

## Screening for iron deficiency anemia in one-year-old infants: Hemoglobin alone or hemoglobin and mean corpuscular volume as predictors of response to iron treatment

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Initial screening for anemia was performed on a capillary blood sample in 800 apparently healthy one-year-old white infants. Twenty-six percent had a Hgb or MCV below the estimated tenth percentile of normal and were asked to return for blood counts on a venous sample. They were then started on a three-month course of oral iron. Of the 151 infants who satisfactorily completed the treatment regimen, 54 had a  $\geq 1$  gm/dl rise in venous Hgb (2.8 infants treated/response). If confirmation of the capillary by the venous values had been a prerequisite for treatment, the size of the treatment group would have decreased from 151 to 87, the response rate would have been improved to 2.0 infants treated per response, but 11 of the infants who had a  $\geq 1$  gm/dl response would have been missed. Since toxicity from iron therapy is rare, either the use of capillary blood counts alone or in combination with venous values constitutes a satisfactory basis for a therapeutic trial of iron in similar populations. Further evaluation can then be reserved for the small number whose Hgb remains below the lower limit of normal after treatment.

Jerry D. Reeves, M.D.,\* David A. Driggers, M.D., Travis Air Force Base, Calif.,  
Edward Y. T. Lo, M.S., and Peter R. Dallman, M.D., San Francisco, Calif.

THE GOAL of screening for iron deficiency is to identify as many individuals who will have an increase in hemoglobin in response to iron treatment as possible, while minimizing the number of unresponsive infants who may be treated unnecessarily. Since iron deficiency is also characterized by a low mean corpuscular volume, the use of both tests may be helpful when an electronic counter is available.

Traditionally, treatment and further evaluation for anemia has been reserved for individuals with hemoglobin values that are below the "normal" range. The common convention is to use the 95% range of a healthy

reference population in which there is no clinical or laboratory evidence of abnormalities such as iron deficiency, thalassemia minor, or infection. For one-year-old infants, the lower limit of the 95% range is estimated to be 11 gm/dl for Hgb.<sup>1,2</sup> However, some individuals who have an Hgb concentration that is within the lower portion of the normal range may also be iron responsive.<sup>3,4</sup> Such individuals, whose Hgb values are below their potential level, would ordinarily be recognized only after treatment with iron.

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| Abbreviations used<br>Hgb: hemoglobin<br>MCV: mean corpuscular volume |
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From Department of Pediatrics, David Grant Medical Center, Travis Air Force Base, and the Department of Pediatrics, University of California, San Francisco.

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\*Reprint address: Department of Pediatrics, David Grant Medical Center, Travis Air Force Base, CA 94535.

The purpose of this prospective study was to determine the response to oral iron treatment in apparently healthy one-year-old white infants whose capillary Hgb or MCV values were below the estimated tenth percentile for iron-sufficient infants.<sup>5</sup> This report deals with the first 18

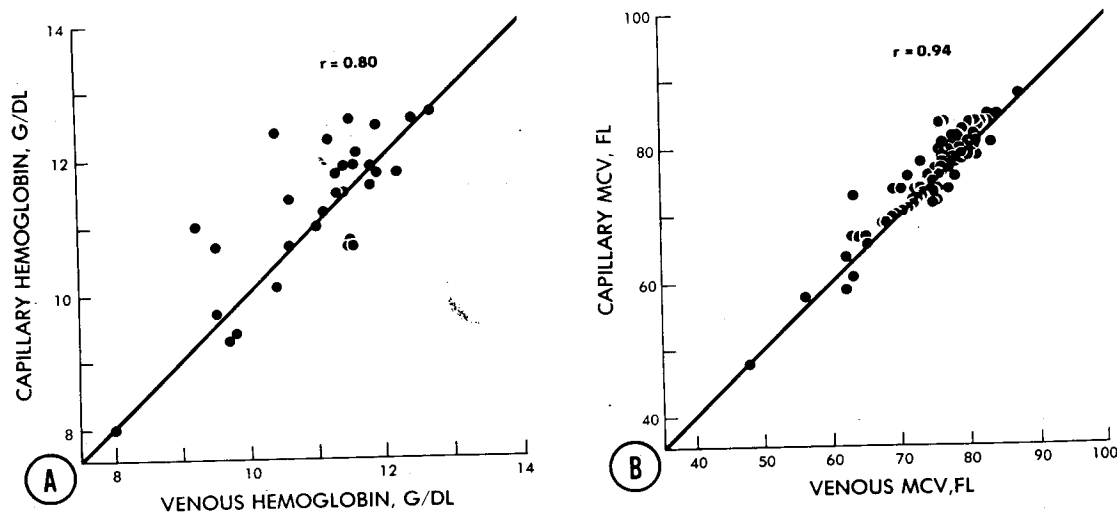


Fig. 1. A, Correspondence between capillary and pretreatment venous Hgb in infants selected for treatment on the basis of MCV. Capillary values are on the vertical axis and venous values on the horizontal. The diagonal line represents identical results. B, Correspondence between capillary and pretreatment venous MCV in infants selected for treatment on the basis of hemoglobin. Capillary values are on the vertical axis and venous values on the horizontal. The diagonal line represents identical results.

months of a four-year study to evaluate laboratory tests and criteria used to predict a hemoglobin response to iron treatment in one-year-old infants.

#### MATERIALS AND METHODS

**Experimental design.** Between March, 1978, and September, 1980, 1,128 apparently healthy one-year-old infants whose racial background was recorded had hematologic analyses performed by a Coulter Model S electronic counter during their routine well-baby visit at Travis Air Force Base, California. This report includes only the data from 800 white subjects because of the possibility that Hgb in blacks is somewhat lower and might require different criteria for therapy.<sup>6-9</sup>

Infants who had an initial capillary Hgb concentration < 11.5 gm/dl or an MCV < 72 fl (Coulter Model S) were requested to return for these laboratory studies to be repeated on venous blood. All of these infants were treated with 3 mg/kg/day of elemental iron as ferrous sulfate (Fer-in-sol, Mead Johnson Laboratories, Evansville, Ind.) orally 30 minutes before breakfast for three months. This dose is recommended for infants and children<sup>9</sup> and is roughly equivalent to that shown to result in an optimal hemoglobin response in the adult.<sup>10</sup> Following treatment with iron, the laboratory tests, including quantitative hemoglobin A<sub>2</sub>, were repeated on a venous blood sample. Compliance was estimated by the amount of medication remaining in the bottle.

**Analysis of results.** An important feature of the experimental design was the use of the initial capillary Hgb as a

basis for starting treatment with iron, whereas the pretreatment venous Hgb constituted the baseline for estimating the change in Hgb during the course of therapy. This design is one method for minimizing errors in interpretation due to a statistical phenomenon termed regression to the mean<sup>11</sup>; i.e., if any population is singled out because of a low or high laboratory value, simply repeating the laboratory test will result in an average value that is closer to the normal mean for that test. This is related to a composite of random factors such as sampling error, laboratory error, and biologic variations that could have been partly responsible for the initial outlying value. Thus, in our design, the pretreatment venous Hgb represents a more reliable baseline than the prior capillary Hgb for estimating a real change in Hgb concentration, even though the capillary value was the basis for treatment.

We estimated that an increase of more than 0.6 gm/dl in venous hemoglobin is statistically significant and should exclude 95% of the differences caused by analytic factors and biologic variations, because 0.5 gm/dl is about twice the estimated variance for sequential analyses of venous Hgb,<sup>12</sup> and the normal developmental increase in Hgb between 12 and 15 months of age is about 0.05 gm/dl.<sup>3</sup> However, an Hgb increase of  $\geq 1.0$  gm/dl was arbitrarily termed a response because we believed it was of sufficient magnitude and physiologic relevance to warrant intervention with iron therapy in a clinical setting. For purposes of comparing various screening regimens, we considered the total response group to represent 100%. However, we recognize the likelihood

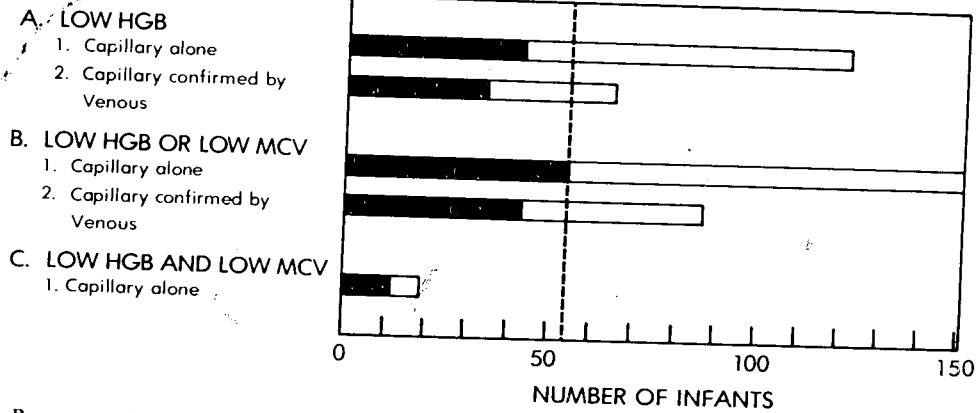


Fig. 2. Response to iron treatment in relation to initial Hgb and MCV. Low values are considered to be Hgb < 11.5 gm/dl and/or MCV < 72 fl. The entire length of the column represents the total number infants meeting each of the laboratory criteria and the dark portion of the column indicates the number of infants with a  $\geq 1$  gm/dl rise in venous Hgb after oral treatment with iron. The vertical dashed line represents the total of 54 infants who had a  $\geq 1$  gm/dl rise in venous Hgb.

that a few potential responders were missed by having neither a capillary Hgb < 11.5 gm/dl nor MCV < 72 fl.

**Methods.** Hgb and MCV were measured on the Coulter Model S electronic counter using the 4 C tripack quality controls with each set of assays. Hemoglobin A<sub>2</sub> was quantified by microcolumn.<sup>13</sup>

## RESULTS

**Findings in the group as a whole.** Of the 800 infants studied, 174 (22%) had an Hgb < 11.5 gm/dl and 66 (8%) had an MCV < 72 fl. Anemia was almost always mild. Only nine infants (1.1%) had an Hgb < 10.0 gm/dl and 71 (9%) had an Hgb < 11.0 gm/dl. The group of 209 infants who had Hgb < 11.5 or MCV < 72 or both represented the infants who qualified for repeat blood studies and treatment with iron; 151 of these infants satisfactorily completed the regimen by having both pre- and post-treatment venipunctures and better than 50% compliance with the iron therapy. Only three infants had gastrointestinal symptoms requiring cessation of therapy. Of the 58 who did not complete the regimen, we estimate that 21 were unavailable because of transfer to other bases; 14 failed to keep one or both appointments; eight had been given insufficient iron medication to be considered in compliance; and 15 had not had sufficient time to complete the iron treatment portion of the regimen.

**Correspondence between capillary and venous Hgb and MCV.** The correspondence between the results obtained on the pretreatment capillary and venous samples is shown in Fig. 1. Only Hgb data from infants selected on the basis of MCV were analyzed; similarly, MCV values were compared only in infants selected on the basis of

Hgb, so as to decrease bias toward higher venous values due to regression to the mean for the low capillary value. As might be anticipated, there was a much closer agreement among the MCV values ( $r = 0.94$ ) than among Hgb values ( $r = 0.80$ ), since measurement of red cell size is independent of sampling or dilution error and should not be subject to much day-to-day variation.

**Comparison of treatment criteria.** We next analyzed our data to determine how the criteria for treatment could be improved by using capillary values alone or in combination with venous values for Hgb and MCV. Such improvements would require including most of the response group while decreasing the total number of infants treated and the number treated per response. In addition, treatment criteria requiring a venipuncture would have to have a clear advantage to justify using this procedure, which is difficult in one-year-old infants.

**Therapeutic trial based on Hgb only.** Using the capillary values alone, of the 151 infants who had either Hgb < 11.5 gm/dl or MCV < 72 fl, 122 would have been selected on only the basis of Hgb < 11.5 gm/dl, and 43 of the 122 would have had a  $\geq 1$  gm/dl rise in venous Hgb. Had only the group of 122 been treated, the number of infants treated per response would have been 2.8 (Fig. 2). The low capillary hemoglobin concentration was confirmed by a low venous Hgb on only 65 of the 122 infants. However, this group of 65 included 34 responders; thus, the therapeutic ratio would have been markedly improved and only nine responders would have been missed by treating only this group (1.9 infants treated/response).

**Therapeutic trial based on low Hgb or MCV.** The total group of 151 infants who completed the regimen met the criterion of a capillary Hgb < 11.5 gm/dl or MCV < 72

f1 (Fig. 2). Fifty-four of these infants responded and represented the total response group (2.8 treated/response). This therapeutic ratio is the same as that in the group of infants who would have been treated on the basis of capillary Hgb alone, but it also includes 11 responding infants who were identified only on the basis of a low capillary MCV.

In 87 infants the initial capillary finding of Hgb < 11.5 gm/dl or MCV < 72 fl was confirmed by meeting one of these criteria on a venous sample. Using this combination as a criterion for treatment would have resulted in an improved therapeutic ratio, treating 2.0 infants per response and including 80% of the total  $\geq 1$  gm/dl response group.

*Therapeutic trial based on low Hgb and MCV.* Of the 19 infants who completed the regimen and who met both Hgb < 11.5 gm/dl and MCV < 72 fl criteria, 12 had a response. Although only 1.6 infants in this group of 19 would have had to be treated per response, less than a third of the total response group would have been included. Thus, by requiring that both Hgb and MCV criteria be met, the disadvantage of missing most responders outweighed the advantage of a high response rate.

*Thalassemia minor.* Thalassemia minor, like iron deficiency, may be associated with mild anemia and microcytosis, but its prevalence was low in our population, and it did not pose as much of a problem in differential diagnosis as we anticipated from previous experience with a different population.<sup>14</sup> Of the 151 infants treated, only six had an elevated Hgb A<sub>2</sub>  $\geq 4.0\%$  after treatment; two of these also had a response to treatment (presumably having had both iron deficiency and thalassemia minor). However, of the four who had no response, only one had pre- or post-treatment venous values below 11 gm/dl for hemoglobin or 70 fl for MCV.

*Other causes of anemia.* Other causes of anemia were rare in our population. Only nine of the 151 infants in the treatment group had a Hgb < 11 gm/dl after treatment. Unexplained microcytosis (MCV < 70 fl) was present in 16 infants after treatment. Ten of these were in the  $\geq 1$  gm/dl Hgb response group and seven of the ten had a marked increase in the MCV values, suggesting that their response to iron therapy was still incomplete.

## DISCUSSION

Our experimental design was intended to approximate routine methods of screening for anemia. Blood was initially obtained by fingerstick, despite the likelihood of greater sampling error, because it is technically much easier than a venipuncture in one-year-old infants. If the initial Hgb, hematocrit, or MCV value is below the reference range, the options include a therapeutic trial

with iron and/or further laboratory studies, often requiring venipuncture.

If the Hgb concentration is determined by electronic counter, the MCV is also obtained, often at no extra cost. The use of a capillary Hgb < 11.5 gm/dl or MCV < 72 fl as a treatment criterion increased the number of responders over that detected by Hgb < 11.5 gm/dl alone. Requiring that both criteria be met was an excellent predictor of response but included far too small a percentage of the responders to be an appropriate alternative.

Confirmation of suspect capillary results by obtaining venous blood had some advantage in decreasing the size of the treatment group and the number of infants treated per response, while including most responders. Whether these advantages outweigh the inconvenience of requiring a second visit and venipuncture in about a quarter of the infants will depend on the clinical setting.

The poor agreement between capillary and venous hemoglobin values helps to explain why results of treatment could be better predicted by the use of both values. Capillary sampling appears to be inherently less reproducible than venous sampling despite careful attention to warming the extremity and avoidance of squeezing the finger (for one thing, the dilution procedure for the Coulter count is more difficult). In addition, it is possible that there is a consistent discrepancy in Hgb concentration between the two types of samples. One-year-old infants are reported to have a higher venous than capillary Hgb values,<sup>15</sup> but we were unable to substantiate this finding in the subpopulation of infants who had a venous sample performed on the basis of their initial MCV. The extent of disagreement between capillary and venous Hgb values was much greater than would have been expected from the results of sequential venous Hgb determinations in adults<sup>12</sup> or from the coefficient of variation of 1.0% for the Hgb method in our laboratory. Thus, uncertainty about the reproducibility of the capillary Hgb seems a factor in favor of initial venous sampling in the screening for anemia, particularly as it becomes technically easier after infancy. In addition, the tendency for venous Hgb values to be higher than capillary values can be partly explained by the phenomenon of regression to the mean. This is an additional factor accounting for the markedly improved prediction of response when the capillary Hgb of < 11.5 gm/DL was confirmed in the venous blood sample.

The predictive value of any screening criterion depends to a very large extent on the prevalence of the abnormality. In populations in which iron deficiency anemia is rare, we would anticipate having to treat too many normal infants for each response if treatment criteria included the lower part of the normal reference range. For example, if

the criterion for iron treatment included all infants below the tenth percentile for hemoglobin reference values, no response could be expected in the 10% of the iron-sufficient population that would be treated. If only 1% of the infants actually had an iron-responsive anemia, it would mean treating about ten infants to obtain a single response. In such populations, a better prediction of response would be expected by decreasing the cutoff values to the lower limits of the 95% reference range (an Hgb of 11 gm/dl and an MCV of 70 fl). Logistic considerations also affect the applicability of our results to other settings. Whether a hemoglobinometer is more available than an electronic counter and the difficulty of performing venipunctures in infants are important factors in influencing selection of a screening procedure. In some clinical settings, the simple procedure of a therapeutic trial of iron after capillary testing of hemoglobin may be the most appropriate routine despite a relatively low therapeutic ratio, since oral iron is well tolerated by most infants.

Assuming that the infants from a nonindigent population at Travis Air Force Base are representative of middle-income populations elsewhere, we believe that several acceptable alternatives in screening for anemia are associated with a reasonable rate of response and identify most potential responders. These regimens, based on routine blood counts and therapeutic trial, are both economical and appealing in their simplicity. Further work-up could be restricted to those rare infants who have residual anemia and/or microcytosis after an adequate trial of therapy.

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