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Diagnosis of Iron Deficiency: Mean Corpuscular Hemoglobin (MCH) as a Predictor of Iron Deficiency in Infants

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Summary

Hematologic variables were measured in 240 apparently healthy infants ranging from 1–12 months of age attending a well baby clinic. There were 20 infants for each month of age. Hematologic parameters were measured in each infant by Coulter Counter Model S. Serum iron, total iron binding capacity, free erythrocyte protoporphyrin (FEP) and serum ferritin levels were measured in most infants. Their weights together with their serum iron, total iron binding capacity, and serum ferritin were judged to be independent variables of iron status, whereas the hematologic variables were considered to be response variables indicative of iron status.

The correlation coefficients among these variables, after excluding redundant variables and transforming to logarithms, were computed. Canonical correlation analysis was applied to the matrix of correlation coefficients to yield the linear function of the independent variables most highly correlated with a linear function of the response variables. The linear function of the response variables was found to be well approximated by the logarithm of the mean corpuscular haemoglobin, which was highly correlated with each of the independent variables.

Abbreviations

FEP, free erythrocyte protoporphyrin
Hb, hemoglogin
HCT, hematocrit
MCH, mean corpuscular hemoglobin
MCHC, mean corpuscular hemoglobin concentration
MCP, mean corpuscular porphyrin
MCPC, mean corpuscular porphyrin concentration
MCV, mean corpuscular volume
RBC, red blood cell
SI, serum iron
TIBC, total iron binding capacity
TP, total porphyrin

Iron deficiency anemia is common during late infancy and in the young child (8). It is important to detect and treat iron deficiency at an early age as the systemic effects may influence the health, growth and development of infants and young children (9, 13).

In iron deficiency the concentration of hemoglobin is determined by the restricted supply of iron. This latter restriction may show a response in the hemoglobin (Hb), total red blood cell count (RBC count), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (1). It may also determine the serum iron (SI), total iron binding capacity (TIBC), iron saturation %, free erythrocyte protoporphyrin (FEP), and serum ferritin levels. There is, however, a considerable degree of overlap between normal and abnormal reference values for these tests in an infant population. Their interpretation during childhood is further compounded by factors such as age and periods of rapid growth. To overcome some of these difficulties, various combinations of tests have been suggested to assist in the diagnosis of mild iron deficiency (3, 7, 10, 12, 13, 14). In our hands they have not proved satisfactory and their cost prohibitive in a developing country.

A statistical exercise was embarked upon to establish the most useful single hematologic parameter for the assessment of the iron status of an infant. It formed part of a study to determine the prevalence of iron deficiency anemia during infancy in a community living in Cape Town.

MATERIALS AND METHODS

Infants. Informed consent and a full history and clinical examination of the infant was obtained in order to see whether the child fulfilled the criteria of the study. Venous blood samples were then collected. Criteria were based on a birthweight of more than 2500 g after a gestational period of more than 37 wk, acceptable developmental milestones and dietary habits, satisfactory gain in weight, and a normal clinical examination. Two hundred and forty apparently healthy infants ranging from 1–12 months of age were studied. There were 20 infants in each month.

The study was approved by the Ethical Review Committee of the Faculty of Medicine of the University of Cape Town. The prevalence of anemia in the community will be reported in a separate publication. The laboratory findings of this study were employed in the present statistical exercise.

Laboratory methods. Hb, RBC count, HCT, MCV, MCH and MCHC were obtained by Coulter Counter Model S.

SI and TIBC were estimated spectrophometrically using the Ferro. Chek II Test (supplied by Hyland, Div. Travenol Laboratories Inc., Costa Weson, CA).

FEP was measured by the fluorometric assay described by Piomelli (11) and Serum Ferrintin by a two-site immunoradiometric assay (4).

Rationale for statistical approach. The iron status of an infant is the net result of iron absorption, utilization, storage, and iron excretion. It may be determined by the measurement of SI and TIBC and serum ferritin, indicative of iron absorption and storage, respectively. The infant's weight will determine its iron requirement. The values obtained from these four variables relate to one another. Their interpretation is difficult but considered jointly, they give some indication of iron status. Changes in their values result in a response in the total RBC count, MCV, MCHC, and FEP levels. The latter, individually or collectively, may be considered as response variables affected by the independent variables and indicative of the iron status of an individual. Hb, HCT, and MCH can be derived from them.

The statistical methods applied seek to find some linear combination of the four independent variables maximally correlated

with some linear combination of the response variables as indicative of iron status in regard to its hematologic response.

Statistical methods. Canonical correlation may be viewed as a multivariate extension of multiple linear regression. In multiple linear regression only one dependent variable is permitted. In canonical correlation a number of dependent variables may be considered collectively.

The data collected were examined by this means using a computer program based on the procedure described by Cooley and Lohnes (2).

Canonical correlation is applied to a matrix of correlation coefficients arising from a set of variables measured on the same subjects. The variables are considered to belong to two subjects: the first, x variables representing the left hand variables and the remaining, y variables the right hand variables.

The analysis seeks to find a linear combination of the left hand variables, which yields maximum correlation, with some linear combination of the right hand variables—the first canonical correlation. The analysis then proceeds to a second and further canonical correlations in decreasing value such that the successive linear combinations of the left hand variables are uncorrelated with other linear combinations of those variables as also are the several linear combinations of the right hand variables.

The analysis is concerned with combinations of variables formed by additions and subtractions. With the data considered here many known relationships are of a multiplicative nature, *e.g.*, the product of RBC count, MCV, and MCHC defines hemoglobin. If the logarithms of the variables replace the variables themselves linearity is ensured for many defined combinations.

In applying the procedure to the present data, the left hand variables are designated independent variables and the right hand variables as response variables.

RESULTS

One hundred and ninety-five (195) of the 240 children who entered the study had measurements for each of the four independent variables, *i.e.*, SI, TIBC, serum ferritin, and weight, and for the response hematologic variables, *i.e.*, RBC count, MCV, MCHC, and FEP. In the remaining 45, one or more variables were missing for technical reasons.

The correlation coefficients among these variables, after transforming to logarithms, are shown in Table 1. Most of the correlation coefficients are significant ($P < 0.05$). Canonical correlation analysis applied to the matrix of correlations of Table 1 reduced the complex of Table 1 to four canonical correlations (Table 2).

The larger of these yielded a value of 0.7366 with $\varkappa^2 = 181.8337$ and 16 degrees of freedom ($P < 0.001$). The second resulted in a value of 0.3779 with $\varkappa^2 = 32.8499$ and 9 degrees of freedom, which is also considered significant ($P < 0.001$). The remaining canonical correlations resulted in $\varkappa^2$ values less than their expected values and may be ignored.

Interest then centered on the larger of the two significant canonical correlations. The corresponding coefficients, applied to the set of response variables, led to the definition of a linear function, Y: $Y = 0.0717y_1 + 0.9728y_2 + 0.2199y_3 - 0.0092y_4$ (1.1), where y_1, y_2, y_3 and y_4 are log (RBC count), log (MCV), log (MCHC), and log (FEP), standardized by measuring them about their respective means and dividing by their respective standard deviations. The corresponding linear function for the independent variables was found to be: $X = -0.2739x_1 + 0.4543x_2 - 0.4170x_3 + 0.2151x_4$ (1.2) where x_1, x_2, x_3, and x_4 are log (weight), log (SI), log (TIBC) and log (serum ferritin), respectively. These also in standardized form. The canonical correlation between X and Y of 0.7366 (Table 2) is the greatest correlation that can be found between any linear combination of the x's and any linear combination of the y's.

A combination of the y variables of the form: $X' = a_1y_1 + a_2y_2 + a_3y_3 + a_4y_4$, where the a's are any arbitrary set of values represents a linear combination. There are clearly an infinite number of these but certain sets of "a" values define quantities which are familiar. Thus if $a_1 = a_2 = a_3 = 1$ and $a_4 = 0$ then X' becomes formally equivalent to log (Hb). Various hematologic variables defined in this manner are shown in Table 3 together with the multiple correlation coefficients obtained by multiple linear regression (5) in which each of the defined hematologic variables was in turn taken as the dependent variable and regressed on the four independent variables. (A canonical correlation where there is only one right hand variable will be equal in value to the multiple correlation coefficient obtained when the right hand variable is regressed on the left hand, *i.e.*, independent variables.)

FEP directly measured is expressed in terms of HCT. It is a measure analogous to MCHC. It might, alternatively, be referred to as mean corpuscular porphyrin concentration (MCPC). In a similar manner (MCV)*(FEP) (ignoring a scaling factor) could be referred to as mean corpuscular porphyrin (MCP) and the product (RBC count)*(MCV)*(FEP) referred to as total prophyrin (TP). As (RBC count)*(MCV) is equivalent to 10*(HCT) the measure (FEP)*(HCT)/100 is a measure of (TP), appropriately scaled. The ratio (TP)/(HB), or equivalently, (FEP)*(HCT)/(100*(HB) has been proposed (10, 14) as a measure of possible utility in the investigation of iron deficiency. It is easy to show that this measure is more simply stated as (FEP)/(MCHC).

The multiple correlation coefficient for log (MCH) was found to be 0.7342 and therefore only marginally less than the maximum value of 0.7366 for the first canonical correlation corresponding to the undefined linear combination of 1.1. Moreover each of the determinate independent variables was found to be significant ($P < 0.01$) as a regressor.

DISCUSSION

The purpose of this paper has been to subsume into a single, coherent system a number of variables, some of which are postulated *a priori* to be determinate of iron status and others responsive

Table 2. *Estimated canonical correlations*

	Canonical correlation	Chi-square	Degrees of freedom
1	0.7366	181.8337	16
2	0.3779	32.8499	9
3	0.1249	3.5005	4
4	0.0515	0.5067	1

Table 1. *Correlation coefficients among the variables*[1]

		1	2	3	4	5	6	7	8
log (weight)	1	1.000							
log (serum iron)	2	−0.245	1.000						
log (total iron binding capacity)	3	0.506	−0.239	1.000					
log (serum ferritin)	4	−0.395	0.312	−0.649	1.000				
log (red blood cell count)	5	0.528	−0.207	0.425	−0.383	1.000			
log (mean corpuscular volume)	6	−0.517	0.475	−0.590	0.519	−0.735	1.000		
log (mean corpuscular hemoglobin concentration)	7	−0.161	0.258	−0.208	0.281	−0.145	0.212	1.000	
log (free erythrocyte protoporphyrin)	8	0.140	−0.322	−0.312	−0.294	0.144	−0.453	−0.335	1.000

[1] Correlation coefficients with absolute values greater than 0.1406 are considered significant ($P < 0.05$).

Table 3. *Linear combinations of the indicator variables with defined equivalents*

	log (RBC count)	log (MCV)	log (MCHC)	log (FEP)	Multiple correlation coefficient
log (hematocrit)	1	1	0	0	0.3897
log (mean corpuscular hemoglobin)	0	1	1	0	0.7342
log (hemoglobin)	1	1	1	0	0.4366
log (mean corpuscular porphyrin)	0	1	0	1	0.2702
log (total porphyrin)	1	1	0	1	0.3732
log (TP/Hb)	0	0	−1	1	0.4287

of iron status insofar as the latter are reflected in hematologic measurements.

The high first canonical correlation suggests the feasibility of expressing the interactions of independent and response variables in this way. The MCH was found to be the best response variable to changes in the independent variables, *i.e.*, SI, TIBC, ferritin, and FEP. MCH is very nearly as well determined in this way as the best possible combination of the hematologic variables corresponding with the first canonical correlation. The independent variables were found to be related to MCH in an intuitively reasonable way in that higher values of serum iron and serum ferritin are associated with higher values of MCH, whereas higher values of weight and TIBC act in the opposite direction. It follows that decreased values of MCH occur with iron deficiency.

Saarinen and Siimes (12) found lower MCH and MCV values in a group of infants studied compared to those who received prolonged iron supplementation, although infants with low ferritin and transferrin saturation or both had been excluded. They concluded that the differences in MCH and MCV values might indicate that these two indices are sensitive indicators of mild iron deficiency. Hershko *et al.* (6) also concluded that MCH was most suitable as an indicator of iron deficiency anemia in a rural population of children aged 1–6 years.

The most reliable criterion of iron deficiency anemia is the hemoglobin response to a one-month therapeutic trial of iron in adequate dosage in an infant with suspected iron deficiency. Compliance in taking the medication correctly, however, is often poor and follow-up of infants difficult and expensive. For these reasons this method cannot be easily applied and alternative methods have to be explored. It is postulated that MCH is the best single hematologic indicator of iron status. It would be necessary to institute studies to establish the usefulness of MCH for the identification of infants who will respond to iron therapy.

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