



Mean Density of Hemoglobin Per Liter of Blood: A New Hematologic Parameter With an Inherent Discriminant Function

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ABSTRACT

This study introduces a new hematologic parameter, the mean density of hemoglobin per liter of blood (MDHL), based on a redefinition of the mean cell hemoglobin concentration (MCHC) as the mean cell hemoglobin density (MCHD). In a retrospective study of 96 well-characterized patients with iron deficiency anemia (IDA; n = 43) and thalassemia trait (TT; n = 53), we show that the MDHL is more effective than 4 other previously described discriminant tests in discriminating between the 2 conditions. It was superior in sensitivity, specificity, and predictive value and has an overall efficacy of 94% when compared with the England and Fraser discriminant function (84%), the discriminant score (82%), and the Mintzer formula (88%). When we excluded 3 patients who had combined IDA and TT, MDHL showed sensitivities, specificities, predictive value, and efficacies of 100%. These findings are highly promising in reducing the costs involved in evaluating patients with hypochromic microcytosis. *Lab Hematol.* 1999;5:149-152.

KEY WORDS: MDHL · MCHD · Microcytic hypochromia · Iron deficiency · Thalassemia

INTRODUCTION

The red blood cell (RBC) indices were first introduced by Wintrobe [1] in 1929. They include the mean cell volume (MCV), mean

cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The MCV describes the average volume of the erythrocytes [2], expressed in femtoliters (fL). MCH describes the amount of hemoglobin contained in the cells [2], expressed in picograms (pg). MCHC is the hemoglobin concentration in the "average" RBC [2], that is, the ratio of weight of hemoglobin to the volume in which it is contained, expressed in grams per liter (g/L) [3].

Wintrobe [3] used the following expression:

hemoglobin (mass)/packed cell volume or PCV (volume)

to describe and calculate the MCHC. The nominator in this expression is the whole blood hemoglobin and the denominator is the PCV. Translating these factors to represent the hemoglobin concentration in the average RBC is not obviously logical, which probably contributes to the difficulty in understanding and teaching the concept of MCHC.

We considered, therefore, that if MCHC is to live up to its name, it would be necessary to either create a new derivation or create a name change. We have elected to do neither, but have considered devising a new RBC index—one that would provide information in a more direct manner on the relation between the amount of hemoglobin and its "container" RBC.

Building on the original thinking of Wintrobe, we felt that a more rational way of expressing the relation between RBC hemoglobin content and its volume should be based on the equation MCH/MCV expressed in pg/fL. However, we have chosen the term MCHD, in which D stands for density.

Building on this revised concept of MCHC–MCHD, we then developed the idea of a new hematologic parameter, which we name the mean density of hemoglobin per liter of blood (MDHL). MDHL is calculated by the formula

$$\text{MCHD (pg/fL)} \times \text{RBC count } (\times 10^{12}/\text{L})$$

and is expressed in g/L.

A new RBC parameter must have not only logical rigor but also clinical usefulness, because the hemogram, as obtained from current automated cell counters, is already very crowded. We elected to assess the usefulness of the new parameter MDHL in the

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evaluation of the common clinical hematologic problem of hypochromic microcytosis: how to discriminate between iron deficiency anemia (IDA) and thalassemia trait (TT). In common clinical practice, differentiation is based on the demonstration of reduced serum iron, increased total iron-binding capacity, and/or reduced serum ferritin (for IDA). For identifying TT or normal iron status, estimation of hemoglobin (eg, by electrophoresis) is required. However, these diagnostic procedures are not inexpensive, especially when, in addition, α -thalassemia trait (α -TT) is considered. In some cases, globin-chain synthesis rate determination or even α -globin gene analysis may be required.

To measure the efficacy of MDHL, we challenged it against several formulas that have been proposed to differentiate between IDA and TT. First, England and Fraser [4,5] introduced a linear discriminant function (DF) derived from the MCV, RBC count (RBCC), and hemoglobin (Hb) concentration. DF was of the form $MCV - RBCC - (5 \times Hb) - k$, where k is a constant determined by the method used to calibrate the cell counter. Positive DF values indicate IDA and negative values indicate TT. Mentzer [6] suggested that an even simpler index, MCV/RBC , was equally useful for distinguishing the 2 conditions, with values below 13 indicating TT. Shine and Lal [7] used $(MCV)^2 \times MCH$ in screening for TT, with values of less than 1530 being regarded as diagnostic of the condition.

Another formula is the discrimination score (DS), [8] based on the following equation:

$$(0.096 \times MCV) + (0.415 \times RDW) - (0.139 \times RBC) - 12.722 \text{ for men,} \\ \text{and } (0.096 \times MCV) + (0.415 \times RDW) - 12.722 \text{ for women,}$$

where RDW indicates red cell distribution width, with 0.3095 as a cutoff point, below which TT is probable and above which IDA is likely. Unfortunately, all of these approaches have significant limitations. First, their incorporation in the complete blood cell (CBC) hemogram is impractical because the mathematical procedures are elaborate. Second, their sensitivity and specificity are not high, resulting in considerable imprecision in discriminating between IDA and TT.

MATERIALS AND METHODS

The patients, retrospectively selected randomly, were older than 12 years with microcytic hypochromic blood picture $Hb >70$ g/L and $MCV <80$ fL. CBC counts of patients and healthy subjects were performed on an STKS automated cell counter (Coulter Electronics, Hialeah, FL). Quantification of serum iron was done on a Hitachi 917 clinical chemistry analyzer (Boehringer Mannheim, Gaithersburg, MD) using the ferrozine method, and ferritin was assayed by chemiluminescence sandwich assay on the ACS:180 (Ciba-Corning), with a reference range of 23-322 $\mu\text{mol/L}$ in men and 10-291 $\mu\text{mol/L}$ in women. Transferrin was measured turbidimetrically on a Cobas Mira Plus/Unimate 3 system (Roche Diagnostics, Somerville, NJ), and the iron-binding capacity was calculated from the transferrin value (by multiplying $\text{g/L} \times 24$), with reference range of 53-92 $\mu\text{mol/L}$. Hemoglobin analysis was performed by hemoglobin electrophoresis on cellulose acetate membrane at pH of 8.4. $Hb-A_2$ quantification was performed by ion exchange column chromatography, using the Beta-Thal Quick column (Helena Laboratories, Beaumont, TX). Hemoglobin F was determined by radial immunodiffusion method using a kit manufactured by Helena Laboratories.

TABLE 1. Normal Mean Cell Hemoglobin Density (MCHD) and Mean Density of Hemoglobin Per Liter of Blood (MDHL)

	Male	Female
MCHD		
Median	0.339	0.339
Mean (+SD)	0.340 (± 0.006)	0.339 (± 0.006)
MDHL		
Median	1.73	1.5
Mean (+SD)	1.75 (± 0.12)	1.50 (± 0.11)

Diagnosis of β -TT was based on demonstrating an $Hb-A_2$ level higher than 3.7%, which is the upper limit of normal in this laboratory. The diagnosis of α -TT was based on demonstrating a normal $Hb-A_2$ level or one below 2.2%, which is our lower limit of normal, with or without HbH inclusions in RBC. IDA was defined in patients with serum iron less than 6, total iron-binding capacity higher than 92, and serum ferritin less than 22 for men and less than 10 for women. Data for the normal reference range for peripheral blood CBC were obtained from a study by Al-Buhairan [9] performed on the same instrument in our laboratory.

For each set of patient and control data, MCHD was calculated from the relation MCH/MCV , and MDHL from the relation $MCHD \times RBC$ count, as previously described. The mean, median, and SD of MCHD and MDHL were then calculated. The previously described DFs were also compared with the new MDHL parameter for efficacy in discriminating between IDA and TT. We followed the recommended cutoff points for the various discriminant procedures as follows: For DF, positive values indicate IDA, whereas negative values indicate thalassemia. For the $(MCV)^2 \times MCH$ formula, values <1530 indicate TT. For the MCV/RBC ratio, values >13 indicate IDA, whereas those <13 indicate TT. For the DS, values >0.3095 indicate IDA and those <0.3095 indicate TT.

Sensitivity and specificity were calculated according to standard formulas, namely: Sensitivity = $(TP) \div (TP + FN)$ and specificity = $(TN) \div (TN + FP)$, where TP = true positives, FN = false-negatives, TN = true negatives, and FP = false-positives [10]. The predictive values (PV), whether positive (+) or negative (-), were similarly calculated, with +PV being $(TP) \div (TP + FP)$ and -PV being $(TN) \div (TN + FN)$. Finally, the efficacy was calculated as follows:

$$(TP + TN) \div (TP + TN + FP + FN).$$

RESULTS

Using data from the study of Al-Buhairan [9], we determined the mean of MDHL of healthy subjects as 1.75 (± 0.24) and a median of 1.73 for men and a mean of 1.5 (± 0.21) and median of 1.5 for women (Table 1). Our study population consisted of 96 adult patients (49 females). Using criteria outlined in Materials and Methods, there were 43 patients with IDA and 53 patients with TT (Table 2). Of the 53 with TT, 35 had β -TT. The remaining 18 had α -TT.

We computed the mean, median, and SD of MDHL, which showed that patients with values equal to or below the mean of healthy subjects were most likely to have IDA (+PV of 84%), and

TABLE 2. Distribution of 96 Cases of Iron Deficiency Anemia and Thalassemia*

	IDA	TT	Total
Male	16 (1) [†]	31	47
Female	27	22 (2) [‡]	49
Total	43	53	96

*IDA indicates iron deficiency anemia; TT, thalassemia trait.

[†]One patient with associated TT.

[‡]Two patients with associated IDA.

those whose MDHL value was greater than the mean were more likely to have TT (+PV of 100%) (Figure 1). When compared with 4 previously reported discriminant formulas, the MDHL showed the highest efficacy in the diagnosis of IDA and TT compared with the others. These comparisons are summarized in Table 3. Although we included the $(MCV)^2 \times MCH$ formula, it is not strictly comparable to the others, because it is, as originally described, intended for identifying thalassemia patients only.

DISCUSSION

We have introduced two new RBC parameters, MCHD and MDHL, to rationalize the concepts of hemoglobin distribution within the average RBC and in blood. MCHD is a more direct way of assessing the amount of hemoglobin in the average RBC, because it indicates how much hemoglobin is contained within the RBC in relation to its volume. MDHL, derived from MCHD, gives an estimate of the hemoglobin density per liter of blood.

How does MCHD differ from MCHC? Apart from the name, by which MCHD more correctly describes the concept without confusion, MCHD differs also by the scale of the unit of measure (a factor of 10^{-12}). Moreover, the expression of MCHD in pg/fL appears much more in line with the other RBC indices, MCV and MCH, and also harmonizes well with MDHL.

The use of the term “density” should not be equated with light scattering by the individual RBC. Light-scatter methods, using laser technique, for example, can determine the hemoglobin con-

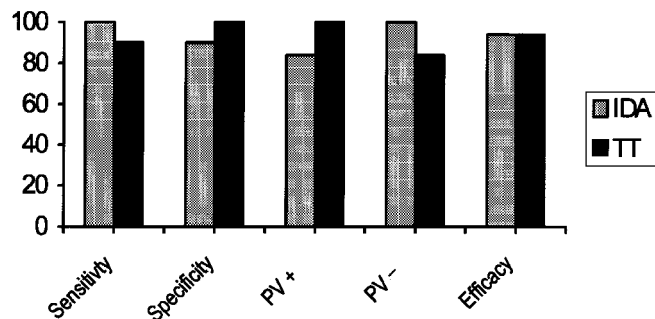


FIGURE 1. Efficacy of MDHL in discriminating between IDA and TT. PV+ indicates positive predictive value; PV-, negative predictive value; IDA, iron deficiency anemia; T, thalassemia trait.

tent of individual RBC accurately, but the information derived has not been applied to the problem of distinguishing between IDA and TT [11]. Likewise, RBC density, with which MDHL is not to be confused, deals with the density of the RBC, as estimated by buoyancy gradient techniques, for example. Significant increases in RBC density are seen in sickle cell syndromes and Hb CC [12], but, again, this parameter has not been applied to distinguish between IDA and TT.

We next assessed the clinical validity of our new parameters in differentiating between IDA and TT without resorting to expensive testing. These entities can sometimes exhibit the same degree of anemia, microcytosis, and hypochromasia, but in only a limited number of cases are CBC parameters helpful in distinguishing between the 2 conditions. For this differentiation exercise, MCV, MCH, and MCHC are most frequently used. However, none is sensitive enough to enable a clear distinction between the 2 types of hypochromic microcytosis, although it is usually claimed that MCHC is more frequently below normal in IDA. Because of this inability of the routine indices to differentiate, formulas have been devised to achieve this objective. Some of these formulas are complicated, however, and not precise enough to be useful in a significant minority of cases.

Using MDHL in this series of 96 patients, we show that those with IDA have an MDHL lower than or equal to the mean

TABLE 3. Comparison Between the New MDHL Parameter and the 4 Reported Formulas*

	Sensitivity (%)		Specificity (%)		PV+ (%)		PV- (%)		Efficacy (%)	
	IDA	TT	IDA	TT	IDA	TT	IDA	TT	IDA	TT
MDHL	100	90	90	100	84	100	100	84	94	94
Formula 1	—	100	—	7.5	—	56.9	—	100	—	58.3
Formula 2	95.3	83	83	95.3	82	95.6	95.6	82	88.5	88.5
DS	81.4	83	83	81.4	79.5	86.6	84	79.5	82.3	82.3
DF	97.7	73.6	73.6	97.7	75	97.5	97.5	75	84.4	84.4

Formula 1: $MCV^2 \times MCH$ [8]; Formula 2: MCV/RBC [7].

*DF indicates discrimination function; DS, discrimination score; IDA, iron deficiency anemia; MCH, mean cell hemoglobin; MCV mean cell volume; MDHL, mean density of hemoglobin per liter of blood; PV, predictive value; RBC, red blood cell; TT, thalassemia trait.

MDHL in a normal reference population, whereas patients with TT have an MDHL higher than the mean. For discrimination between IDA and TT, we evaluated MDHL for sensitivity, specificity, predictive value, and efficacy, compared with the previously reported formulas (Figure 1). As shown in Table 3, sensitivity, specificity, predictive value, and efficacy were superior to those obtained with the other formulas. Seven female patients who had IDA and were under specific therapy were correctly classified by a low MDHL. What is extraordinary about MDHL is that when we exclude 3 patients (2 women and 1 man) who had combined IDA and β -TT (IDA prevailing in 2 patients and TT in the other patient), the sensitivities, specificities (negative and a positive PV) reached 100%. This discriminating ability holds whether β -TT or α -TT is considered.

An intriguing observation is that, although the mean MDHL provided a clear discriminator between IDA and TT, 50% of the healthy subjects must, by definition, have a lower MDHL, whereas the other 50% must have a higher MDHL. Thus, MDHL is of no apparent value in subjects with normal MCV and MCH. But, when appropriately applied, MDHL provides an extremely powerful screening tool for discriminating between IDA and TT. Programming new or even present-generation counters would be a relatively simple matter that could result in considerable savings in the general practice of hematology.

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