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Iron status in pregnant women: which measurements are valid?

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Summary

Anaemia in pregnancy in developing countries continues to be a public health problem of significant proportion. At least 50% of the anaemia has been blamed on iron deficiency. In populations where chronic inflammation and iron deficiency anaemia coexist, the criteria to accurately define iron status are not always clear. Similarly, in pregnancy, with marked

physiological changes, cut-off points for biochemical parameters need to be re-examined. In this study we examined the diagnostic accuracy of iron parameters including mean cellular volume (MCV), serum iron, transferrin, total iron binding capacity (TIBC) and its saturation, zinc protoporphyrin (ZPP), ferritin and serum transferrin receptor (TfR) for the assessment of iron status in a population of anaemic pregnant women in Malawi. Stained bone marrow aspirates were used as the standard for comparison.

Results show that for the purpose of screening, serum ferritin is the best single indicator of storage iron provided a cut-off point of 30 micro g/l is used. A number of other commonly used parameters of iron status were shown to have limited diagnostic accuracy. Logistic regression was used to obtain mathematical models for the prediction of bone marrow iron status using a combination of available parameters.

Keywords: anaemia, iron, parameters, pregnancy, inflammation.

Estimates of prevalence of anaemia in pregnancy in developing countries range from 35% to 75% (W.H.O., 1992) [46]. At least 50% of the anaemia has been attributed to iron deficiency (DeMaeyer & Adiels-Tegeman, 1985; DeMaeyer, 1989), [14,15] but there are only a few published studies to substantiate this (Assami et al, 1988; Fleming, 1989; Hercberg et al, 1987; Isah et al, 1985; Liljestrand et al, 1986; Mayet, 1985; Prual et al, 1988) [1,17,22,23,28,32,34]. Criteria that have been used to define iron deficiency vary and have often not been stringent enough (MacPhail & Bothwell, 1992) [31].

Assessment of iron status is notoriously difficult, especially in pregnancy and in conditions associated with inflammation. In pregnancy, changes in maternal physiology include expansion of plasma volume, increased erythropoiesis and increased demands of the fetoplacental unit for iron. This occurs throughout gestation and can differ markedly between individuals. The resulting changes in serum levels of biochemical markers for iron status make it necessary to re-define cut-off values for the diagnosis of iron deficiency (Bentley, 1985; deLeeuw et al, 1966; Meeks et al, 1987) [3,13,33]. In the presence of inflammation, serum ferritin can be elevated and then no longer reflects iron stores proportionately. Furthermore, plasma transferrin, MCV and MCHC are low to normal and serum iron is often decreased (Dabbagh et al, 1993; Lipschitz et al, 1974) [11,30].

Examination of a stained aspirate of bone marrow for haemosiderin is still considered the 'gold standard' method for the evaluation of iron status (Cook, 1994; Hansen, 1983) [8,21]. This technique is invasive and not suitable for screening purposes. There are currently a large number of relatively non-invasive biochemical measurements available for the detection of iron deficiency and the assessment of its severity (Cook & Skikne, 1989; Cook et al, 1992) [10,9]. Although these have not all been validated against bone marrow iron content, they are now used in preference to this method. To improve sensitivity and specificity, a combination of biochemical tests may be used. However, for the accurate measurement of iron status in pregnancy and/or in conditions complicated by chronic or acute inflammation, there is no consensus about the best combination (vandenBroek, 1996; Cook, et al, 1992; Letsky, 1998; Puolakka et al, 1980; Thompson, 1988) [42,9,27,36,41]. There is therefore a need to accurately define valid parameters for confirmation of the diagnosis of iron deficiency anaemia in pregnancy and to assess the degree of iron depletion. Moreover there is a need to differentiate anaemia due to iron deficiency from that caused by other nutritional deficiencies or failure of incorporation of iron as a result of inflammatory processes (Prual et al, 1988; Yip & Dallman,

1988) [34,48].

In this study we examine the diagnostic accuracy of iron parameters including mean cellular volume (MCV), serum iron, transferrin, total iron binding capacity (TIBC) and its saturation, zinc protoporphyrin (ZPP), ferritin and serum transferrin receptor (TfR) for the assessment of iron status in a population of anaemic pregnant women in Malawi. C-reactive protein (CRP) was used as a marker of inflammation. In order to examine which combination of biochemical parameters best reflected iron status, logistic regression models were fitted. Bone marrow aspirates stained for haemosiderin were obtained to serve as a standard for comparison. The population studied is representative of others in developing countries where anaemia and chronic inflammation are highly prevalent and often coexist.

METHODS [†]

Pregnant women attending the antenatal clinic at the Queen Elizabeth Central Hospital in Blantyre were screened for anaemia by examination of a capillary blood sample (fingerprick) using a battery operated HemoCue haemoglobinometer. In accordance with the W.H.O. definition of anaemia for pregnant women in and beyond the second trimester of pregnancy, anaemia was defined as haemoglobin [Hb] <10.5 g/dl in this study (vandenBroek, 1998; W.H.O., 1993) [43,47]. A general medical and obstetric examination was carried out in each case. Gestational age was estimated from the date of the last menstrual period and from measurement of the fundal height. After obtaining informed and written consent a second venous blood sample was taken for further haematological and biochemical analysis.

Haematological indices were obtained by Onyx Coulter counter (model Onyx/CP).

Serum iron was measured by Ferrozine colourimetric assay (Boehringer-Mannheim, Hitachi 911 analyser, detection limit 2 micro mol/l). Transferrin was measured by Tina-quant immunoturbidimetric assay (Boehringer-Mannheim, Hitachi 911 analyser, detection limit 0.2 g/l). Serum ferritin and serum transferrin receptors were determined by immunoenzymometric assay (Ramco, U.S.A., detection limit 0.6 micro g/l and 0.7 micro g/ml respectively). The ratio was calculated as: (serum transferrin receptor x 1000)/ferritin (Skikne et al, 1990b) [39]. C-reactive protein (CRP) was measured by Tina-quant immunoturbidimetric assay (Boehringer-Mannheim, Mannheim, Hitachi 911 analyser, detection limit 3 mg/l). HIV status was determined in duplicate using an immunoenzymometric assay (WellcoZyme).

DNA was obtained from bone marrow aspirate material by standard phenol chloroform extraction. Southern blot procedure was carried out to determine thalassaemia status and PCR to determine sickle cell status (Molecular Haematology Unit, Institute of Molecular Medicine, Oxford).

Written consent for bone marrow aspiration was obtained separately after the procedure had been explained. Aspirates were obtained by sterile procedure under local anaesthesia from the anterior iliac crest. Patients were admitted for observation on the antenatal unit for 24 h following bone marrow aspiration. Bone marrow films were stained for iron using Prussian blue (Perls' method) (Dacie & Lewis, 1995) [12]. All bone marrow aspirates included in the analysis were representative and contained sufficient stroma. The stained films were examined by a haematologist blinded to the biochemical and haematological results and classified as: 0, no iron present; 1, traces of iron only; 2, moderate amounts of iron present; 3, abundant iron present. Ethical permission for this study was obtained from the Health Sciences Research Committee in

Malawi.

RESULTS

A total of 93 women consented to bone marrow aspiration. Their mean gestational age was 31 weeks. 76% of women were in the third, 23% in the second and 1% in the first trimester.

Summary statistics (mean, standard deviation, median, range, skewness) for haematological and biochemical parameters obtained in the study population are presented in [Table I](#).

	Mean	SD	Median	Min.	Max.	Skewness	n
Hb (g/dl)	8.3	1.6	8.6	2.8	10.6	-1.24	93
CRP (mg/l)	40.5	53.1	11.5	1	174	1.3	90
MCV (fl)	84.5	19.9	90	52	136	0.01	93
Serum iron (μ mol/l)	3.6	2.8	2.7	0.1	10.9	1.0	90
Transferrin (g/l)	3.2	1.0	3.2	0.06	5.34	-0.3	90
IBC (μ mol/l)	61.6	12.8	61.5	22	88	-0.3	90
Saturation (%)	34.1	27.5	24.0	2.0	105	1.1	90
ZPP (μ mol/l)	2.8	1.4	2.6	1.1	9.2	1.9	92
Ferritin (μ g/l)	157.1	148	30.5	5.6	2657	4.9	88
Transferrin receptor (TR) (mg/l)	11.6	6.2	9.8	3.1	38.0	1.5	90
Ratio (TR \times 1000)/ferritin	57.9	71.3	30.1	2.1	333.3	1.9	88

CRP, C-reactive protein; MCV, mean cellular volume; IBC, iron binding capacity; ZPP, zinc protoporphyrin.

Table I. Haematological and biochemical parameters in 93 anaemic pregnant women.

The maximum value of haemoglobin concentration of 10.6 g/dl was obtained in a patient with Hb of 10.4 g/dl as measured by a HemoCue haemoglobinometer during the initial screening procedure at antenatal visit.

Of the 93 patients, 44 were HIV positive and 48 HIV negative and in one case HIV status was unknown. There was no statistically significant difference between HIV-positive and HIV-negative subjects in terms of bone marrow iron status ($P > 0.90$).

There was no patient with sickle cell disease, i.e. HbSS, HbSC or HbS-beta-thalassaemia.

There was no alpha degrees thalassaemia or beta thalassaemia, but homozygous alpha⁺ (alpha^{3.7}/alpha^{3.7}) was present in 14% (12/85) and alpha⁺ (alpha alpha/alpha^{3.7}) in 24.7% (21/85).

The mean [Hb] for 0⁺ and 0⁺ homozygous patients was 8.6 and 8.2 g/dl respectively. The mean [Hb] for all patients is 8.3 g/dl. Using one-way analysis of variance this difference is shown to be non-significant ($F = 0.347$, $df 2,79$, $P = 0.71$).

Similarly, mean MCV for 0⁺ and 0⁺ homozygous patients was 81.6 and 80.7 respectively, with a mean MCV of 84.3 for all patients. One-way analysis of variance shows this to be non-significant ($F = 0.625$, $df 2,79$, $P = 0.54$).

Ninety-three representative bone marrow aspirates were obtained and examined for iron content ([Table II](#)). In 43 (46.2%) cases there was no demonstrable iron present, in 15 (16.2%) traces of iron only and in 35 (37.7%) of women there was sufficient or abundant iron in the form of haemosiderin.

	Category	<i>n</i>	%
No stainable iron	0	43	46.2
Trace of iron	1	15	16.1
Moderate amount of iron	2	17	18.3
Abundant iron	3	18	19.4
		93	100

Table II. Results of assessment of iron status from bone marrow aspirates in 93 anaemic pregnant women.

Diagnostic value of individual indicators of iron status The 43 bone marrow aspirates with no demonstrable iron present (iron = 0) were considered indicative of true iron deficiency and used as a 'gold standard' against which biochemical measurements were compared. The performance of the various biochemical indicators of iron status was evaluated in terms of sensitivity, specificity, positive and negative predictive values and accuracy using commonly accepted cut-off points. In addition, Likelihood Ratios (LR), indicating by how much a given test result would raise the pretest probability of iron deficiency, were calculated (Jaescke et al, 1994) [24] (Table III).

Parameter	Cut-off point	Sens.	Spec.	Acc.	PPV	NPV	LR	<i>n</i>
MCV (fl)	<85	58.1	60.0	59.1	55.5	37.5	1.5	93
	<80	41.9	66.0	54.8	51.4	66.0	1.2	93
	<70	41.9	74.0	59.1	58.1	59.7	1.6	93
Serum iron ($\mu\text{mol/l}$)	≤ 5.4	76.2	19.1	46.1	45.7	19.1	1.0	89
	≤ 9.0	95.2	6.4	48.3	47.6	6.4	1.0	89
Transferrin ($\mu\text{g/l}$)	≥ 3	88.1	72.3	79.8	74.0	87.2	3.2	89
IBC ($\mu\text{mol/l}$)	<70	47.6	91.7	71.1	83.3	66.7	5.7	90
Saturation (%)	<15	38.1	76.6	58.4	59.3	58.1	1.6	90
ZPP ($\mu\text{mol/l}$)	1.2	90.5	4.1	44.0	44.7	33.3	0.9	92
Ferritin ($\mu\text{g/l}$)	≤ 30	90.0	85.1	87.4	83.7	90.9	6.0	88
	≤ 12	37.5	93.7	68.2	83.3	35.2	6.0	88
Transferrin receptor (TfR) (ng/l)	≥ 8.5	76.1	45.8	60.0	55.2	68.8	1.4	90
	≥ 9.0	71.4	52.1	61.1	56.6	67.6	1.4	90
Ratio (TfR $\times 1000$)/ferritin	≥ 300	85.0	79.2	81.8	77.3	86.4	4.1	88
	≥ 500	70.0	93.8	83.0	90.3	78.9	11.2	88

MCV, mean cellular volume; IBC, iron binding capacity; ZPP, zinc protoporphyrin.

Table III. Sensitivity (Sens.), specificity (Spec.), accuracy (Acc.), positive and negative predictive values (PPV, NPV) and likelihood ratio (LR) for diagnosing iron deficiency (bone marrow iron = 0) in 93 anaemic pregnant women.

Receiver operating characteristic curves (ROC) \pm

ROC curves were constructed for serum ferritin, transferrin receptor and the ratio (Figure 1). The optimal cut-off point for ferritin in the population studied was found to be 30 micro g/l resulting in a sensitivity of 90.0% and a specificity of 85.1%. The likelihood ratio for diagnosing iron deficiency (defined as bone marrow iron = 0) at this cut-off point was 6.0. The ROC curve for transferrin receptor was found to be most useful when this indicator was expressed as the

ratio. Cut-off points of 300 and 500 were considered. For a ratio \geq 300 sensitivity was 85% and specificity 79%. A cut-off point of \geq 500 is associated with a higher specificity of 94% but lower sensitivity of 70% (Table III, Figure 1).

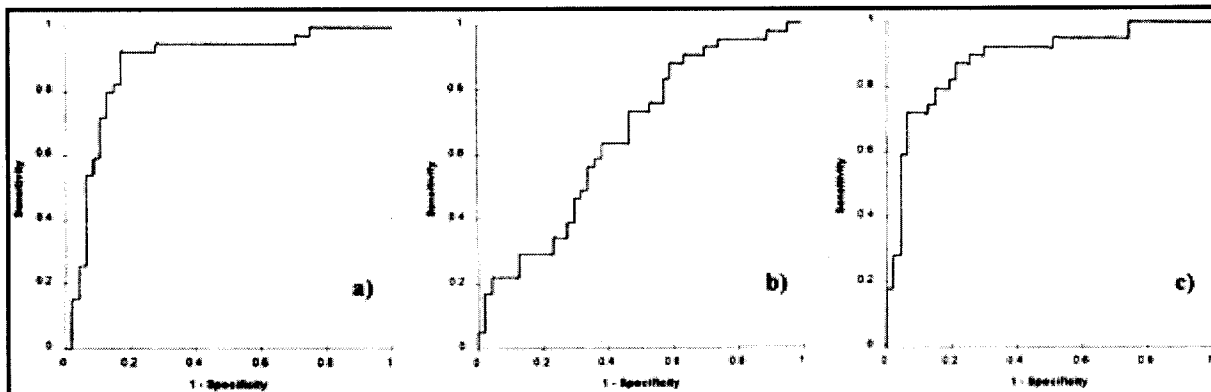


Figure 1. ROC curves for (a) ferritin, (b) transferrin receptor, and (c) the ratio (n = 88).

Logistic regression models \pm

In order to examine which biochemical parameters best predicted the absence/presence of bone marrow iron, logistic regression models were fitted using various combinations of parameters, including: C-reactive protein (CRP), ferritin, serum transferrin receptor, the ratio ((serum transferrin receptor x 1000)/ferritin), transferrin, total iron binding capacity (TIBC), serum iron, mean cell volume (MCV), zinc protoporphyrin (ZPP), saturation and HIV status (Table IV). With this method, all measurements are treated as continuous variables and results obtained are not influenced by cut-off points.

	Deviation	df	Δ Deviation	Δ df	P
Null model	118.48	85			
Ferritin	86.65	84	31.83	1	< 0.005
Transferrin receptor (TfR)	112.55	84	5.93	1	0.015
Ratio	77.97	84	40.51	1	<0.0005
Ferritin + TfR	85.41	83	1.24	1	0.27
Ferritin + ratio	73.68	83	12.97	1	<0.0005
Ferritin + CRP	83.92	83	2.73	1	0.10
Ratio + CRP	73.72	83	4.25	1	0.04
Ferritin + ratio + CRP	72.02	82	1.66	1	0.20
Ferritin + ratio + CRP + transferrin	68.65	81	3.37	1	0.07
Ferritin + ratio + CRP + IBC	68.81	81	3.21	1	0.07
Ferritin + ratio + CRP + serum iron	72.02	81	0.00	1	1.00
Ferritin + ratio + CRP + MCV	71.99	81	0.03	1	0.86
Ferritin + ratio + CRP + ZPP	69.08	81	2.94	1	0.09
Ferritin + ratio + CRP + saturation	71.81	81	0.21	1	0.65
Ferritin + ratio + CRP + HIV	71.66	81	0.36	1	0.55
ZPP	115.84	84	2.64	1	0.10
ZPP + ferritin	86.43	83	0.22	1	0.64

TfR, serum transferrin receptor; CRP, C-reactive protein; IBC, iron binding capacity; MCV, mean cellular volume; ZPP, zinc protoporphyrin; ratio, (TfR × 1000)/ferritin; df, degrees of freedom.

Table IV. Analysis of deviance for logistic regression models (n = 86).

The models fitted are of the form: $\ln[p/(1-p)] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_j x_j$, where p denotes the probability that there is no bone marrow iron, the β_i 's are unknown constants and the x_i 's are the parameters considered for inclusion.

For each set of parameters, a formula was obtained which could be used to estimate the probability of having no stainable storage iron. A classification rule can be specified from the formula, by classifying (or predicting) a subject to have no stainable storage iron if the estimated probability p is >0.5 . To assess the validity of the models fitted, the number of subjects thus correctly classified could be considered. The importance of each set of variables considered was assessed using likelihood ratio tests (deviances). The null model is one which involves no parameters, therefore the probability, p , is a constant.

The deviance for a model reflects how much variation in the data remains unexplained. The reduction in deviance from that of the null model is an indication of how important the variables are in explaining the variation or, in this case, in predicting bone marrow iron status.

Ferritin and the ratio were the most important predictive variables ($P < 0.0005$). Among the other variables considered, ZPP, transferrin and IBC were the only ones which showed some evidence ($P < 0.10$) of being useful in predicting the bone marrow iron status of subjects. These analyses indicate that ferritin, the ratio, transferrin receptor and CRP are relevant in predicting bone marrow iron status and that transferrin and IBC may be useful. Results are presented in Table IV.

From the analyses, the classification formulae derived are: treat as absence of stainable storage

iron (bone marrow iron =0) if:

$$(1) 0.34 + 0.0043 \times \text{ferritin} - (2.7 \times \text{tFR})/\text{ferritin} + 0.00696 \times \text{CRP} + 0.05 \times \text{tFR} < 0$$

$$(2) 3.30 + 0.0029 \times \text{ferritin} - (1.85 \times \text{tFR})/\text{ferritin} + 0.00598 \times \text{CRP} + 0.025 \times \text{tFR} - 0.902 \times \text{transferrin} < 0$$

$$(3) 4.69 + 0.0029 \times \text{ferritin} - (1.88 \times \text{tFR})/\text{ferritin} + 0.0060 \times \text{CRP} + 0.026 \times \text{tFR} - 0.0693 \times \text{IBC} < 0$$

Using a combination of the measurements, ferritin, TfR, CRP and the ratio resulted in a sensitivity of 77% (30/39) and a specificity of 89% (42/47) (formula 1).

With the addition of either transferrin or IBC a sensitivity of 79% and specificity of 87% was obtained (formulae 2 and 3).

Sequential test strategy ¶

Sequential approaches to testing were also examined. Using a test with high sensitivity such as ferritin (cut-off point 30 micro g/l), followed by a test with high specificity such as the ratio (cut-off point 500), resulted in a sensitivity of 70% (28/40) and specificity of 94% (45/48). Alternatively, determination of serum ferritin may be followed by the use of, for example, formula 2 above.

When this method is used, 43/88 patients were classified as iron deficient using a cut-off point of 30 micro g/l. Of these, six would be identified out as not iron deficient if a ferritin test was followed by formula 2. Of these six patients, four had no iron in the bone marrow and two patients had bone marrow iron = 1 and = 2. Therefore two will have been correctly reclassified as not being iron deficient whereas four will be wrongly reclassified.

This approach could also be used with the intention to confirm iron sufficiency: 45/88 patients would be classified as iron sufficient using serum ferritin as an initial test. If formula 2 is then used on this group for confirmation, one patient will be reclassified as iron deplete. This would have been correct as this patient had no stainable bone marrow iron present.

DISCUSSION ¶

Haemoglobin concentration [Hb] has been used extensively as an index of iron deficiency. This provides a quantitative measure of the severity of iron deficiency once anaemia has developed, but lacks sensitivity and specificity and should not be used as the sole index of iron status (Hallberg, 1994) [20]. Furthermore, measurements of haemoglobin concentration alone are unreliable in pregnancy as a result of marked physiological changes in plasma volume and red cell mass (deLeeuw et al, 1966; Hall, 1974; Lind et al, 1975) [13,19,29]. Assessment of the amount of stainable iron in the form of haemosiderin, i.e. aggregates of degraded ferritin, in bone marrow aspirates is considered to be the most accurate method available to determine iron status. Although failure to detect marrow iron is not unequivocal proof of iron deficiency anaemia, its presence excludes iron depletion (Cook, 1994) [8]. We have therefore used stained bone marrow aspirates as a 'gold standard' against which other haematological and biochemical parameters were compared. In this population of anaemic pregnant women, iron deficiency as defined by the absence of stainable iron in bone marrow aspirates was present in 46% of cases. This confirms

that iron deficiency is an important contributor to anaemia. Only one other published study has described bone marrow iron status in anaemia of pregnancy in sub-Saharan Africa in which 38% of severely anaemic Zambian women were found to be bone marrow iron depleted (Fleming, 1989) [17].

Diagnostic accuracy of measurements of iron status ¹

(Table III) Analysis of the results of biochemical tests for the diagnosis of iron deficiency shows clearly that sensitivity and specificity depend on the cut-off points which are chosen to define iron deficiency. In the population studied a number of commonly recommended tests of iron status were shown to have low diagnostic accuracy. The possible reasons for this are as follows:

A reduction in mean corpuscular volume (MCV) to < 80 fl, reflecting reduced haemoglobin synthesis, has commonly been advocated as an index of iron deficiency even though it occurs late in its development. In pregnancy, as a result of stimulated erythropoiesis, there is a relative increase in circulating larger young erythrocytes and the MCV is no longer thought to be an accurate index of iron deficiency (Celada, 1982; Chanarin et al, 1977; Letsky, 1998; Thompson, 1988) [5,6,27,41]. This is reflected in our data which showed a low diagnostic accuracy even when cut-off points of 85 fl or 70 fl were chosen.

Serum iron is of limited use because of diurnal variation, the effect of recently ingested iron and a decrease in patients with an acute-phase reaction (Tietz et al, 1994) [40].

Both TIBC and transferrin are measurements of transport iron. Immunochemical assays of transferrin are more accurate than chemical assays of TIBC. Since TIBC and transferrin are increased in iron deficiency and decreased in inflammation, measurements may be within the normal range when both conditions co-exist. Transferrin is known to decrease in the presence of generalized malnutrition. This was not found to be a problem in the population studied.

Elevation of zinc protoporphyrin (ZPP) is noted several weeks after the onset of iron deficiency and as ZPP can be measured easily and cheaply using haemofluorometry it has been used in several studies to assess iron status. With the usual laboratory cut-off point of 1.2 micro mol/l, specificity was only 4.1%. This is therefore an exceptionally poor test of iron status and overdiagnosis of iron deficiency using this measurement is common.

In the published literature, serum ferritin is the most frequently used indicator of iron status. The range of values for serum ferritin found in non-anaemic individuals is 12-300 micro g/l. In the presence of anaemia values of <20 micro g/l are commonly taken as evidence of iron deficiency (Cook, 1994) [8]. However, patients with levels of up to 37 micro g/l have been shown to have absent marrow haemosiderin (Lipschitz et al, 1974) [30]. In pregnancy serum ferritin is lower and mean values are close to the iron-deficient range (Hallberg, 1994) [20]. Romslo et al (1983) [37] found serum ferritin to be a more sensitive and specific test of iron deficiency than serum iron, TIBC or ZPP as measured by the response to iron supplements. Although low serum ferritin is a reliable index of iron deficiency, in the presence of infection, normal or raised levels may be seen in deficient subjects (Lipschitz et al, 1974) [30]. More recently the measurement of serum transferrin receptors and the serum transferrin receptor:ferritin ratio have been proposed as new indices of iron deficiency (Flowers et al, 1989; Skikne et al, 1990b) [18,39] Receptor synthesis is up-regulated in iron-deprived tissues. It can be argued that assessment of iron status at the tissue level is of more functional importance when examining the effects of iron depletion on the body than an assessment of stores. Furthermore, the serum transferrin receptor

concentration is elevated earlier than erythrocyte protoporphyrin or a reduction in MCV. A close inverse relationship can be demonstrated between serum receptor and an induced deficit in functional iron (Skikne et al, 1990b), [39] and serum transferrin receptor concentration is reported to remain normal in the majority of patients with inflammation (Punnonen et al, 1997; Skikne et al, 1990a) [35,38]. Levels have been shown to reflect iron status in pregnancy (Carriaga et al, 1991) [4]. Levels of serum transferrin receptor concentration >8.5 mg/l were found only in women with depleted iron stores. The present study is the first to publish data for serum transferrin receptor measurements as an indicator of iron status in pregnancy with bone marrow assessment as comparison.

ROC curves

In our population the optimal cut-off point for serum ferritin was found to be 30 micro g/l. This is significantly higher than the cut-off points previously suggested for the diagnosis of iron deficiency anaemia in pregnancy (DeMaeyer, 1989; W.H.O., 1991) [15,45]. Only one other study, from Nigeria, measured iron stores directly by estimating stainable iron in bone marrow (Fleming, 1982) [16]. Non-pregnant women and children with markedly reduced iron stores had serum ferritin levels <50 micro g/l. Workers in Chad and Zaire also recommended the use of a higher cut-off point of 50 micro g/dl in the presence of chronic inflammation (Prual et al, 1988; Kuvibidila et al, 1994) [34,26]. When ferritin levels are low, iron stores are likely to be depleted, but slightly higher levels may mask underlying iron depletion. Cut-off points for ferritin in other population groups need to be carefully established to reflect the local situation and especially the underlying degree of chronic inflammation. The extent to which inflammation is present and contributes to the anaemia of pregnancy has not been comprehensively assessed but is likely to be underestimated. Similarly, cut-off points for the ratio may also have to be adapted to take into account the presence of coexisting infection. We observed that a cut-off point of 500 had a higher diagnostic accuracy than the previously recommended cut-off point of 300.

Logistic regression models

(Table IV) Logistic analysis showed that prediction of bone marrow iron in Malawian anaemic pregnant women is significantly improved when both serum ferritin and the serum transferrin receptor are determined as compared to serum ferritin alone or ferritin in combination with CRP. Overall, the most accurate prediction of bone marrow iron was obtained using a combination of biochemical markers which included ferritin, serum transferrin receptor, CRP and transferrin. Serum iron, MCV, ZPP, saturation and/or HIV status did not significantly improve bone marrow iron prediction when included in the formulae. Addition of a measure of chronic inflammation, in our study C-reactive-protein, improved prediction only slightly. Whereas Witte et al (1986) [44] advocated a nomogram combining CRP and serum ferritin for estimation of iron status, two other groups (Baumann Kurer et al, 1995; Coenen et al, 1991) [2,7] in subsequent studies also found that the addition of a marker of inflammation did not significantly improve bone marrow iron prediction. The method of deriving the classification formulae presented assumes linearity in the influence of the iron parameters studied. Logarithmic transformations were considered for individual variables, but this resulted in no substantial improvement. These formulae provide a mechanism for assessing the evidence that any pregnant woman for whom the necessary data are available has no bone marrow iron. Application of these to other population groups needs to be evaluated by further study.

Strategy for diagnostic testing

The value of a diagnostic test will depend on the planned intervention. In an area with a high prevalence of iron deficiency it may be more important to have a very sensitive screening test so that iron supplements are given to all that appear to be deficient. Alternatively, a more specific test may be chosen to target the most at-risk population for more intensive management. A sequential approach may be used, for example a test with high sensitivity such as serum ferritin may be followed by a test with high specificity such as the ratio. Determination of serum ferritin and serum transferrin receptor would reflect iron status at both the storage and tissue levels. Both can be measured relatively simply using an ELISA. However, it is misleading to think that two tests when combined always lead to improved diagnostic performance, as their error rates are also combined (Jones & Payne, 1997) [25]. However, when used correctly, a combination of tests can be the best strategy. If, for example, the aim is to have a high degree of accuracy in confirming iron sufficiency, an approach using ferritin combined with a formula derived from logistic regression could be used. This is outlined in the Results section under 'sequential approach'.

In conclusion, our results show that for the purpose of screening, in the population studied, serum ferritin is the best single indicator of storage iron provided a cut-off point of 30 micro g/l is used (sensitivity 90%, specificity 85%). A number of other commonly used parameters of iron status such as MCV, serum iron and ZPP can be shown to be of little or no value as single parameters. Although not more accurate per se, the value of using a model with multiple biochemical parameters may be that a more complete estimate of total body iron composition is obtained as transport, storage and tissue compartments are reflected. Each of the formulae (models) provided in this paper have a poorer sensitivity than using ferritin <30 micro g/l, but the specificity is slightly better. The combination of ferritin, serum transferrin receptor, the ratio and CRP result in a sensitivity of 77% and specificity of 89%. The addition of other biochemical parameters to the prediction formula, e.g. transferrin or TIBC, can improve accuracy further, but only to a marginal extent.

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