Screening for childhood anaemia using copper sulphate densitometry

by

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submitted in partial fulfillment of requirements for the Master of Science (Clinical Epidemiology) degree

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DECLARATION

I declare that the dissertation "Screening for childhood anaemia using copper sulphate densitometry", which I hereby submit in partial fulfillment of the Master of Science (Clinical Epidemiology) degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another University.

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Key words:

Anaemia, child, screening, copper sulphate

SUMMARY

The objective of this study was to evaluate copper sulphate densitometry as a screening method for anaemia in children. The accuracy of copper sulphate densitometry was also compared to clinical assessment for the presence of pallor and haemoglobin measurement with a BMS-haemoglobinometer. Different observers performed these three screening tests independently. For the purposes of this study, anaemia was defined as a laboratory haemoglobin (Hb) concentration below 10 g/dl. A cross-sectional screening study was undertaken, where the results of the different screening tests were compared to laboratory haemoglobin determination (gold standard).

The study sample consisted of one hundred consecutive children, aged between 6 months and 6 years, whose parents had given informed written consent for participation. The study was conducted in the Paediatric Outpatient Department of Pretoria Academic Hospital (73 children) and a local crèche (27 children).

In this study sample, the prevalence of anaemia (Hb < 10 g/dl) was 17% (95% Confidence Interval (95%CI) 10.2; 25.8). Clinical assessment by students for the presence of pallor had a sensitivity of 41.2% (95%CI 19.4; 66.5), specificity of 81.9% (95%CI 71.6; 89.2), positive predictive value of 31.8% (95% CI 14.7; 54.9) and negative predictive value of 87.2%(95%CI 77.2; 93.3). The likelihood ratio for detection of anaemia by clinical assessment was 2.3. Copper sulphate densitometry had a sensitivity of 88.2% (95%CI 62.3; 97.9), specificity of 89.2% (95%CI 79.9; 94.6), positive predictive value of 62.5% (95% CI 40.8; 80.5) and negative predictive value of 97.4% (95%CI 90.0; 99.5) to screen for anaemia. The Likelihood Ratio of a positive copper sulphate-screening test was 8.17. On average, haemoglobin concentration was underestimated by 0.29 g/dl with the BMS-haemoglobinometer, with the 95% limits of agreement ranging from underestimation by 1.3 g/dl to over-estimation by 1.9 g/dl.

Logistic regression analysis revealed that both the copper sulphate test and measurements with the BMS-haemoglobinometer predicted anaemia accurately. The area under the Receiver Operating Characteristic (ROC) curve for the haemoglobinometer was 0.94 (95%CI 0.87; 1), while the area under the curve for copper sulphate densitometry was 0.89 (95% CI 0.73; 1). Used together, the area under the ROC curve was 0.95 (95% CI 0.89; 1). In resource-poor settings, copper sulphate densitometry could be an accurate, inexpensive and simple screening method for anaemia in children.

ABSTRACT

A cross-sectional screening study was undertaken to evaluate copper sulphate densitometry as a screening method for anaemia in children. The accuracy of copper sulphate densitometry was compared to clinical assessment for the presence of pallor and haemoglobin measurements with a BMS-haemoglobinometer. Different observers performed these three screening tests independently. The results of these screening tests were compared against laboratory determination of haemoglobin concentration, the gold standard. Anaemia was defined as a laboratory haemoglobin (Hb) concentration below 10 g/dl.

The study sample consisted of one hundred consecutive children, aged between 6 months and 6 years, whose parents had given informed written consent for participation. The study was conducted in the Paediatric Outpatient Department of Pretoria Academic Hospital (73 children) and a local crèche (27 children).

In this study sample, the prevalence of anaemia (Hb < 10 g/dl) was 17% (95% Confidence Interval (95%CI) 10.2; 25.8). Clinical assessment by senior medical students for the presence of pallor had a sensitivity of 41.2% (95%CI 19.4; 66.5), specificity of 81.9% (95%CI 71.6; 89.2), positive predictive value of 31.8% (95% CI 14.7; 54.9) and negative predictive value of 87.2%(95%CI 77.2; 93.3). The likelihood ratio for detection of anaemia by clinical assessment was 2.3. Copper sulphate densitometry had a sensitivity of 88.2% (95%CI 62.3; 97.9), specificity of 89.2% (95%CI 79.9; 94.6), positive predictive value of 62.5% (95% CI 40.8;

80.5) and negative predictive value of 97.4% (95%CI 90.0; 99.5) to screen for anaemia. The Likelihood Ratio of a positive copper sulphate-screening test was 8.17. On average, haemoglobin concentration was underestimated by 0.29 g/dl with the BMS-haemoglobinometer, with the 95% limits of agreement ranging from underestimation by 1.3 g/dl to over-estimation by 1.9 g/dl.

Logistic regression analysis revealed that both the copper sulphate test and measurements with the BMS-haemoglobinometer predicted anaemia accurately. The area under the Receiver Operating Characteristic (ROC) curve for the haemoglobinometer was 0.94 (95%CI 0.87; 1), while the area under the curve for copper sulphate densitometry was 0.89 (95% CI 0.73; 1). Used together, the area under the ROC curve was 0.95 (95% CI 0.89; 1). In resource-poor settings, copper sulphate densitometry could be an accurate, inexpensive and simple screening method for anaemia in children.

Key words:

Anaemia, child, screening, copper sulphate

ABSTRAK

Kopersulfaat-digtheidsmeting is evalueer as 'n siftingsmetode vir anemie by kinders, deur middel van 'n dwarssnit siftings-studie. Die akkuraatheid van kopersulfaat-digtheidsmeting is vergelyk met kliniese evaluasie vir die teenwoordigheid van bleekheid en hemoglobien bepalings met 'n BMS-hemoglobinometer. Verskillende ondersoekers het die drie siftings-ondersoeke onafhanklik uitgevoer. Die resultate van die siftings-ondersoeke is vergelyk met laboratorium bepaling van hemoglobien konsentrasie, die goud standard. 'n Laboratorium hemoglobien (Hb) konsentrasie van minder as 10 g/dl was aanduidend van anemie.

Die steekproef vir die studie is saamgestel uit 100 opeenvolgende kinders tussen 6 maande en 6 jaar oud met geskrewe, ingeligte toestemming deur ouers vir deelname. Die studie is uitgevoer in die Pediatrie Buitepasiënte Afdeling van die Pretoria Akademiese Hospitaal (73 kinders) en 'n nabygeleë kleuterskool (27 kinders). In hierdie studie was die voorkoms (prevalensie) van anemie 17% (95% vertrouensinterval 10.2; 25.8). Kliniese evaluasie deur senior mediese studente vir die teenwoordigheid van bleekheid het 'n sensitiwiteit van 41.2% (95% vertrouensinterval 19.4; 66.5), spesifisiteit van 81.9% (95% vertrouensinterval 71.6; 89.2), positiewe voorspellingswaarde van 31.8% (95% vertrouensinterval 14.7; 54.9) en negatiewe voorspellingswaarde van 87.2% (95% vertrouensinterval 77.2; 93.3) gehad. Die waarskynlikheidsverhouding ("Likelihood Ratio") om anemie klinies te diagnoseer was 2.3. Kopersulfaat-digtheidsmeting het 'n sensitiwiteit van 88.2% (95% vertrouensinterval 62.3; 97.9), spesifisiteit van 89.2% (95% vertrouensinterval 79.9; 94.6), positiewe voorspellingswaarde van 62.5% (95% vertrouensinterval 40.8; 80.5) en negatiewe voorspellingswaarde van 97.4% (95% vertrouensinterval 90.0; 99.5) gehad as 'n siftingsmetode vir anemie. Die waarskynlikheidsverhouding van 'n positiewe kopersulfaat siftingstoets was 8.17. Die gemiddelde verskil tussen laboratorium hemoglobien bepalings en metings met die BMS-hemoglobienmeter was 'n onderskatting van 0.29 g/dl met die hemoglobinometer. Die 95% verwysingsreikwydte vir die verskil tussen die metodes het varieer van onderskatting met 1.3 g/dl tot oorskatting met 1.9 g/dl met die hemoglobinometer. Met logistiese regressie analise was beide kopersulfaat-digtheidsmeting en metings met die BMS-hemoglobienmeter akkurate voorspellers van anemie. Die oppervlakte onder die "Receiver Operating Characteristic (ROC)"- kurwe vir die hemoglobienmeter was 0.94 (95% vertrouensinterval 0.87;1), terwyl dit 0.89 (95% vertrouensinterval 0.73; 1) vir die kopersulfaat-digtheidsbepaling was. Waar hulpbronne beperk is, kan kopersulfaat digtheidsbepaling aangewend word as 'n akkurate, goedkoop en eenvoudige siftingsmetode vir anemie by kinders.

Sleutelwoorde:

Anemie, kind, sifting, kopersulfaat

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LIST OF ABBREVIATIONS

95%CI	-	95% Confidence Interval
g/dl	-	grams/deciliter
g/l	-	grams/liter
Hb	-	haemoglobin
LR+	-	Likelihood ratio for a positive test (Sensitivity/1-Specificity)
MCHC	-	Mean corpuscular haemaglobin concentration
PPV	-	Positive predictive value
NPV	-	Negative predictive value
SG	-	Specific gravity
ROC Curve	-	Receiver operating characteristic curve

CHAPTER 1

BACKGROUND

Anaemia is the most common haematologic disease of infancy and childhood. Iron deficiency anaemia due to inadequate intake is common, especially between 9 and 24 months of age. [1] It may lead to tiredness, irritability and anorexia, as well as dramatic manifestations such as cardiac failure, breath-holding spells and even stroke. [1,2]
The decreased attention span and learning associated with iron deficiency may have adverse effects on cognitive and psychomotor development. [1,3] Furthermore, appropriate treatment of iron deficiency is effective and safe. It would therefore seem worthwhile to screen for anaemia in young children.

Many health care workers would prefer to perform non-invasive screening for anaemia, especially in young children. The clinical assessment of anaemia is however subjective with wide inter-observer variability. [4] The ideal screening test should be objective and accurate, but also simple, inexpensive and performed on site. A test not requiring the availability of electricity or batteries is preferable, especially in developing countries. [5]

Copper sulphate densitometry has been shown to fulfill all these requirements as a screening test for anaemia in adult populations. [6, 7, 8] It is based on Archimedes' principle of relative densities, which states that an object will sink in a solution of lower specific gravity (SG), or float in a solution of higher SG. The major determinants of SG of blood are haemoglobin concentration and plasma protein concentration. When a drop of blood falls into a solution of copper sulphate, a sac of copper proteinate forms on the surface of the drop, preventing a change in its SG for 5-10 seconds. The momentum of its fall carries the drop 1-2 cm below the surface. During the next 5 seconds, the drop will either rise (indicating a lower SG of the blood compared to the solution), continue to sink (indicating a blood SG higher than that of the solution), or remain stationary for a few seconds and then sink (indicating that the blood SG equals that of the solution).

The copper sulphate test is accurate and reproducible in environmental temperatures ranging between 4°C and 40 °C, provided that evaporation of the solution is avoided. [6] The size of blood droplets does not affect the accuracy of the copper sulphate test, as long as the technique is correct (described in Chapter 3). In addition, quite marked variation in mean corpuscular haemoglobin concentration (MCHC) does not affect the accuracy of the copper sulphate test significantly. [7]

Although the copper sulphate test was originally devised during World War II to determine haemoglobin concentration accurately [6, 7] – using a range of solutions with different specific gravities – the test could be used to detect haemoglobin concentrations below a specific screening level.

To our knowledge, there are no published evaluations of copper sulphate densitometry as a screening test for childhood anaemia. If accurate, copper sulphate densitometry could facilitate earlier diagnosis, investigation and treatment of anaemia in childhood. One could even monitor response to treatment using this test.

CHAPTER 2

OBJECTIVES

The main objective of the study was to determine the accuracy of copper sulphate densitometry as a screening method for anaemia in children between 6 months and 6 years of age. The accuracy of copper sulphate densitometry was compared to clinical assessment for pallor and measurement of haemoglobin (Hb) concentration using a haemoglobinometer. The gold standard for the diagnosis of anaemia was a laboratory-determined Hb concentration below 10 g/dl.

The effects of variations in plasma protein and albumin concentrations on the accuracy of the copper sulphate test were explored.

As a secondary objective, the prevalence and characteristics of anaemia was determined in the study population.

CHAPTER 3

METHODS AND MEASUREMENTS

A cross-sectional screening study was undertaken in the Outpatient department of Pretoria Academic Hospital and a local crèche ("Die Eike"). One hundred consecutive children aged between 6 months and 6 years were studied, after the investigators had obtained informed written consent from their parents for participation in the study. The Research Ethics Committee of the University of Pretoria approved the study.

All children were weighed on a standardized scale in the Outpatient clinic or at the crèche. The length of children below three years of age was measured supine on a measuring board and children older than three years were measured standing up. The investigators also made a clinical nutritional assessment.

A medical student and a registrar independently assessed children included in the study for pallor, based on the colour of the mucosae and nail beds.

Venous blood samples were then collected for full blood count. The haemoglobin (Hb) concentration measured in the laboratory was used as the gold standard to diagnose anaemia. Children with a laboratory

Hb concentration of less than 10 g/dl were classified as anaemic, while those with a Hb concentration of 10 g/dl or more were classified as non-anaemic. Laboratory technicians performed these tests using the ADVIA® 120 Hematology System. The measurement in this system is a modification of the manual cyanmethaemoglobin method, as developed by the International Committee for Standardization in Hematology. Plasma protein and albumin concentrations were also measured, as these contribute to the SG of blood.

At the time of blood collection, a drop of blood was provided for copper sulphate densitometry and another drop was used for haemoglobin measurement with the BMS-haemoglobinometer. The investigators performing these tests were unaware of the clinical assessments, and they were unaware of each other's findings.

A medical student performed the copper sulphate tests using a drop of each child's blood sample, according to the method described by Phillips and Van Slyke [6] (1946). A summary of the method is appended at the end of this chapter. We used a copper sulphate solution with SG 1.048 to screen for Hb concentrations below 10g/dl, in accordance with the nomogram of Van Slyke and Plillips [7] and assuming a plasma protein concentration within the range 60-85 g/l.

Data were recorded and analyzed using the Epi Info and STATA (version 7) statistical software packages. Anaemia was defined as a laboratory Hb concentration below 10 g/dl. The accuracy of clinical assessment of anaemia, as well as copper sulphate densitometry were determined, using laboratory measurements of Hb concentration as the gold standard. Sensitivity, specificity, positive and negative predictive values were calculated, as well as the likelihood ratios for a positive screening test for anaemia.

Plasma protein concentration affects the SG of blood and therefore the accuracy of the copper sulphate test. The associations between false positive test results and low plasma protein concentration (<60 g/l), as well as false negative test results and high plasma protein concentration (> 80 g/l) were analyzed.

For the measurements made with the BMS-haemoglobinometer, a Bland and Altman plot [9] was made (difference vs. mean of laboratory Hb measurements and BMS-haemoglobinometer measurements) to determine the mean difference in measurements between the two methods, as well as the 95% limits of agreement, i.e. the 95% reference range for the difference.

Receiver Operating Characteristic (ROC) Curves were constructed for each of the screening tests for anaemia separately and in combination. The area under the curve, indicating the ability of the test(s) to distinguish between anaemic and non-anaemic children, was also calculated.

Logistic regression models were fitted to find the best combination of variables to explain anaemia in children. The variables that were considered in the logistic regression models were: age, nutritional status (weight for age), clinical assessment of anaemia, copper sulphate densitometry and BMS-haemoglobinometer measurement. Finally, ROC curves were used to identify a subgroup of children at high risk of anaemia, i.e. with a high prevalence of anaemia.

Appendix:

Copper sulphate densitometry method to screen for anaemia

1. Remove stopper of bottle containing 100 ml copper sulphate solution (SG 1.048).
2. Let a drop of blood fall into the solution from a distance of 1 cm above the surface.
3. The drop will sink beneath the surface from the momentum of its fall (first 3 seconds).
4. Observe the behaviour of the drop during the 10 seconds after it has lost momentum:

There are 3 possibilities:

- If the drop rises at all before beginning to sink: Hb < 10 g%
- If the drop remains stationary for a few seconds, and then falls to the bottom: Hb = 10 g%
- If the drop continues to fall: Hb > 10 g%

5. Replace the stopper of the bottle.
6. After 20 drops of blood had been tested, replace the test solution with 100 ml fresh copper sulphate solution.

CHAPTER 4

RESULTS

Seventy-three children were recruited from the Outpatient department and 27 from "Die Eike" crèche. The characteristics of the study population are summarized in Table I. Eleven percent of the study participants were stunted (length for age < 90% of expected), while 6% were wasted (weight for height < 80% of expected).

The prevalence of anaemia (Hb concentration below 10 g/dl) was 17% (95% CI 10.2; 25.8) in the study population, but varied between 7.4% in crèche children and 20.5% in children attending the Outpatient department. These proportions were not statistically significantly different (Fisher exact test). Twenty-five percent of children aged 6–36 months were anaemic. Thirty-one percent of children with a weight for age less than 80% of expected, had laboratory Hb concentrations below 10 g/dl.

Table I * Characteristics of study population

	Outpatient department *n=73*	Crèche *n=27*	Total study population *n=100*
Age (months)	30.9 (17.0)	30.3 (15.9)	30.8 (16.6)
Gender			
Male (%)	38	12	50
Female (%)	35	15	50
Nutritional status (weight for age)			
Normal (%)	59	24	83
Underweight (%)	11	3	14
Marasmus (%)	2	0	2
Overweight for age (%)	1	0	1
Hb (g/dl)	11.18 (1.63)	12.07 (1.15)	11.42 (2.43)
MCV (fl)	77.46 (7.37)	77.61 (5.62)	77.50 (6.90)
Total serum protein (g/l)	72.06 (7.61)	65.70 (4.07)	70.34 (7.38)
Total serum albumin (g/l)	36.47 (5.36)	36.89 (3.96)	36.58 (5.00)
Number with Hb < 10 g/dl	15	2	17

* Data are presented as mean (SD), or as percentage of the total study population

The accuracy of clinical assessment of anaemia is depicted in Tables II and III. Assessment by the medical students had a sensitivity of 41.2% (95%CI 19.4;66.5), specificity of 81.9% (95%CI 71.6; 89.2), positive predictive value of 31.8% (95%CI 14.7; 54.9) and a negative predictive value of 87.2% (95%CI 77.2; 93.3). The assessment by registrars had a sensitivity of 35.3% (95%CI 15.3; 61.4), specificity of 80.7% (95%CI 70.36; 88.3), positive predictive value of 27.3% (95%CI 11.6; 50.4) and negative predictive value of 85.9% (95%CI 75.7; 92.4). The likelihood ratio for detection of anaemia by students was 2.3 and by registrars was 1.8. The observed agreement between students and registrars on the presence or absence of anaemia was 82%. Chance expected agreement between two observers would be 66%. In this study, more than two observers assessed children for anaemia. The Kappa statistic for the case of more than two raters was 0.48. [10] Agreement between observers was therefore moderate (Landis and Koch). [11]

Table II * Accuracy of clinical assessment of anaemia by students

Clinical assessment by medical student	Laboratory haemoglobin concentration (g/dl)		
	Hb < 10 g/dl	Hb ≥ 10 g/dl	Total
Pallor present	7	15	22
Pallor absent	10	68	78
Total	17	83	100

* Data are presented as numbers (n)

Sensitivity=41.2% (95%CI 19.4; 66.5) **PPV=31.8% (95%CI 14.7; 54.9)**

Specificity=81.9% (95%CI 71.6; 89.2) **NPV=87.2% (95%CI 77.2; 93.3)**

Likelihood Ratio (+) = 2.3 **Overall accuracy=75%**

Table III * Accuracy of clinical assessment of anaemia by registrars

Clinical assessment by registrar	Laboratory haemoglobin concentration (g/dl)		
	Hb < 10 g/dl	Hb ≥ 10 g/dl	Total
Pallor present	6	16	22
Pallor absent	11	67	78
Total	17	83	100

* Data are presented as numbers (n)

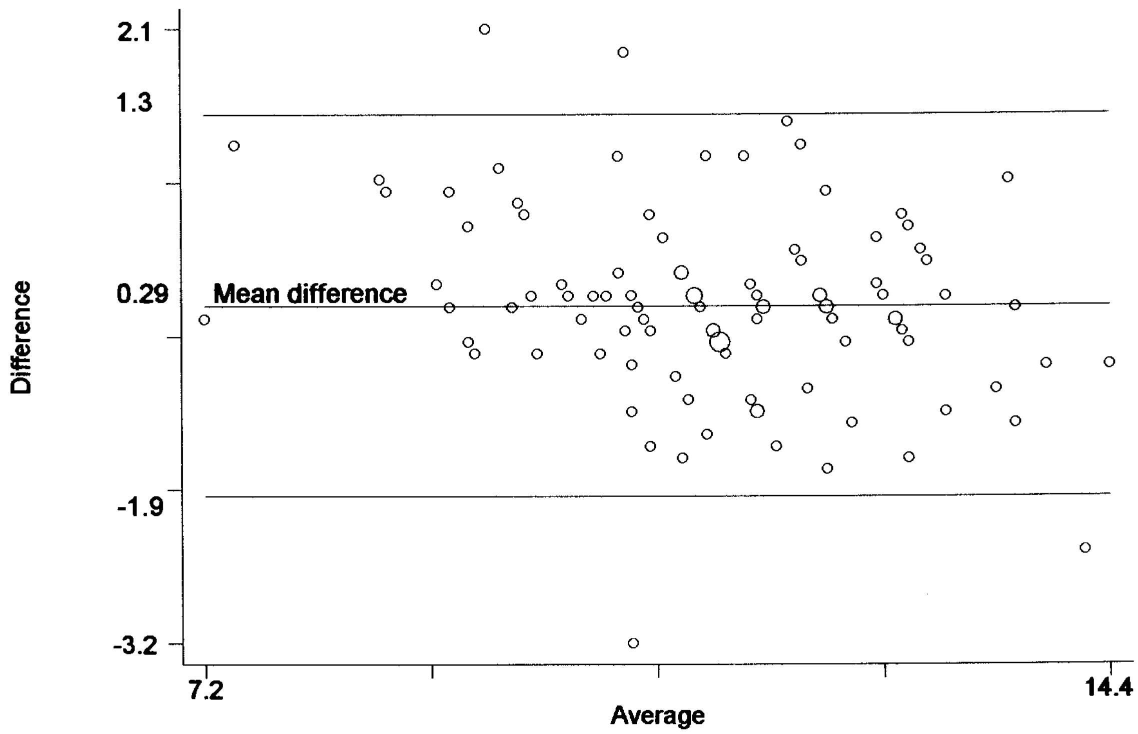
Sensitivity=35.3% (95%CI 15.3; 61.4) **PPV=27.3% (95%CI 11.6; 50.4)**

Specificity=80.7% (95%CI 70.4; 88.3) **NPV=85.9% (95%CI 75.7; 92.4)**

Likelihood Ratio (+) = 1.8 **Overall accuracy=73%**

To assess agreement between laboratory determinations of Hb concentration and Hb measurements with the BMS Hb-meter, a Bland and Altman plot was constructed (Figure 1). The mean difference between laboratory haemoglobin measurements and BMS Hb-meter measurements indicated that Hb concentration was underestimated on average by 0.29 g/dl with the BMS Hb-meter. The 95% limits of agreement (95% reference range for the difference) ranged between –1.9 and +1.3 g/dl. In other words, Hb concentration measurements with the Hb meter ranged between overestimation by 1.9 g/dl and underestimation by 1.3 g/dl.

Fig. 1. Bland and Altman plot of difference vs. average of haemoglobin measurements by the laboratory and the BMS haemoglobinometer



In order to compare the accuracy of screening with the BMS Hb-meter to other methods in this study, the measurements obtained with the BMS Hb-meter were categorized in two groups, with measurements below 10g/dl indicating the anaemic group. This categorized analysis is presented in Table IV.

Table IV[*] Accuracy of Hb-meter measurements to detect anaemia

	Laboratory haemoglobin concentration (g/dl)		
Hb-meter	Hb < 10 g/dl	Hb ≥ 10 g/dl	**Total**
Hb-meter < 10 g/dl	12	2	14
Hb-meter ≥ 10 g/dl	5	81	86
Total	17	83	100

[*] Data are presented as numbers (n)

Sensitivity=70.6% (95%CI 44.0; 88.6)

Specificity=97.6% (95%CI 90.8; 99.6)

Likelihood Ratio (+) = 29.4

PPV=85.7% (95%CI 56.2; 97.5)

NPV=94.2% (95%CI 86.3; 97.8)

Overall accuracy=93%

The results obtained with the copper sulphate test are presented in Table V. Copper sulphate densitometry had a sensitivity of 88.2% (95% CI 62.3; 97.9), specificity of 89.2% (95%CI 79.9; 94.6), positive predictive value of 62.5% (95% CI 40.8; 80.5) and negative predictive value of 97.4% (95%CI 90.0; 99.5) to screen for a Hb concentration below 10 g/dl. The likelihood ratio of a positive screening test was 8.17. Therefore, a child with anaemia had an 8.17 times greater probability of a positive screening test, than a child without anaemia.

Table V * Accuracy of copper sulphate densitometry to detect anaemia

Copper sulphate test	Laboratory haemoglobin concentration (g/dl)		
	Hb < 10 g/dl	Hb ≥ 10 g/dl	**Total**
Drop rose in solution	15	9	24
Drop hovered or sank	2	74	76
Total	17	83	100

* Data are presented as numbers (n)

Sensitivity=88.2% (95%CI 62.3; 97.9)

Specificity=89.2% (95%CI 79.9; 94.6)

Likelihood Ratio (+) = 8.2

PPV=62.5% (95%CI 40.8; 80.5)

NPV=97.4% (95%CI 90.0; 99.5)

Overall accuracy=89%

There were 2 false negative screening tests, both with a laboratory Hb concentration of 9.6 g/dl. One of these children had an elevated total plasma protein concentration of 97 g/l, which probably increased the SG of his blood. The other child's plasma protein concentration was 70 g/l. The haemoglobin concentrations of the 9 children with false positive copper sulphate tests ranged from 10.1 to 11.4 g/dl. Four of these children had Hb concentrations above 10.5 g/dl. Plasma protein concentrations of these 9 children were in the normal range, ranging from 57 to 76 g/l.

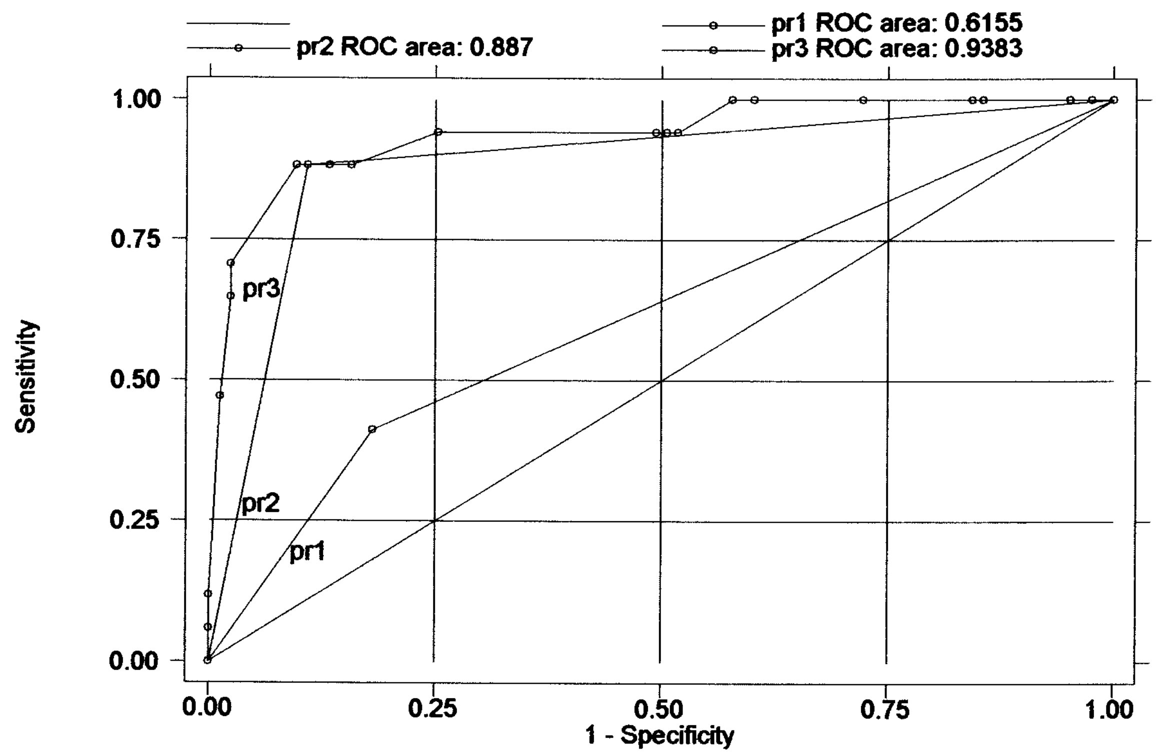
Logistic regression analysis was performed for each explanatory variable individually (Table VI), before more complicated models were constructed to explain the presence of anaemia.

Table VI Logistic regression analysis of individual explanatory variables for anaemia

Variable	Odds Ratio	95% CI	p-value	Area under ROC curve
Age	0.93	0.88; 0.98	0.004	0.74
Weight for age	0.94	0.91; 0.98	0.006	0.75
Student assessment	3.17	1.04; 9.69	0.04	0.62
Registrar assessment	2.28	0.73; 7.10	0.15	0.58
Hb-meter	0.07	0.02; 0.26	<0.001	0.94
Copper sulphate densitometry	61.67	12.09; 314.61	<0.001	0.89

The Receiver Operating Characteristic (ROC) curves for clinical assessment of anaemia by students, Hb measurement with the Hb-meter and copper sulphate densitometry were compared in Figure 2. When these areas were compared, they were statistically significantly different ($\chi^2 = 25.23$; $p<0.001$).

Fig. 2. Receiver Operating Characteristic Curves comparing clinical assessment by students (pr1), copper sulphate densitometry (pr2) and measurements with the Hb-meter (pr3)

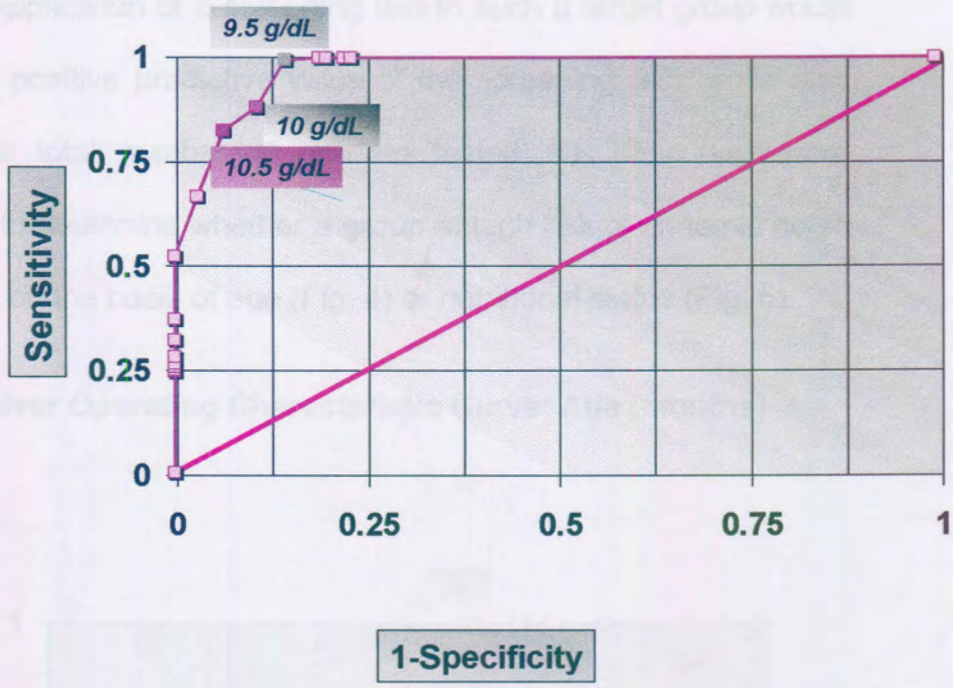


The different variables were then systematically combined to find a reliable model to explain childhood anaemia. Copper sulphate densitometry and measurements with the BMS Hb-meter were included in the final model, as they were the only reliable predictors of anaemia. The goodness-of-fit test indicated adequate fit of the model.

The Receiver Operating Characteristic (ROC) Curve constructed for the copper sulphate test - using a solution of SG 1.048 - had an area of 0.89 (95%CI 0.73; 1) under the curve. This indicated good discriminative ability between anaemic and non-anaemic children. The best trade-off between sensitivity and specificity was at a haemoglobin cut-off value of 10 g/dl (Fig. 3).

The addition of weight-for-age to copper sulphate densitometry improved the area under the ROC curve to 0.91 (95%CI 0.82; 0.99). The combination of copper sulphate densitometry and student assessment for anaemia also had an area under the ROC curve of 0.91 (95%CI 0.86; 0.96). Adding age to the model with copper sulphate densitometry, improved the area under the ROC curve to 0.93 (95%CI 0.87; 0.99). There was however no statistically significant difference in the area under the ROC curves when copper sulphate densitometry alone was compared to these combinations (χ^2=6.59; p=0.16).

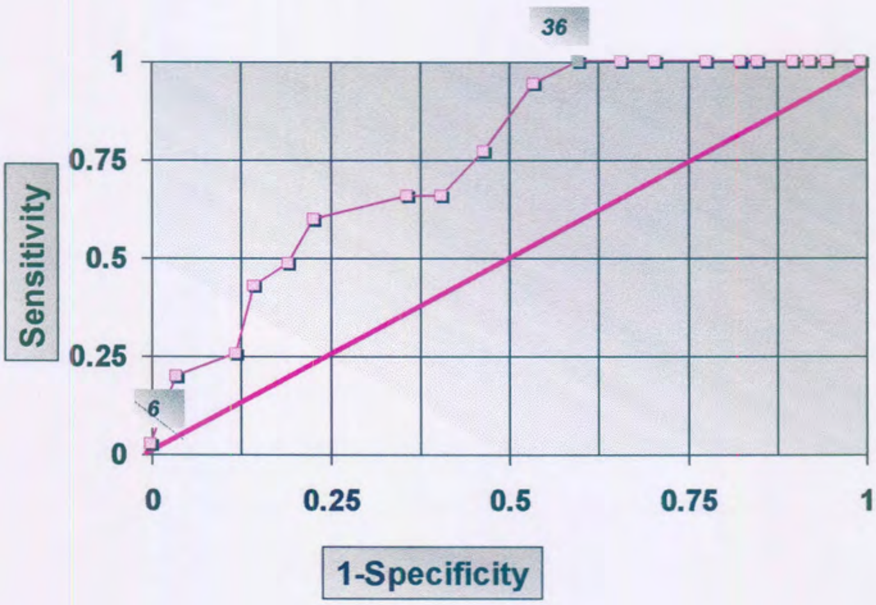
Fig. 3. Receiver Operating Characteristic Curve:
Copper Sulphate Densitometry (SG 1.048)



The area under the ROC curve for the model with the Hb-meter alone was 0.94 (95%CI 0.87; 1); with both the Hb-meter and copper sulphate densitometry it was 0.95 (95% CI 0.89; 1). Comparison of the area under the curve for copper sulphate densitometry, Hb-meter measurement, and a combination of these two methods showed no statistically significant difference (χ^2=5.12; p=0.08).

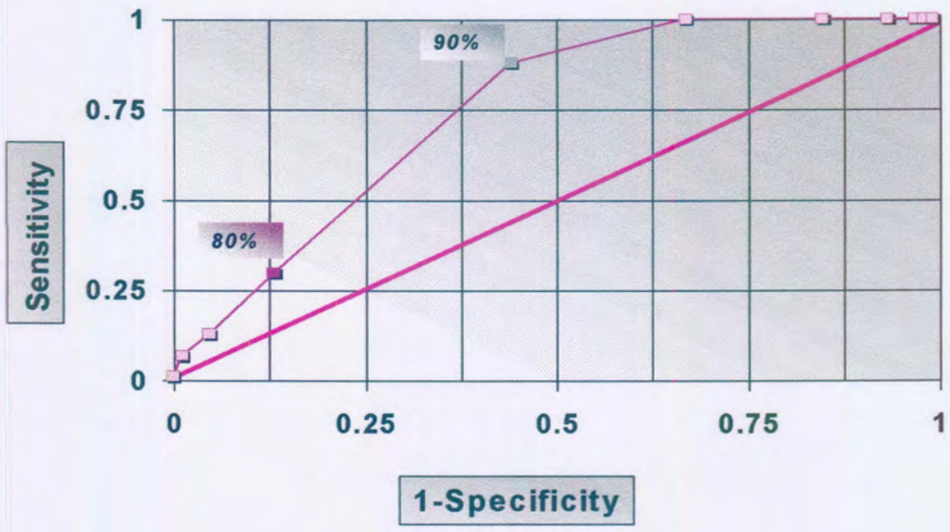
In order to improve the cost-effectiveness of a screening programme, a subgroup of children with a high prevalence of anaemia should be screened. Application of a screening test to such a target group would improve the positive predictive value of the screening test, while also reducing the total number of children tested. ROC curves were constructed to determine whether a group at high risk of anaemia could be identified on the basis of age (Fig. 4) or nutritional status (Fig. 5).

Fig. 4. Receiver Operating Characteristic Curve: Age (months)



Screening only the 67 children aged between 6 and 36 months, all 17 anaemic children would have received screening tests. The prevalence of anaemia in this age group was 25% (95% CI 15.5; 37.5).

Fig.5. Receiver Operating Characteristic Curve: Weight for Age (% of Median)



Thirty-one percent (95% CI 11.0; 58.7), i.e. five of the 16 underweight and marasmic children were anaemic. If only underweight children (weight for age less than 80% of expected) were screened, 71% (95% CI 44.0; 89.7) of all anaemic children would not have been tested.

Screening children between 6 and 36 months of age for anaemia seems a more reasonable strategy to target a high prevalence group and improve the positive predictive value of a screening test for anaemia.

CHAPTER 5

DISCUSSION

Nutritional anaemia fulfills the usual conditions to qualify for screening: the condition must be common, a cheap and reliable screening test must be available, as well as a cost-effective intervention to prevent deterioration and complications. In the present study we set out to evaluate a screening test for anaemia, which could be utilized in a primary care setting.

Suspicion of anaemia is usually based on the clinical sign of pallor. This is known to be a weak clinical sign with low sensitivity. [12] In a study from Lagos [13], the sensitivity of conjunctival pallor to detect Hb concentration below 10 g/dl was 25%, specificity was 89%, positive predictive value was 46.9% and negative predictive value was 75.4% in a population with a 29.9% prevalence of anaemia. We also found the sign of pallor to have a low sensitivity and specificity. More than half of the anaemic children in the present study were considered to be "not pale" by both senior medical students and registrars in Paediatrics. Yet, 25% of children aged less than 36 months had a haemoglobin concentration less than 10 g/dl. Even among the crèche children without medical symptomatology, 2 of 27 children were anaemic. Consequently, mild to moderate anaemia is regularly missed if the haemoglobin is not measured.

Iron deficiency has adverse effects on cognitive function and work energy, apart from its effect on red cells and haemoglobin. The main benefit of early diagnosis lies in the potential for giving appropriate dietary advice and iron supplementation. As iron deficiency is so common, it is acceptable practice to provide short-term empiric iron therapy to young children with moderate, uncomplicated anaemia. Those not responding to this empiric therapy within a short period of time require investigation.

Screening children for anaemia by means of laboratory Hb determination would entail considerable cost. With a bedside haemoglobinometer, the patient's specific haemoglobin value is known within minutes, provided the test is performed accurately. [14] In the present study, the accuracy of the Hb-meter was again confirmed. An initial capital outlay is however required, as well as ongoing costs for haemolysing sticks and batteries. The frustrations of unworkable or incomplete equipment result in the sending of blood samples to the laboratory.

The use of copper sulphate densitometry as a screening test would be much cheaper. At present prices, the cost of one screening test would be 8 cents if 50 tests were done per 100 ml solution as suggested by Phillips et al. [6] In our study, we found that interpretation of the tests became difficult due to murkiness after about 20 drops of blood in 100 ml solution. Replacement of 100 ml of copper sulphate solution after every 20 tests would therefore increase the cost to 19 cents per test. The cost of screening for anaemia using copper sulphate densitometry is then about 2% of the cost of laboratory haemoglobin determination. After initial instruction, the medical students found the test easy to perform. The test results were accurate, simple to interpret and immediately available.

Presently, a fresh 100 ml bottle of copper sulphate solution would cost in the region of R3.89 daily. A single blood drop into the bottle would rapidly and effectively identify those children requiring more careful clinical evaluation. No judgment decisions are necessary on the level of Hb obtained. The specific gravity is set at the level at which action is required: either treatment or referral. In the present study, we chose the solution specific gravity to correspond with a Hb level of 10 g/dl, but any other level could be chosen to link to action policy decisions. In the IMCI programme, such a screening test would also remind the health provider to prescribe an iron tonic and to emphasize the importance of a balanced diet.

A potential disadvantage of copper sulphate densitometry lies in the gradual change of the SG of the copper sulfate solution with repeated blood drops. A fresh 100 ml solution must be available after every 20 patient tests. In practice, this means that a fresh bottle should be available every day. Consequently, the logistics of a steady supply of copper sulphate solution must be worked out for each testing site.

CHAPTER 6

RECOMMENDATION

Copper sulphate densitometry screening for anaemia can be incorporated in the IMCI programme at primary care level. Any child under the age of 36 months attending for incidental health care should be screened. Patients screening positive should receive a month's treatment with iron, and then re-attend for a further screen. Patients still screening positive should be referred for evaluation of a non-responsive anaemia. Children attending for routine well-baby care and immunization at 6 and 9 months of age could similarly be screened.

CHAPTER 7

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