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An added value for the hemoglobin content in reticulocytes (CHr) and the mean corpuscular volume (MCV) in the diagnosis of iron deficiency in postpartum anemic women

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SUMMARY

Introduction: To evaluate the use of reticulocyte hemoglobin content (CHr) and mean corpuscular volume (MCV) to identify truly iron-deficient women with postpartum anemia (PPA), in order to reduce unnecessary iron supplementation.

Methods: Three hundred women with more than 500 mL of blood loss or clinical signs of anemia were divided in a control (Hb $\geq$ 10.5 g/dL, $N = 150$) and postpartum anemia group (PPA, Hb $<$ 10.5 g/dL; $N = 150$). PPA women were given ferrous fumarate for a period of 4 weeks. Efficacy of the treatment was evaluated by comparing Hb, CHr, and MCV at baseline (T_0) and after 4 weeks (T_4). Using standard iron deficiency cut off values for MCV (80 fL) and CHr (28 pg) at T_0, we divided the PPA group of both parameters into two subgroups, one suggestive for iron deficiency and one suggestive for noniron deficiency.

Results: Irrespective of the parameter or the subdivision, delta Hb concentrations (T_4–T_0) showed a similar increase in all PPA subgroups investigated. Both parameters in the PPA subgroups below their respective cut off value showed a significant improvement toward normalization, while the MCV and CHr in the PPA subgroups above their respective cut off value did not show any significant increase.

Conclusion: Our data suggest that the etiology of the anemia in postpartum anemic women is not always iron deficiency. Using a combination of Hb, MCV and CHr, we increased the stringency to identify truly iron-deficient postpartum anemic women, thereby reducing unnecessary iron supplementation in those women with sufficient iron stores.

INTRODUCTION

Postpartum anemia (PPA) occurs frequently and is an important risk factor for maternal morbidity [1–3]. The prevalence of PPA varies considerably in the different studies, ranging from 22% to 27% [4, 5], with the highest prevalence in women with a low socioeconomic status [5]. Anemia in nonpregnant women is defined by the World Health Organization as a hemoglobin concentration (Hb) of <12 g/dL (7.5 mM × 1.611) [6]. Although there is no clear cut off value for PPA in literature, the Dutch Society of Obstetrics and Gynecology-guideline for anemia uses a cut off value of 10.5 g/dL [7]. The difference in cut off value between nonpregnant and pregnant women is explained by the attempt to correct for the physiological hemodilution as present in pregnancy [8] and, more importantly, because of blood loss during labor. Because of this, added to the increased demand for iron during pregnancy, it is assumed that the most probable cause of anemia in the postpartum period is because of iron deficiency. While in our hospital, severe PPA (Hb < 6.5 g/dL) is treated with transfusion of packed red blood cells, treatment of mild PPA ($6.5 \leq$ Hb < 10.5 g/dL) consists of oral iron supplementation. Ferrous fumarate is the most commonly used iron compound in Dutch obstetric practice. While severe side effects of iron supplementation (i.e., iron overload) are restricted to rare entities such as thalassemia's or hemochromatosis (either congenital or acquired), most side effects are mild but nevertheless inconvenient, resulting in gastro-intestinal complaints such as nausea, diarrhea, or constipation [9].

Most anemia protocols make use of multiple laboratory parameters to unravel the etiology of the anemia at hand. However, in dealing with mild PPA, it is assumed that the most probable cause of the anemia is iron deficiency and consequently is treated as such. This assumption disregards the fact that some PPA women with sufficient iron stores have no beneficial effect of supplementation.

The reticulocyte hemoglobin content (CHr) is a relatively new measure for iron deficiency. Using the Prussian blue staining of a bone marrow aspirate to define iron deficiency, CHr outperformed the traditional markers like ferritin, transferrin saturation, and mean corpuscular volume (MCV) for the diagnosis of iron deficiency [10]. CHr is a parameter that can be measured on an Advia platform (Siemens Healhcare Diagnostics, Breda, Netherlands). However, comparable measurements of the reticulocyte hemoglobin content are available on other platforms [11, 12].

In this study, we investigate the use of the CHr together with the MCV to establish whether or not iron deficiency is the true cause of the PPA, thereby preventing unnecessary supplementation and as a consequence averting potential side effects of iron supplementation in noniron-deficient women. Ferritin is also a well-established parameter for diagnosing iron deficiency anemia. However, in concurrence with the literature, we decided against using ferritin as a marker for iron deficiency in PPA women because of its prominent role in the acute phase response during parturition [13, 14].

METHODS

Population

The source population consisted of women who delivered in the TweeSteden hospital in the period between January 2007 and December 2008. Table 1 shows an overview of all inclusion and exclusion criteria. Women aged 18 years or older, with more than 500 mL of blood loss during labor or with clinical signs of anemia (e.g., fatigue, loss of stamina, headache, faintness, pale skin, pale mucous membranes, hypotension, or tachycardia) were asked to join the study. After obtaining informed consent, all included women undergone blood testing (see below). Based on their Hb concentration, subjects were divided in a nonanemic control group (Hb $\geq$ 10.5 g/dL; $N = 150$) and a PPA index group ($6.5 \leq$ Hb < 10.5 g/dL; $N = 150$). Women with extreme PPA (Hb < 6.5 g/dL) were excluded because of packed red blood cells transfusion. The nonanemic control group did not receive any prescribed medication. The PPA index group received ferrous fumarate (200 mg × 3 tablets a day) for a period of 4 weeks.

Laboratory parameters

Both the nonanemic control and the PPA index group were subjected to blood testing at the time of inclusion ($T = 0$ weeks, T_0, being 0–48 h after delivery)

Table 1. Overview of all inclusion and exclusion criteria. Criteria were checked during a thorough interview

Inclusion	Exclusion
Women aged >18 years	Vitamin B12 deficiency (<160 pM)
Delivery in a clinical obstetric setting	Packed cell infusion in the previous 3 months
0–48 h after delivery	Alcohol/drug addiction
>500 mL blood loss or clinical signs of anemia	Chronic infection
Thorough grasp of the Dutch language	Gastro-intestinal disease
Informed consent acceptance	Thalassemia/hemoglobinopathy
	Aplastic, megaloblastic, or hemolytic anemia
	Malignant disease
	Kidney failure
	Liver failure
	Bone marrow disease
	Methothrexate use (interaction with folic acid)
	Contra-indications folic acid use
	Contra-indications ferrous fumarate use

and during follow-up ($T = 4$ weeks, T_4). The following parameters were tested: Hb, mean corpuscular volume (MCV), and reticulocyte hemoglobin content (CHr). Red blood cell parameters (Hb, MCV, and CHr) were measured in K_2EDTA anti-coagulated blood on an ADVIA2120i or ADVIA120 Hematology platform (Siemens Healthcare Diagnostics, Breda, The Netherlands). All parameters were measured in control and analyzed in the normal diagnostic practice of our laboratory. For the MCV and CHr, the respective coefficients of variation of control material were 1.55 (target value 85 fL) and 1.00% (target value 23 pg).

Cut off values for MCV and CHr for iron deficiency

The MCV is a widely accepted erythrocyte index and is one of the first parameters to be analyzed in most anemia protocols. With the prerequisite that the Hb concentration is below the reference interval, an MCV of below 80 fL is indicative for iron deficiency [15]. Note that severe microcytosis (i.e., <72 fL) is more suggestive for hemoglobinopathies [16].

The hemoglobin content in reticulocytes (CHr) is the product of the cellular volume and the Hb concentration of reticulocytes [17]. Compared to the slow turn over of erythrocytes (~120 days), reticulocytes have an average life span of 4 days, making the CHr a much more dynamic parameter compared to the standard erythrocytes indices. For adults, a CHr cut off value of <28 pg is used for the diagnosis of iron deficiency [10].

Group analysis

Based on literature and clinical guidelines regarding iron deficiency anemia [6, 10, 18], cut off values for MCV (<80 fL) and CHr (<28 pg, i.e., 1.74 fmol) were used to create PPA subgroups. For both parameters, this created three groups: a nonanemic control group and two ferrous fumarate-supplemented PPA groups, one with patients below the above mentioned cut off values and the other with patients above the cut off values. For all patients, parameter changes between T_0 and T_4 were calculated and expressed as delta-values (T_4–T_0, dHb, dMCV, dCHr). Normality of distribution of the individual parameters was verified using a one-sample Kolmogorov–Smirnov test. Statistical analysis was performed using a paired (T_0 *vs.* T_4) *t*-test (SPSS PASW 17.0.2; IBM, New York, NY, USA).

RESULTS

Nonanemic *vs.* anemic women

Table 2 shows an overview of the changes in Hb, MCV, and CHr between T_0 and T_4 in the nonanemic control and PPA group. Both the nonanemic and supplemented anemic group showed a significant increase in Hb at T_4 when compared to baseline (T_0). This increase was more prominent in the anemic group compared to the nonanemic control group, 3.4 *vs.* 1.6 g/dL, respectively. The increase in MCV in the PPA group at T_4 is statistically significant compared to T_0. However, the delta MCV of +1.2 fL falls within the 1.55% coefficient of variation (CV) of the MCV assay. In the PPA group, CHr at T_4 did not (significantly) change compared to T_0 (dCHr 0.0 pg).

Table 2. Changes in iron deficiency laboratory parameters between $T = 0$ weeks (T_0) and $T = 4$ weeks (T_4) in the nonanemic control and PPA index group. Data in columns T_0 and T_4 are shown as mean (SD)

Parameter	Group	N	T_0	T_4	Delta (T_4–T_0)
Hb (g/dL)	Nonanemic	101	11.4 (0.8)	13.0 (0.8)*	+1.6
	PPA	115	9.0 (1.0)	12.4 (1.0)*	+3.4
MCV (fL)	Nonanemic	101	86.2 (4)	85.5 (4)	−0.7
	PPA	115	83.2 (6)	84.4 (6)*	+1.2
CHr (pg)	Nonanemic	90	31.6 (2.4)	30.8 (2.1)*	−0.8
	PPA	105	29.8 (2.9)	29.8 (2.6)	0.0

*$P < 0.001$ *vs.* T_0. Note: although at T_0 150 women were included, some were lost to the study during follow-up at T_4. Because of the preferred paired statistical analysis between T_0 and T_4, this lowered the group size (N) of the parameters.

Table 3. Changes in Hb and MCV between $T = 0$ weeks (T_0) and $T = 4$ weeks (T_4) in the nonanemic control, low MCV (<80 fL) PPA, and normal MCV (≥80 fL) PPA group

Parameter	Group	N	T_0	T_4	Delta (T_4–T_0)
Hb (g/dL)	Nonanemic	101	11.4 (0.8)	13.0 (0.8)*	+1.6
	PPA (MCV < 80 fL)	30	8.9 (1.0)	11.8 (1.1)*	+2.9
	PPA (MCV ≥ 80 fL)	85	9.2 (1.0)	12.6 (0.8)*	+3.4
MCV (fL)	Nonanemic	101	86.2 (4)	85.5 (4)	−0.7
	PPA (MCV < 80 fL)	30	75.5 (3)	78.2 (3)*	+2.7
	PPA (MCV ≥ 80 fL)	85	85.8 (4)	86.5 (4)	+0.7

Data in columns T_0 and T_4 are shown as mean (SD). *$P < 0.001$ *vs.* T_0.

PPA MCV subgroups

By dividing the PPA group at T_0 in a microcytic (MCV < 80 fL) and normocytic (MCV ≥ 80 fL) anemia subgroup, we created two MCV subgroups. Irrespective of the subdivision, both subgroups showed an increase in Hb at T_4 of +2.9 and +3.4 g/dL for microcytic and normocytic PPA women, respectively (Table 3). However, in microcytic PPA women, the increase in MCV (dMVC +2.7 fL) was significant and well outside the 1.55% CV of the assay. In contrast, the MCV of normocytic PPA women showed an insignificant increase of +0.7 fL (Table 3, Figure 1a).

PPA CHr subgroups

When anemic women were divided based on their CHr [e.g., low CHr (<28 pg) and normal CHr (≥28 pg)], no difference was found in the increase in Hb concentration at T_4 (Table 4). The mean CHr at T_0 in the low CHr subgroup was 26.1 pg and increased by +1.9 pg reaching the CHr cut off value of 28 pg at T_4. Conversely, the CHr in the normal CHr subgroup (T_0, 31.4 pg) decreased by −1.0 pg (Figure 1b). The decrease in the PPA normal CHr subgroup was similar to the decrease in the nonanemic control group (dCHr −0.8 pg, Table 4).

MCV vs. CHr correlation

The baseline MCV and CHr values (T_0) of all PPA women show a good correlation ($R^2 = 0.663$, $P < 0.001$, Figure 2). Despite an Hb of <10.5 g/dL, 64% of the PPA women (89/140) (Figure 2, open dots) had baseline MCV and CHr values above their respective cut off values. 36% of the PPA women (51/140) (Figure 2, closed dots) had a microcytic MCV, a CHr below 28 pg, or both.

DISCUSSION

In postpartum anemia (PPA), it is assumed that the etiology of the anemia is attributed to iron

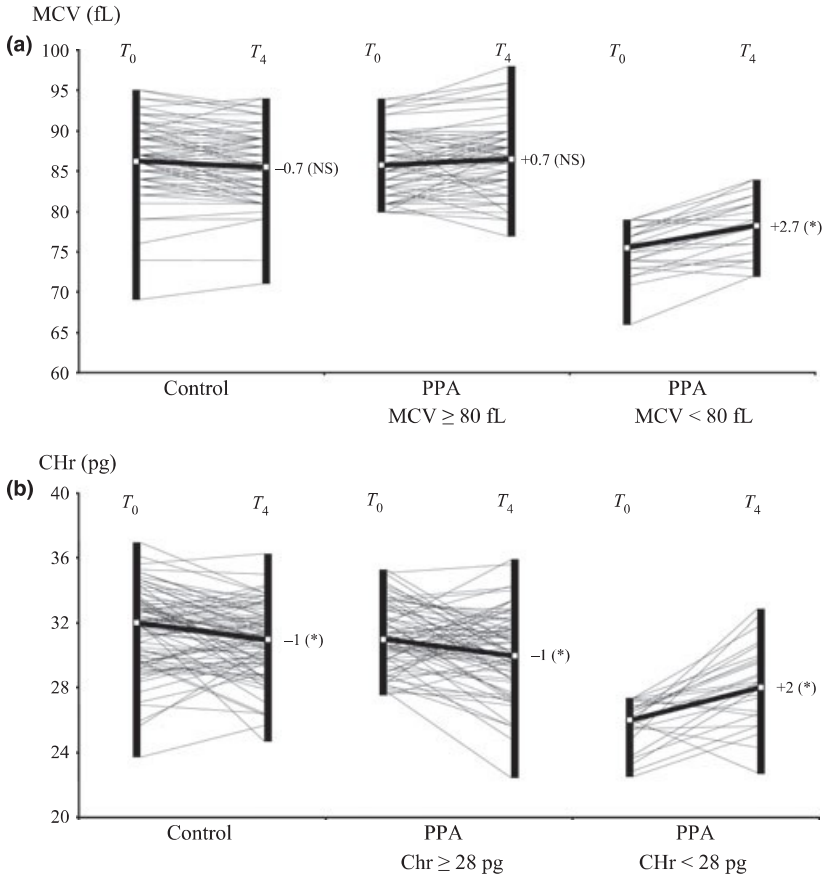

Figure 1. (a) Mean (thick black lines) and individual (thin black lines) delta MCV (dMCV, T_4–T_0) in nonanemic control, PPA normal MCV (MCV ≥ 80 fL), and PPA low MCV subgroups (MCV < 80 fL). T_0: T = 0 weeks, T_4: T = 4 weeks, NS: not significant, *P < 0.001 *vs.* T_0. (b) Mean (Thick black lines) and individual (thin black lines) delta CHr (dCHr, T_4–T_0) in nonanemic control, PPA normal CHr (CHr ≥ 28 pg), and PPA low CHr subgroups (CHr < 28 pg). T_0: T = 0 weeks, T_4: T = 4 weeks, *P < 0.001 *vs.* T_0. Note: the nonanemic control and PPA normal CHr showed a significant decrease.

Table 4. Changes in Hb and CHr between T = 0 weeks (T_0) and T = 4 weeks (T_4) in the nonanemic control, low CHr (<28 pg) PPA, and normal CHr (≥28 pg) PPA group

Parameter	Group	N	T_0	T_4	Delta (T_4–T_0)
Hb (g/dL)	Nonanemic	90	11.4 (0.8)	13.0 (0.8)*	+1.6
	PPA (CHr < 28 pg)	30	8.9 (1.1)	11.9 (1.1)*	+3.0
	PPA (CHr ≥ 28 pg)	75	9.2 (1.0)	12.6 (1.0)*	+3.4
CHr (pg)	Nonanemic	90	31.6 (2.4)	30.8 (2.1)*	−0.8
	PPA (CHr < 28 pg)	30	26.1 (1.4)	28.0 (2.6)*	+1.9
	PPA (CHr ≥ 28 pg)	75	31.4 (1.8)	30.4 (2.3)*	−1.0

Data in columns T_0 and T_4 are shown as mean (SD). *P < 0.001 *vs.* T_0.

deficiency. Consequently, all PPA women with an Hb concentration between 6.5 and 10.5 g/dL are orally supplemented with iron. This potentially results in unnecessary iron supplementation in those women with sufficient iron stores. To evaluate the need for iron supplementation in women with mild PPA, we followed the therapeutical response to iron supplementation using accomplished cut off values for MCV and CHr.

Irrespective of baseline iron deficiency parameters, the Hb concentration in all PPA women improved significantly after 4 weeks (T_4). This might be interpreted as a beneficial therapeutical response to iron supplementation for all women with PPA.

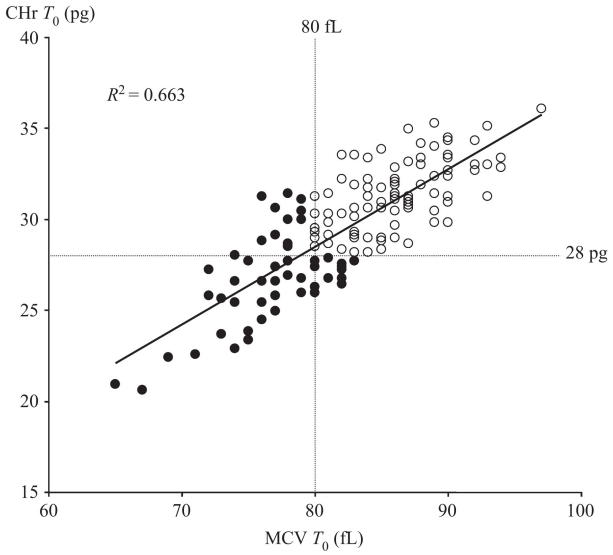

Figure 2. Correlation between baseline MCV (*x*-axis) and CHr values (*y*-axis) in PPA women. Coefficient of correlation (R^2) is 0.663 ($P < 0.001$). The vertical and horizontal dotted lines represent the respective cut off values of the MCV (80 fL) and CHr (28 pg). Open dots: normocytic MCV and CHr above cut off value. Filled dots: microcytic MCV, CHr below cut off value, or both.

Indeed, the PPA MCV and CHr subgroups suggestive for depleted iron stores at T_0 showed a significant improvement for both MCV and CHr toward normalization at T_4 indicating a beneficial therapeutical response to iron supplementation. However, in the PPA MCV and CHr subgroups arguing against iron deficiency, no improvement in the respective parameters at T_4 was seen. The lack of improvement of the MCV and CHr in these subgroups might be explained by the fact that the iron stores at T_0 were already adequate and that no additional iron was taken up via the intestines.

The study is limited by the fact that all women with PPA were orally supplemented with iron, lacking a control group with PPA and normal iron stores who were not supplemented with iron. Because intestinal iron uptake is inhibited by hepcidin which in turn is upregulated by adequate intracellular iron stores [19, 20], we assume that in the PPA group with sufficient iron stores, no intestinal iron was taken up. Unfortunately, this remains an assumption because hepcidin concentrations were not measured in this study. Future studies are needed to confirm our findings. One such study would involve randomizing PPA women with adequate baseline iron stores (i.e., normal MCV and CHr $\geq$ 28 pg) creating PPA groups with and without iron supplementation. Confirming our results, one would expect both groups to show a similar increase in hemoglobin, irrespective of supplementation.

The data of our study suggest that the assumption of iron deficiency as etiology of PPA is not always justified. Indeed, the majority of PPA women in our study (64%) had baseline laboratory parameters (i.e., normocytic MCV and CHr $\geq$ 28 pg) arguing against iron deficiency as the cause of the anemia and as a result were unnecessarily supplemented with iron.

The MCV and CHr can be readily analyzed in conjunction with an Hb concentration from the same sample. Therefore, next to Hb, we propose to include measurement of MCV and CHr, using standard cut off values, for identifying truly iron-deficient PPA women who will have a beneficial therapeutical response to iron supplementation, thereby reducing the needless supplementation of iron in those PPA women with sufficient iron stores.

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REFERENCES

1. Gibbs R.Clinical risk factors for puerperal infections. Obstet Gynecol 1980; 55 (55 Suppl): 178S–84S.
2. Gilbert L, Porter W, Brown VA. Postpartum haemorrhage – a continuing problem. BJOG 1987;94:67–71.
3. Vora M, Gruslin A. Erythropoietin in obstetrics. Obstetrical & Gynecological Survey 1998;53:500–8.
4. Bergmann RL, Richter R, Bergmann KE, Dudenhausen JW. Prevalence and risk factors for early postpartum anemia. Eur J Obstet Gynecol Reprod Biol 2010;150: 126–31.

5. Bodnar LM, Scanlon KS, Freedman DS, Siega-Riz AM, Cogswell ME. High prevelance of postpartum anemia among low-income women in the United States. Am J Obstet Gynecol 2001;185:438–43.
6. WHO/UNICEF/United Nations University. Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Program Managers (document WHO/NHD/01.3). Geneva: World Health Organization; 2001: 1–132.
7. Jans S, Beentjes M. Anemia in Obstetrics: Guidelines for Prevention, Diagnosis and Treatment. Utrecht, The Netherlands: Dutch Society of Obstetrics and Gynecology; 2001: 31–3.
8. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280S–4S.
9. Melamed N, Ben-Haroush A, Kaplan B, Yogev Y. Iron supplementation in pregnancy – does the preparation matter? Arch Gynecol Obstet 2007;276:601–4.
10. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 2002;99:1489–91.
11. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assesment of iron-deficient states. Clin Lab Haematol 2006;28:303–8.
12. Ermens AAM, Hoffmann JJML, Krockenberger M, van Wijk EM. New erythrocyte and reticulocyte parameters on Cell-Dyn Sapphire: analytical and preanalytical aspects. Int J Lab Hematol 2012; in press
13. Breymann C, Honegger C, Holzgreve W, Surbek D. Diagnosis and treatment of iron-deficiency anaemia during pregnancy and postpartum. Arch Gynecol Obstet 2010;282:577–80.
14. Cicarelli LM, Perroni AG, Zugaib M, de Albuquerque PB, Campa A. Maternal and cord-blood levels of serum amyloid A, C-reactive protein, tumor necrosis factor alpha, interleukin-1beta and interleukin-8 IL8 during and after delivery. Mediators Inflamm 2005;2005:96–100.
15. Okuno T, Chou A. The significance of small erythrocytes. Am J Pathol 1975;64:48–52.
16. Lafferty JD, Crowther MA, Ali MA, Levine ML. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. Am J Clin Pathol 1996;106:201–5.
17. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. Am J Hematol 2008;83:307–10.
18. Kolnaar BGM, van Wijk MAM, Pijnenborg L, Assendelft WJJ. Summary of the Dutch College of General Practitioners' practice guideline ''Anemia''. Ned Tijdschr Geneeskd 2003;147:1956–61.
19. Ganz T. Hepcidin and iron regulation: 10 years later. Blood 2011;117:4425–33.
20. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, McVey-Ward D, Ganz T, Kaplan J Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306:2090–3.