
**Alpha-thalassemia should be considered
in the differential diagnosis
of a child with anemia**

Dear Editor,

We have read with special interest the article recently published in this journal, in which the authors analyzed the relative contribution of iron deficiency to the prevalence of anemia in a nested cohort of children from Rio Grande do Sul, the southernmost state of Brazil, at two assessment points, at age 12-16 months and later at age 3-4 years.¹

We congratulate the authors on the quality of the article and for providing information for better understanding an issue often seen in pediatric practice: asymptomatic children, older than 2 years, not rarely present with clinical features of mild anemia, including mild microcytosis and/or hypochromia, or only with abnormal red blood cell (RBC) counts, without anemia, detected in routine blood count. "Normal" results in tests assessing iron kinetics or even failure of iron supplementation therapy lead the pediatrician to consider other causes to explain this clinical picture.

In the article, the authors found, as expected, that 95% of anemia cases in infants (hemoglobin < 11 g/dL) were associated with iron deficiency (ferritin < 15 µg/L). At age 3-4 years, this proportion was only 19.3%. In the Discussion, based on other published results, the authors listed several hypotheses, such as deficiency of folic acid, retinol, riboflavin, or vitamin C. Regarding hemoglobinopathies, including "thalassemia", the authors ruled out these conditions as possible causes due to their low prevalence as detected by a newborn screening program conducted statewide.¹ The possibility of these children having infections at blood collection was also ruled out by measuring C-reactive protein (CRP) concentration (note that there is a typographical error in the *Methods*, since children with CRP levels above - rather than below - the cutoff value were excluded from the analysis). Interesting information that was left out of the article is the frequency with which the 114 anemic preschoolers were also considered anemic as infants.

We suggest two additional hypotheses for the difference in the prevalence of iron deficiency as the etiology of anemia between the two age groups.

The first hypothesis concerns the statistical definition of anemia and, especially, of iron deficiency, since the distribution of values defining the assessments represents a continuum and the definition of cutoff points is necessarily arbitrary. Since children were assessed at different ages, the distribution of values is unlikely to be the same for both age groups. The definition of iron deficiency could have been more specific if

serum iron, transferrin saturation, and transferrin receptors had been measured.

Second, it seems reasonable to hypothesize that the main cause of anemia in this case might be alpha-thalassemia with one or two inactive alpha-globin genes, a common occurrence in Brazil and in other countries as demonstrated below. A reliable detection of these two forms of thalassemia is not feasible through newborn screening. Deletion of three or four alpha genes leads to hemoglobin H disease and hydrops fetalis, respectively.² Newborn screening can detect increased concentration of Bart's hemoglobin.

The prevalence of $\alpha^{3,7}$ deletion, the most common type of alpha-thalassemia in Brazil, may vary across regions, but it is usually above 20%. In a study conducted in the state of Minas Gerais, southeastern Brazil, we demonstrated coinheritance of alpha-thalassemia in 30% of children with sickle cell anemia from a random sample of newborn screening.³ This prevalence rate was also observed in other Brazilian states^{4,5} (see also references 9, 37, and 38 within our reference³). Rates are also high in Caucasian populations, such as in Ontario, Canada, where the prevalence of some form of $\alpha^{3,7}$ deletion was 24.4% in adult patients referred for microcytosis investigation, defined as mean corpuscular volume (MCV) < 80 fL, after exclusion of iron deficiency.⁶

In Campinas, southeastern Brazil, a study involving 339 adults without anemia, but with microcytosis (MCV \leq 80 fL) and hypochromia (mean corpuscular hemoglobin, MCH \leq 27 pg), after iron deficiency was ruled out by ferritin measurement, demonstrated that 69.8% of 98 Afro-Brazilian participants had $\alpha^{3,7}$ deletion. Among 241 Caucasian participants, the prevalence was 39.4%.⁴

We may infer from those studies that the prevalence of a silent carrier state (deletion of one gene) and alpha-thalassemia trait (deletion of two genes) is high in the Brazilian population. This assumption is particularly true for individuals of African descent, represented in different studies by patients with sickle cell disease or by black or brown-skinned subjects, but it also applies to "Caucasians", who may actually be representatives of the ethnic mix characteristic of Brazil. Moreover, it is well known that the prevalence of alpha-thalassemia is high in Mediterranean countries.² In Rio Grande do Sul, state where the study was conducted,¹ a significant proportion of individuals is of Italian descent, which reinforces our hypothesis.

Another clinical evidence available is that, after the introduction of the molecular diagnostic method for deletional thalassemia at Hemominas Foundation, Brazil, a current requirement for conducting studies in children with sickle anemia at Universidade Federal de Minas Gerais (UFMG),³ Brazil, several children treated at UFMG Pediatric Hematology Outpatient Unit had their diagnostic status clarified. These children presented persistent microcytic and/or mild hypochromic anemia that was "unresponsive" to iron replacement therapy or they had changes suggestive of iron deficiency despite normal iron kinetics. Beta-

thalassemia minor was unlikely to occur because hemoglobin A2 was within normal levels. Several children had the $-\alpha^{3.7}/\alpha\alpha$ form and some had $-\alpha^{3.7}/-\alpha^{3.7}$. Two families had the deletional type, which is more rare, $-\alpha^{4.2}$, and none of them, so far, has showed the variants found in the Mediterranean ($--^{MED}/\alpha\alpha$) or in Southeast Asia ($--^{SEA}/\alpha\alpha$).

In the study analyzed herein,¹ in order to confirm our hypothesis, at least preschoolers considered anemic, without evidence of iron deficiency, should undergo molecular genetic testing for detection of alpha-thalassemia.³ It is obvious that infants, in addition to iron deficiency, may show inheritance of alpha-thalassemia. Indirect evidence could be obtained in the study if MCV and/or MCH (note that the authors did not use MCH and did not provide the definition of microcytosis in the *Methods*) were significantly lower in the anemic non-iron deficient preschoolers than in non-anemic children.

In conclusion, we believe that alpha-thalassemia is probably one of the main reasons underlying the results found by the authors.

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Authors' reply

Dear Editor,

We would like to thank the researchers from the Department of Pediatrics of Universidade Federal de Minas Gerais (UFMG), Brazil, for their contributions to the discussion on the etiology of anemia in children. The main purpose of our study was to promote debate about the fact that iron deficiency is not always the main cause of anemia, as classically understood, and that other known or unknown causes may be present. Thus, we believe that the results of our study,¹ published in this journal, provide some support for pediatricians and other health professionals to decide about the assessment and management strategy to be adopted in clinical practice in the diagnosis of anemia.

Both hypotheses proposed by the researchers are based on significant evidence and enrich the discussion of the results found in our study.¹

The first hypothesis regarding the possible inadequacy of the cutoff points for anemia and iron deficiency is consistent with the complexity of iron metabolism and the presence of various hematological and biochemical parameters that reflect the stages of iron deficiency. Therefore, a combination of different parameters is recommended in order to increase the specificity of the diagnosis of iron deficiency.^{2,3} The choice of parameters to be used should take into account inherent characteristics of individuals or populations, age, prevalence and severity of iron deficiency, incidence of inflammatory and infectious diseases, frequency of hematologic diseases, blood sample volume required, costs and complexity of the methodology used, and susceptibility to laboratory error.² The World Health Organization, together with the U.S. Center for Disease Control and Prevention (CDC), suggested that the best indicator for measuring iron nutritional status in populations is the combination of hemoglobin, ferritin, and soluble transferrin receptor measurements associated with parameters that assess infection (C-reactive protein or alpha-1 glycoprotein).^{2,3} Unfortunately, in our study, we did not analyze transferrin receptors, which were also mentioned by the researchers as an important parameter for differential diagnosis. We agree that the cutoff points used for anemia and iron deficiency are limitations of the study, and further analyses are therefore warranted to evaluate the distribution of values in our study sample.¹

The second hypothesis that children with anemia, but without iron deficiency, are carriers of alpha-thalassemia provides a wider perspective on investigations in this field, including additional genetic testing. As suggested by the researchers, we comparatively analyzed mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) between groups of anemic children without iron deficiency and non-anemic children. According to Table 1, values were significantly lower for the anemic group, which substantiates the hypothesis of the researchers. As further support for the discussion, we have included Table 2 showing the prevalence of microcytosis in children with and without iron deficiency, using a cutoff point of 74 fL. According to Table 2, 75.5% of children with microcytosis did not have iron deficiency. Accordingly, we may

ask: Is there any other hypothesis that might explain such a high prevalence of microcytosis without iron deficiency in children aged 3-4 years?

It is important to emphasize that the hypothesis of alpha-thalassemia might explain part of our findings and another part may result from deficiency of other micronutrients required for iron mobilization due to the low socioeconomic status of the study population.

Table 1 - Mean and standard deviation of MCV and MCH values of children with anemia and without anemia (children with iron deficiency were excluded)

	Patients with anemia (n = 92)*	Patients without anemia (n = 158)†	p
MCH	25.6±1.7	26.4±1.2	< 0.001
MCV	73.8±5.2	76.4±4.0	< 0.001

MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume.

* Hemoglobin < 11 g/dL and ferritin ≥ 15 µg/L.

† Hemoglobin ≥ 11 g/dL and ferritin ≥ 15 µg/L.

Table 2 - Prevalence of microcytosis according to the presence or absence of iron deficiency in children

ID	Microcytosis		p
	Yes*	No*	
Yes	24.5 (24)	12.0 (24)	0.006
No	75.5 (74)	88.0 (88)	

ID = iron deficiency.

* Values expressed as % (n).

In conclusion, the additional considerations submitted by the researchers to this journal regarding our article have certainly added new scientific knowledge on the etiology of anemia and contribute to a better management of the disease in clinical practice.

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