSAT (%): 38	ESA dose: 71 IU/kg/wk (141 patients) Iron washout: No washout
Tsuchiya, 2003[19] [12608554] Japan	HD CKD	202 / 149	Male (%): 38 Age (yr): 55 Race (%): NR	NR	Hb (g/dL): 10.2 Hct (%): 32.4 ferritin (ng/mL): 98 TSAT (%): 23	ESA dose: 93.1 IU/kg/wk (n=120, patients received EPO treatment) Iron washout: no washout

%HYPO=percent of hypochronic red blood cell; CKD=chronic kidney disease; CI=confidence interval; Dx=diagnosis; ESA=erythropoiesis stimulating agents; ESRD=end stage renal disease; Hb=hemoglobin; HD=hemodialysis; Hct=hematocrit; IV=intravenous; IU=international units; NR=not reported; TSAT=transferrin saturation; wk=week; yr=year

Table D5. Summary of results for the test agreements between percent hypochromic red blood cell (%HYPO) and classical markers of iron status

			TSAT ≤15%	TSAT ≤20%	Ferritin ≤100 ng/mL
Study	**$N_{analyzed}$**	**%HYPO cutoff**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**
Adult HD CKD					
Cullen, 1999[9]	25	>2.5	0.36 (-0.08, 0.51)		
Cullen, 1999[9]	25	>5	0.51 (0.05, 0.67)		
Matuszkiewicz-Rowinska 2003[17]	186			0.07 (-0.01, 0.11)	
Cullen, 1999[9]	25	>10	0.41 (-0.07, 0.75)		
Matuszkiewicz-Rowinska 2003[17]	186			0.13 (0.01, 0.22)	
Tsuchiya, 2003[19]	48 vs. 54	1.6-4.0 vs. ≤1.5		OR=2.20 (CI 0.88, 5.48)[a]	
Tsuchiya, 2003[19]	47 vs. 54	≥4.0 vs. ≤1.5		OR=8.53 (CI 3.42, 21.3)[a]	
Bhandari, 1998[7]	72	"Each % increase"		-0.04 (-0.24, 0.17)	0.11 (-0.28, 0.07)

%HYPO=percent of hypochronic red blood cell; CI=95% confidence interval; CKD=chronic kidney disease; HD=hemodialysis; OR=odds ratio

[a] %HYPO was not a significant predictor in the multivariate model.
[b] Calculated Cohen kappa statistics based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity.

Table D6. Study results for percent hypochromic red blood cell (%HYPO) – iron status as defined by classical laboratory markers

Study, Year [Pubmed ID]	Lab Analysis or Assay	Index Test Cut-Off, %	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sens, %	Spec, %	Other Results	Risk of Bias[a]
Adult HD CKD									
Matuszkiewicz-Rowinska 2003[17] [14682204] Poland	Blue dye conventional smears and calculated on the basis of light microscope	>5	TSAT <20%	13	186	84	34	NR	Medium
		>10	TSAT <20%	13	186	68	58		
Tsuchiya, 2003[19] [12608554] Japan	ADVIA 120 autoanalyzer (Bayer Diagnostic)	≤1.5	TSAT <20%	38	149	NR	NR	OR: 1.00 [cutoff ≤1.5%]	Medium
		1.6-4.0						OR: 2.20 [cutoff 1.6%-4.0%], P=0.090	
		≥4.						OR: 8.53 [cutoff ≥4.1%], P<0.001	
Bhandari, 1998[7] [9589378] UK	Tehnicon H3 analyser	"Each % increase"	TSAT ≤20%	51	72	76	21	From ROC analysis, %HYPO "failed to show as a strong performance as measure of iron deficiency"	High
		"Each % increase"	ferritin ≤100 µg/L	58	72	18	70	From ROC analysis, %HYPO "failed to show as a strong performance as measure of iron deficiency"	
Cullen, 1999[9] [10193816] Germany	Technicon H*3 hematology analyzer, Bayer Diagnostic	>2.5	TSAT <15%	44	25	91	54	NR	High
		>5	TSAT <15%	44	25	91	62		
		>10	TSAT <15%	44	25	64	77		

%HYPO=percent of hypochronic red blood cell; HD=hemodialysis;NR= not reported; OR=odds ratio Sens=sensitivity; Spec=specificity; TSAT=transferrin saturation

[a] Based on QUADAS.

Reticulocyte Hemoglobin Equivalent

Key Points

Three cross-sectional studies, enrolling more than 270 adult HD CKD patients (one study included 1500 samples from an unclear number of patients),[8,14,18] and one cross-sectional study with 19 PD CKD patients and an unclear number of ND CKD patients,[11] evaluated the test performance of RetHe for assessing iron status as defined by classical laboratory markers. Studies enrolled primarily older patients who received maintenance ESA treatment, although the maintenance ESA doses varied across studies. Baseline iron status, based on mean serum ferritin and TSTA concentrations, also varied across studies.

One study examined the test performance of RetHe using two different reference standards, and showed that the test performance of RetHe was less favorable for assessing functional iron deficiency than for assessing traditional parameters for iron deficiency in HD CKD patients.[8]

Detailed Synthesis (Table D7 to D9)

Three studies, enrolling more than 270 adult HD CKD patients (one study included 1500 samples from an unclear number of patients),[8,14,18] and one cross-sectional study with 19 PD CKD patients and an unclear number of ND CKD patients[11] evaluated the test performance of RetHe for assessing iron status as defined by classical laboratory markers. Two studies were conducted in Japan, one in the U.S., and one in Bosnia and Herzegovia. Studies enrolled primarily older men and women (mean age ranged from 49 to 65 years old). One study did not report any information on patients' background treatment or anemia or iron status.[8] In the other three studies, baseline mean Hb concentrations ranged from 9.8 to 11.3 g/dL. One study reported a mean ferritin concentration of less than 100 ng/mL (indicating an insufficient iron store status),[18] while the other two reported a mean ferritin concentration greater than 100 ng/mL at baseline (139 and 559 ng/mL).[11,14] Baseline mean TSAT ranged from 20 to 39 percent across studies. All patients received maintenance ESA treatment, although the maintenance ESA doses varied across studies (**Table D7**).

The test performance of RetHe was assessed using four different reference standards of iron status (as defined by classical laboratory markers): 1) TSAT ≤20% and ferritin ≤100 ng/mL;[11,14] 2) TSAT ≤20% or ferritin ≤100 ng/mL;[18] 3) serum iron < 40 µg/dL, TSAT<20%, ferritin <100 ng/mL, and Hb <11 g/dL;[8] and 4) TSAT<20%, ferritin 100-800 ng/mL, and Hb <11 g/dL.[8] No two studies used the same reference standard in the same patient population (i.e., HD CKD, PD CKD, or ND CKD patients). The RetHe cutoffs that were used to define iron deficiency ranged from <27.2 to < 35 pg, and no studies used the same cutoffs of RetHe. Thus, the consistencies of study findings cannot be assessed.

The reported sensitivity and specificity pairs at different RetHe cutoffs, as well as the estimated agreements between RetHe and classical markers of iron status are summarized in **Tables D8 and D9**. The three studies in HD CKD patients showed poor to moderate test agreements between RetHe and classical markers of iron status.[8,14,18] One study examined the test performance of RetHe using two different reference standards: "traditional parameters for iron deficiency" (serum iron < 40 µg/dL, TSAT<20%, ferritin <100 ng/mL, and Hb <11 g/dL) and "functional iron deficiency" (TSAT<20%, ferritin 100-800 ng/mL, and Hb <11 g/dL). This study showed that the test performance of RetHe was less favorable for assessing functional iron deficiency than for assessing traditional parameters for iron deficiency in HD CKD patients

(areas under the curve were 0.657 vs. 0.913, respectively).[8] However, this study had an unclear descriptions of the study population and incorrect statistical analyses due to nonadjustment for within-patient correlation.

One cross-sectional study evaluated the test performance of RetHe for assessing iron status in 19 PD CKD and 84 ND CKD patients, separately.[11] The test agreement between RetHe and classical markers of iron status was poor. However, had a potential for selection bias, an unclear description of the study population (including how 85 samples were drawn from 19 patients), and a bias in results due to nonadjustment for within-patient correlation.

Table D7. Characteristics of cross-sectional studies evaluating the test performance of reticulocyte hemoglobin equivalent (RetHe) for assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Adult HD CKD						
Brugnara, 2006[8] [16999719] USA	HD CKD	NR / 1500 samples	Male (%): 56 Age (yr): 63.5 Race (%):NR	NR	Hb (g/dL): NR Hct (%): NR ferritin (ng/mL): NR TSAT (%):NR	ESA dose: NR Iron washout: NR
Hukic, 2010[14] [21246919] Bosnia and Herzegovina	HD CKD and continuing hospital peritoneal dialysis	53 / 53	Male (%): NR Age (yr): 53 Race (%): NR	NR	Hb (g/dL): 11.0 Hct (%): 0.36 ferritin (ng/mL): 559.22 TSAT (%):38.35	ESA dose: NR (all patients were on ESA therapy) Iron washout: No. Additional iron therapy was given to all patients to maintain ferritin between 300 and 500 ng/mL
Miwa, 2010[18] [19624802] Japan	HD CKD	217 / 752 samples (multiple samples from single subject)	Male (%): 59 Age (yr): 58.4 Race (%): NR	NR	Hb (g/dL): 10.3 Hct (%): 33 ferritin (ng/mL): 74.4 TSAT (%): 20.8	ESA dose: 5069 IU/week Iron washout: No washout
Adult PD CKD and ND CKD						
Eguchi, 2010[11] [20415234] Japan	PD CKD	19 / 85 samples (multiple samples from single subject)	Male (%): 26 Age (yr): 48.6 Race (%): NR	BUN (mg/dL): 56 Creatinine (mg/dL): 11.6 eGFR (mL/min): NR	Hb (g/dL): 9.8 Hct (%): 29.9 ferritin (ng/mL): 197.8 TSAT (%): 32.6	ESA dose: 17,833 IU/ month (Epoetin-beta); 88.2 µg/ month (darbepoetin-alpha) Iron washout: NR

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
	ND CKD	84/ NR	Male (%): 44 Age (yr): 65 Race (%):NR	BUN (mg/dL): NR Creatinine (mg/dL): 3.0 eGFR (mL/min): 24	Hb (g/dL): 11.3 Hct (%): 34.6 ferritin (ng/mL): 138.6 TSAT (%): 28.1	ESA dose: 6393 IU/ month Iron washout: NR

CKD=chronic kidney disease; Dx=diagnosis; ESA=erythropoiesis stimulating agents; Hb=hemoglobin; HD=hemodialysis; Hct=hematocrit; IU=international units; NR=not reported; PD=peritoneal dialysis; TSAT=transferrin saturation; yr=year

Table D8. Summary of results for the test agreements between reticulocyte hemoglobin equivalent (RetHe) and classical markers

			Serum Iron <40 µg/dL, TSAT <20%, Ferritin <100 mg/mL, and Hb < 11 g/dL	TSAT <20%, Ferritin 100-800 mg/mL, and Hb < 11 g/dL	TSAT ≤20% and Ferritin ≤100 ng/mL	TSAT ≤20% or Ferritin ≤100 ng/mL
Study	**$N_{analyzed}$**	**RetHe Cutoff (pg)**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**	***K* (CI)[b]**
Adult HD CKD						
Brugnara, 2006[8]	1500	<27.2	0.60-0.80[a]			
Brugnara, 2006[8]	1500	<27.9		0.20[a]		
Miwa, 2010[18]	752 samples	<30				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<31				0.20-0.40[a]
Hukic, 2010[14]	53	<31.1			0.47 (0.20, 0.54)	
Miwa, 2010[18]	752 samples	<32				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<33				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<34				0.20-0.40[a]
Miwa, 2010[18]	752 samples	<35				0.20[a]
Adult PD CKD						
Eguchi, 2010[11]	85 samples	<32.7				0.20-0.40[a]
Adult ND CKD						
Eguchi, 2010[11]	NR	<31				0.20[a]

CI=95% confidence interval; CKD=chronic kidney disease; HD=hemodialysis; ND=nondialysis; PD=peritoneal dialysis; RetHe=reticulocyte hemoglobin equivalent; SD=standard deviation

[a] Kappa was estimated based on the location of the reported sensitivity and specificity pair in Figure 3 (Chpater 2) depicting the relationship between statistical measures of test performance.
[b] Calculated Cohen kappa statistics based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity.

Table D9. Study results for reticulocyte hemoglobin equivalent (RetHe) – iron status as defined by classical laboratory markers

Study, Year [Pubmed ID]	Lab Analysis or Assay	Index Test Cut-Off (pg)	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sens, %	Spec, %	Other Results	Risk of Bias[a]
Adult HD CKD									
Hukic, 2010[14] [21246919]	Sysmex XE 2100	<31.1	TSAT <20% and ferritin <100 µg/l	49	53	50	96	AUC (CI): 0.73 (0.59, 0.84)	Medium
Miwa, 2010[18] [19624802]	XE-2100 with upgraded software (XE RET MASTER, Sysmex)	<30	TSAT ≤ 20% or ferritin ≤ 100 ng/mL	45	752 samples (from 153 patients)	42.8	91.8	NR	High
		<31	TSAT ≤ 20% or ferritin ≤ 100 ng/mL			51.9	85.7		
		<32	TSAT ≤ 20% or ferritin ≤ 100 ng/mL			60.8	76.8		
		<33	TSAT ≤ 20% or ferritin ≤ 100 ng/mL			74.3	64.9		
		<34	TSAT ≤ 20% or ferritin ≤ 100 ng/mL			83.5	53		
		<35	TSAT ≤ 20% or ferritin ≤ 100 ng/mL			89.7	37.5		
Brugnara, 2006[8] [16999719]	XE 2100	<27.2	serum iron < 40 µg/dL, TSAT<20%, ferritin <100 ng/mL and Hb <11 g/dL (“traditional iron deficiency”)	NR	1500	93.3	83.2	AUC (CI): 0.913 (NR)	High
		<27.9	TSAT<20%, ferritin 100-800 ng/mL and Hb <11 g/dL (“functional iron deficiency anemia”)			40.2	80.3	AUC (CI): 0.657 (NR)	
Adult PD CKD									
Eguchi, 2010[11] [20415234]	Sysmex XE 2100 automated blood cell counter	<32.7	TSAT <20% and ferritin <100ng/mL	15.5	85	80	56	NR	High
Adult ND CKD									

Study, Year [Pubmed ID]	Lab Analysis or Assay	Index Test Cut-Off (pg)	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sens, %	Spec, %	Other Results	Risk of Bias[a]
Eguchi, 2010[11] [20415234]	Sysmex XE 2100 automated blood cell counter	<31	TSAT <20% and ferritin <100ng/mL	15.5	NR	54	70	NR	High

AUC=area under the curve; CI=95% confidence interval; Dx=diagnosis; HD=hemodialysis; NR=not reported; RetHe=reticulocyte hemoglobin equivalent; Sens=sensitivity; Spec=specificity; TSAT=transferrin saturation

[a] Based on QUADAS.

Soluble Transferrin Receptor

Key Points

Four cross-sectional studies, enrolling a total of 325 adult HD CKD patients,[5,12,13,19] and 1 cross-sectional study with 27 pediatric HD and PD CKD patients[10] evaluated the test performance of sTfR for assessing iron status as defined by classical laboratory markers. Of these studies, two were rated as being at a medium risk of bias, and three at a high risk of bias. Four studies enrolled primarily older HD CKD patients (mean age ranged from 43 to 62 years old), and one study enrolled 11 pediatric HD CKD patients (mean age 16 years old) and 16 pediatric PD CKD patients (mean age 13 years old). Baseline iron status, based on mean serum ferritin and TSTA concentrations, varied across studies.

Overall, sTfR and classical laboratory markers (TSAT or ferritin) have poor to fair agreements for assessing iron status in adult HD CKD patients.

Detailed Synthesis (Table D10 to D12)

Four cross-sectional studies, enrolling a total of 325 adult HD CKD patients,[5,12,13,19] and 1 cross-sectional study with 27 pediatric HD and PD CKD patients[10] evaluated the test performance of sTfR for assessing iron status as defined by classical laboratory markers. Studies were conducted in different countries: Belgium, Italy, India, Japan, and Germany. Four studies enrolled primarily older HD CKD patients (mean age ranged from 43 to 62 years old),[5,12,13,19] and one study enrolled 11 pediatric HD CKD patients (mean age 16 years old) and 16 pediatric PD CKD patients (mean age 13 years old).[10] One study did not report any information on background treatment or patients' anemia or iron status.[13] In the other four studies, baseline mean Hb concentrations ranged from 10.2 to 11.5 g/dL. One of the four studies reported a mean ferritin concentration less than 100 ng/mL (indicating an insufficient iron store status),[19] while the other three reported a mean ferritin concentration greater than 100 ng/mL at baseline (ranging from 124 to 353 ng/mL).[5,10,12,13] Patients in two studies received maintenance ESA treatment,[12,19] while the other three[5,10,13] did not report information on background ESA treatment (**Table D10**).

The test performance of sTfR was assessed using three different reference standards of iron status (as defined by classical laboratory markers): 1) TSAT <20% and ferritin <100 ng/mL;[12,13,19] 2) TSAT <20%;[5,12] and 3) TSAT <16% and ferritin <12 ng/mL.[13] Studies used variable CHr cutoffs to define iron deficiency: >1.5 mg/L, > 3.05 nmol/L, >8.5 mg/L, and >28 nmol/L (pediatric CKD). One study analyzed the test performance of sTfR as a continuous measure.[19]

The reported sensitivity and specificity pairs at different sTfR cutoffs, as well as the estimated agreements between sTfR and classical markers of iron status, are summarized in **Tables D11 and D12**. Among adult HD CKD patients, three studies showed poor to fair test agreements between sTfR and classical markers of iron status.[5,12,13] Another study performed multivariate analyses and showed that sTfR remained a significant predictor among other markers (including hematocrit, CHr, %HYPO, log ferritin, and epoetin user status). The multivariate analyses also showed that each 0.1 mg/mL increase in sTfR and one standard deviation increase (equivalent to 4.28 mg/L) in sTfR were associated with a respective 1.88 (95 percent CI, 1.29 to 2.53) and 1.69 (95 percent CI, 1.03 to 2.76) fold increase in the odds of iron deficiency (defined as TSAT < 20 percent).[19] However, CHr was the strongest predictor in this multivariate model.

Among pediatric CKD patients, one cross-sectional study with a total of 16 pediatric PD CKD patients and 11 pediatric HD CKD patients evaluated the test performance of sTfR for assessing iron status.[10] The test agreement between sTfR (a cutoff of > 28 nM/L to define iron deficiency) and classical laboratory markers of iron deficiency (TSAT < 20% and ferritin <100 ng/mL) was poor and of large uncertainty (wide confidence interval) due to small sample size.

Table D10. Characteristics of studies evaluating the test performance of soluble transferring receptor (sTfR) for assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Sampling Population	$N_{enrolled}$ / $N_{analyzed}$	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Adult HD CKD						
Baldus, 1998[5] [9543601] Belgium	HD CKD	95 / 87	Male (%): NR Age (yr): 61 Race (%): NR	NR	Hb (g/dL): 10.2 Hct (%): NR ferritin (ng/mL): 353.3 TSAT (%): 24	ESA dose: NR Iron washout: 3 months
Fusaro, 2005[12] [15772926] Italy	HD CKD	39 / 39	Male (%): 54 Age (yr): 62 Race (%): NR	NR	Hb (g/dL): 11.1 Hct (%): 34.4 ferritin (ng/mL): 204 TSAT (%): 30	ESA dose: 122 IU/kg/wk (among 29 patients treated with EPO) Iron washout: 2-7 days
Gupta, 2003[13] [15025343] India	HD CKD	40 / 40	Male (%): 53 Age (yr): 43 Race (%): NR	NR	Hb (g/dL): NR Hct (%): NR ferritin (ng/mL): NR TSAT (%): NR	ESA dose: NR Iron washout: NR
Tsuchiya, 2003[19] [12608554] Japan	HD CKD	149 / 149	Male (%): 38 Age (yr): 55 Race (%): NR	NR	Hb (g/dL): 10.2 Hct (%): 32.4 ferritin (ng/mL): 98.1 TSAT (%): 23	ESA dose: 93.1 IU/kg/wk (n=120, patients received EPO treatment) Iron washout: No washout and no change allowed in dose
Pediatric CKD						
Daschner, 1999[10] [10543327] Germany	HD CKD	11/ 11	Male (%): 64 Age (yr): 16.1 Race (%): NR	NR	Hb (g/dL): 11.3 Hct (%): NR ferritin (ng/mL): 280 TSAT (%): 37.0	ESA dose: 166.4 IU/kg Iron washout: no washout (HD CKD patients received intravenous iron; PD CKD patients received oral iron)
	PD CKD	16 / 16	Male (%): 75 Age (yr): 12.7 Race (%): NR	NR	Hb (g/dL): 11.5 Hct (%):NR ferritin (ng/mL): 124.0 TSAT (%): 28.4	

CKD=chronic kidney disease; CI=95% confidence interval; Dx=diagnosis; ESA=erythropoiesis stimulating agents; Hb=hemoglobin; HD=hemodialysis; Hct=hematocrit; IU=international units; NR=not reported; sTfR=soluble transferrin receptor; TSAT=transferrin saturation; wk=week; yr=year

Table D11. Summary of results for the test agreements between soluble transferring receptor (sTfR) and classical markers

Study	$N_{analyzed}$	sTfR Cutoff	TSAT<16% and Ferritin <12 ng/mL *K* (CI)[b]	TSAT <20% *K* (CI)[b]	TSAT ≤20% and Ferritin ≤100 ng/mL *K* (CI)[b]
Adult HD CKD					
Fusaro, 2005[12]	39	>1.5 mg/L		0.35 (0.06, 0.35)	0.06 (-0.28, 0.36)
Gupta, 2003[13]	40	>8.5 mg/L	0.50 (0.16, 0.66)		
Baldus, 1998[5]	87	>3.05 nmol/L		0.16 (-0.03, 0.31)	
Tsuchiya, 2003[19]	149	Each 0.1 mg/L increase			Adj OR: 1.88 (1.29, 2.53)[a]
Tsuchiya, 2003[19]	149	Each SD (4.28 mg/L) increase			Adj OR: 1.69 (1.03, 2.76)[a]
Pediatric HD and PD CKD					
Daschner, 1999[10]	27	>28 nmol/L			0.10 (-0.30, 0.46)

Adj OR=adjusted odds ratio; CI=95% confidence interval; CKD=chronic kidney disease; HD=hemodialysis; PD=peritoneal dialysis; SD=standard deviation; sTfR=soluable transferring receptor

[a] Multivariate model included the following covariates: age, gender, duration of hemodialysis, hematocrit, CHr, %HYPO, log10 ferritin, sTfR, and erythropoietin user status.
[b] Calculated Cohen kappa statistics based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity.

Table D12. Study results for soluble transferring receptor (sTfR) – iron status as defined by classical laboratory markers

Study, Year [Pubmed ID]	Lab Analysis or Assay	Index Test Cut-Off	Reference Standard (Iron Deficiency)	Iron Deficiency, %	Total $N_{analyzed}$	Sensitivity, %	Specificity, %	Other Results	Risk of Bias[a]
Adult HD CKD									
Fusaro, 2005 [15772926]	Enzyme-linked immunosorbent assay (ELISA)- R&D systems (Minneapolis, MN, USA)	>1.5 mg/L	TSAT <20% and ferritin <100 ng/mL	41	39	63	43	NR	Medium
		>1.5 mg/L	TSAT <20%	23	39	100	53		
Tsuchiya, 2003 [12608554]	ELISA kit (Eiken Kagaku, Tokyo)	Per 10 g/L increase	TSAT <20% and ferritin <100 ng/mL	NR	149	NR	NR	OR: 2.26 (1.72, 2.98) P<0.001 AdjOR[b]: 1.88 (1.29, 2.53) P<0.001	Medium
		Per 1 SD (424.8 g/L) increase	TSAT <20% and ferritin <100 ng/mL	NR	149	NR	NR	OR: 2.33 (1.55, 3.50) AdjOR[b]: 1.69 (1.03, 2.76)	
Baldus, 1998 [9543601]	ELISA - Amgen Diagnostics.	>3.05 µmol/L	TSAT <20%	45	87	26	90	NR	High
Gupta, 2003 [15025343]	ELISA kit from Bio Plus	>8.5 µg/mL	ferritin <12 ng/mL and TSAT<16%	35	40	86	69	NR	High
Pediatric HD CKD and PD CKD									
Daschner, 1999 [10543327]	Enzyme-linked immunosorbent assay -- R&D systems (Wiesbaden, Germany)	> 28 nmol/L	ferritin < 100 ng/mL and TSAT < 20%	48	27	38	71	NR	High

AUC=area under the curve; AdjOR=adjusted odds ratio; CI=confidence interval; HD=hemodialysis; NKF-DOQI=National Kidney Foundation's kidney disease outcomes quality initiative; NR=not reported; OR=odds ratio; Sens=sensitivity; sTfR=soluble transferrin receptor; Spec=specificity; TSAT=transferrin saturation

[a] Based on QUADAS.
[b] Adjusted for age, gender, duration of hemodialysis, hematocrit, CHr, %HYPO, $\log_{10}$ ferritin, sTfR, and epoetin user status.

Erythrocyte Zinc Protoporphyrin

Key Points

Two cross-sectional studies, enrolling a total of 281 adult HD CKD patients[5,17] evaluated the test performance of CHr for assessing iron status as defined by classical laboratory markers. Of these studies, one was rated as being at a medium risk of bias, and the other at a high risk of bias. Studies enrolled primarily older patients with sufficient iron store (based on mean serum ferritin concentration).

Overall, ZPP and classical laboratory markers (TSAT<20%) have poor to fair agreements for assessing iron status in HD CKD patients with normal iron store as indicated by mean serum ferritin concentrations >100 ng/mL.

Detailed Synthesis (Table D13 to D15)

Two cross-sectional studies, enrolling a total of 281 adult HD CKD patients, evaluated the test performance of CHR for assessing iron status as defined by classical laboratory markers. [5,17] These studies used a TSAT less than 20 percent to define ID. One study was conducted in Poland,[17] and the other conducted in Belgium.[5]

Both studies enrolled primarily older men and women (mean age 61 years old; age range 18 to 75 years old). Baseline mean Hb concentrations were 10.2 and 8.8 g/d. Both studies reported a mean ferritin concentration greater than 100 ng/mL at baseline (353 and 274 ng/mL, respectively). Reported baseline mean TSATs were 24 to 38 percent, respectively. Both studies reported different background treatments (**Table D13**).

Both studies assessed the test performance of ZPP using TSAT <20% as the reference standard of iron status. The reported sensitivity and specificity pairs at different ZPP cutoffs, as well as the estimated agreements between CHr and classical markers of iron status are summarized in **Tables D14 and D15**. The studies showed poor to fair test agreements between ZPP and TSAT, and there were consistent threshold effects across studies. Higher ZPP cutoffs were associated with better agreements with TSAT <20%. One study also assessed the test performance of ZPP to log ferritin index ratio for assessing iron deficiency as defined by TSAT <20%. The reported sensitivity and specificity pairs for ZPP to log ferritin index ≥40 were 76% and 83%, respectively, and the sensitivity and specificity pairs for ZPP to log ferritin index ≥45 were 72% and 86%, respectively.[17]

Table D13. Characteristics of cross-sectional studies evaluating the test performance of erythrocyte zinc protoporphyrin (ZPP) for assessing iron status as defined by classical laboratory markers

Study, Year [Pubmed ID] Country	Sampling Population	Nenrolled / Nanalyzed	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Adult HD CKD						
Baldus, 1998[5] [9543601] Belgium	HD CKD	95 / 87	Male (%): NR Age (yr): 61 Race (%):NR	NR	Hb (g/dL): 10.2 Hct (%):NR ferritin (ng/mL): 353.3 TSAT (%): 24	ESA dose: NR Iron washout: 3 months

Study, Year [Pubmed ID] Country	Sampling Population	Nenrolled / Nanalyzed	Demographics	Kidney Function Indices	Anemia and Iron Status Indices	Background Treatment
Matuszkiewicz-Rowinska 2003[17] [14682204] Poland	HD CKD	186 / 186	Male (%): 55 Age (yr): 18 to 75 Race (%): NR	NR	Hb (g/dL): 8.8 Hct (%): 26.7 ferritin (ng/mL): 274 TSAT (%): 38.3	ESA dose: 71 IU/kg/wk (141 patients) Iron washout: No washout

CKD=chronic kidney disease; Dx=diagnosis; ESA=erythropoiesis stimulating agents; Hb=hemoglobin; HD=hemodialysis; Hct=hematocrit; IV=intravenous; IU=international units; NR=not reported; TSAT=transferrin saturation; wk=week; yr=year

Table D14. Summary of results for the test agreements between erythrocyte zinc protoporphyrin (ZPP) and classical markers

Study	$N_{analyzed}$	ZPP Cutoff (µmol/mol)	TSAT <20% *K* (CI)[a]
Adult HD CKD			
Baldus, 1998[5]	87	>40	0.14 (-0.08, 0.36)
Matuszkiewicz-Rowinska 2003[17]	186	>65	0.25 (0.11, 0.35)
Matuszkiewicz-Rowinska 2003[17]	186	>80	0.34 (0.16, 0.5)
Matuszkiewicz-Rowinska 2003[17]	186	>90	0.40 (0.21, 0.57)

CI=95% confidence interval; CKD=chronic kidney disease; HD=hemodialysis

[a] Calculated Cohen kappa statistics based on the 2x2 contingency tables that were used to estimate the sensitivity and specificity.

Table D15. Summary results for erythrocyte zinc protoporphyrin (ZPP) – iron status as defined by classical laboratory markers

Study, Year [Pubmed ID]	Lab Analysis or Assay	Index Test Cut-Off (µmol/Mol Heme)	Reference Standard (Iron Deficiency)	Iron Deficiency, %	$N_{analyzed}$	Sens	Spec	Other Results	Risk of Bias[a]
Adult HD CKD									
Matuszkiewicz-Rowinska 2003[17] [14682204]	Hematofluorometer 206 D, AVIV Biomedicals	>65	TSAT < 20%	13	186	72	70	NR	Medium
		>80	TSAT < 20%			56	85		
		>90	TSAT < 20%			56	89		
		ZPP/log ferritin index ≥40	TSAT < 20%			76	83		
		ZPP/log ferritin index ≥45	TSAT < 20%			72	86		
Baldus, 1998[5] [9543601]	Hematofluorometer 206 D, AVIV Biomedicals	>40	TSAT < 20%	44	87	41	73	NR	High

AUC=area under the curve; CI=95 % confidence interval; Dx=diagnosis; NR= not reported; SE=standard error; Sens=sensitivity; Spec=specificity; TSAT=transferrin saturation

[a] Based on QUADAS.

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Appendix E: Quality Criteria and Individual Study Grades

Table D-1. Quality criteria and individual study grades for Key Question 2

Author Year PMID	Prosp (y/n/NA)	Consecutive (y/n/nd)	Eligibility defined (y/n/nd)	Pop defined (y/n/nd)	Recruit defined (y/n/nd)	Verification bias unlikely (y/n/nd)	Test Readers blinded (y/n/nd)	Time interval (y/n/nd)	No analytic problem (y/n)	Uncertainty (y/n)	Withdrawals explained (y/n/nd/na)	%Data loss	Overall Risk of Bias[a]
Baldus, 1998 [9543601]	N (cross sectional)	ND	N	N	N	Y	ND	N	Y	Y	NA	0%	HIGH
Bhandari, 1997 [9398126]	N (cross sectional)	ND	Y	N	N	Y	ND	N	Y	N	NA	0%	HIGH
Bhandari, 1998 [9589378]	N (cross sectional)	ND	Y	N	N	Y	ND	N	Y	N	NA	0%	HIGH
Bovy, 2007 [17237481]	Y	N	Y	Y	Y	Y	ND	Y	Y	Y	NA	0%	MEDIUM
Brugnara, 2006 [16999719]	N (Retrospective)	N	N	N	N	Y	ND	N	Y	N	NA	0%	HIGH
Buttarello, 2010 [20472854]	y	ND	Y	N	N	Y	ND	Y	Y	Y	Y	14%	MEDIUM
Chuang, 2003 [12543894]	Y	ND	Y	Y	N	Y	ND	N	Unclear	Y	NA	0%	HIGH
Cullen, 1999 [10193816]	N (cross sectional)	Y	Y	Y	Y	N	ND	N	N	N	N	30%	HIGH
Daschner, 1999 [10543327]	N (cross sectional)	ND	N	Y	N	Y	ND	N	Y	N	NA	0%	HIGH
Domrongkitchaiporn, 1999 [10401012]	Y	N	Y	Y	N	Y	ND	Y	Y	N	Y	8.7%	MEDIUM
Eguchi, 2010 [20415234]	Y	ND	Y	N	N	Unclear (n analyzed is not specified)	ND	ND	N (multiple sampling from same patient)	N	N	ND	HIGH
Fishbane, 1995 [7872320]	Y	N	Y	N	N	Y	ND	Y	N (incorporation bias)	N	Y	19%	HIGH

Author Year PMID	Prosp (y/n/NA)	Consecutive (y/n/nd)	Eligibility defined (y/n/nd)	Pop defined (y/n/nd)	Recruit defined (y/n/nd)	Verification bias unlikely (y/n/nd)	Test Readers blinded (y/n/nd)	Time interval (y/n/nd)	No analytic problem (y/n)	Uncertainty (y/n)	Withdrawals explained (y/n/nd/na)	%Data loss	Overall Risk of Bias[a]
Fishbane, 1997 [9211366]	Y	N	Y	N	N	Y	ND	ND	Y	N	Y	36%	HIGH
Fusaro, 2005 [15772926]	N (cross sectional)	Y	Y	Y	N	Y	ND	N	Y	Y	NA	0%	MEDIUM
Gupta, 2003 [15025343]	ND	ND	Y	N	N	Y	ND	N	Y	N	NA	0%	HIGH
Hukic, 2010 [21246919]	Y	N	Y	N	N	Y	ND	N	Y	Y	N	0%	MEDIUM
Kaneko, 2003 [12631092]	Y	NA	Y	Y	Y	Y	N	Y	Y	N	Y	8%	MEDIUM
Kim, 2008 [18190467]	N (cross sectional)	ND	Y	Y	N	Y	ND	N	Y	N	NA	0%	MEDIUM
Matuszkiewicz-Rowinska, 2003 [14682204]	N (cross sectional)	Y	Y	Y	N	Y	ND	Y	Y	Y	NA	0%	MEDIUM
Mitsuiki, 2003 [14586744]	N (Retrospective)	ND	Y	Y	N	Y	ND	Y	Y	Y	NA	0%	MEDIUM
Mittman, 1997 [9398141]	Y	ND	Y	Y	N	Y	ND	Y	Y	N	N	0%	MEDIUM
Miwa, 2010 [19624802]	N (cross sectional)	N	Y	Y	N	Y	ND	N	N (multiple sampling from same patient)	N	NA	29%	HIGH
Silva, 1998 [9794562]	Y	N	Y	Y	N	Y	ND	Y	N (incorporation bias)	N	Y	40%	HIGH
Tessitore, 2001 [11427634]	ND	N	Y	N	N	Y	ND	N	Y	Y	NA	0%	HIGH
Tessitore, 2010 [20538788]	Y	ND	Y	Y	Y	Y	ND	Y	Y	Y	Y	0%	LOW

Author Year PMID	Prosp (y/n/NA)	Consecutive (y/n/nd)	Eligibility defined (y/n/nd)	Pop defined (y/n/nd)	Recruit defined (y/n/nd)	Verification bias unlikely (y/n/nd)	Test Readers blinded (y/n/nd)	Time interval (y/n/nd)	No analytic problem (y/n)	Uncertainty (y/n)	Withdrawals explained (y/n/nd/na)	%Data loss	Overall Risk of Bias[a]
Tsuchiya, 2003 [12608554]	N (cross sectional)	ND	Y	Y	N	Y	ND	N	Y	Y	NA	0%	MEDIUM
Van Wyck, 2005 [16316362]	Y	Y	Y	Y	N	Y	ND	Y	N	N	Y	14%	MEDIUM

N = No; NA = not applicable; ND = not described; Y= Yes
[a] Criteria derived from QUADAS [36]

Table D-2. Quality criteria and individual study grades for Key Question 3

Author Year PMID	Sequence generation [Y, N, Unclear]	Allocation concealment [Y, N, Unclear]	Blinding of participants, personnel and outcome assessors [Y, N, Unclear]	Incomplete outcome data [Y, N, Unclear]	Free of selective outcome reporting [Y, N, Unclear]	Overall Quality[a]
Fishbane, 2001 [11737617]	Unclear	Unclear	Y	N	N	MEDIUM
Kaneko, 2003 [12631092]	Unclear	Unclear	N	N	N	MEDIUM

KQ = Key Question; N = No; Y= Yes
[a] Criteria derived from Cochrane risk of bias tool for intervention studies[37]

Table D-3. Quality criteria and individual study grades for Key Question 4

Author Year PMID	Prosp (y/n/NA)	Consecutive (y/n/nd)	Eligibility defined (y/n/nd)	Pop defined (y/n/nd)	Recruit defined (y/n/nd)	Verification bias unlikely (y/n/nd)	Test Readers blinded (y/n/nd)	Time interval (y/n/nd)	No analytic problem (y/n)	Uncertainty (y/n)	Withdrawals explained (y/n/nd/na)	%Data loss	Overall Risk of Bias
Ahluwalia, 1997 [9328369]	Y	ND	Y	Y	N	Y	ND	Y	Y	N	Y	10.8 %	MEDIUM

Author Year PMID	Prosp (y/n/NA)	Consecutive (y/n/nd)	Eligibility defined (y/n/nd)	Pop defined (y/n/nd)	Recruit defined (y/n/nd)	Verification bias unlikely (y/n/nd)	Test Readers blinded (y/n/nd)	Time interval (y/n/nd)	No analytic problem (y/n)	Uncertainty (y/n)	Withdrawals explained (y/n/nd/na)	%Data loss	Overall Risk of Bias
Singh, 2007 [17396118]	Y	Y	Y	Y	Y	Y	ND	N	Y	N	N	28%	MEDIUM

N = No; NA = not applicable; ND = not described; Y= Yes
[a] Criteria derived from QUADAS [36]