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Scientific Comment

The importance of hemoglobin A_2 determination☆

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Hemoglobin (Hb) A_2 ($\alpha_2\delta_2$) constitutes less than 3% of the total hemoglobin (Hb) in adults and has almost no physiological importance.[1] On the other hand, the determination of Hb A_2 is an important tool to diagnose the beta-thalassemia trait (BTT).[1,2] Although individuals with BTT do not need treatment, the accurate detection of the carrier state is important in genetic counseling to determine risk of having a child with a major disease.[3]

Elevated levels of Hb A_2 and microcytosis are suggestive of the diagnosis of BTT. However, BTT may be present with normal levels of Hb A_2 as a few cases of β-thalassemia are not associated with elevated Hb A_2, and because of the association of BTT with iron deficiency or with α-thalassemia (α-Thal).[1,2,4–6] There are many other factors, inherited or acquired, that can interfere in Hb A_2 levels (Table 1).[3,4]

Hb A_2 can be measured by several laboratorial methods, but these methods have differences in accuracy.[7] Cation exchange high performance liquid chromatography (HPLC), microcolumn chromatography, and cellulose acetate electrophoresis with elution are considered acceptable methods to diagnose BTT, whereas cellulose acetate electrophoresis followed by scanning densitometry is not.[2] The accuracy of cellulose acetate electrophoresis with elution depends on the training and experience of the laboratory technician who performs the test, and microcolumn chromatography can give problems with co-elution of some Hb variants.[7]

Recent studies have confirmed the higher quality of automated HPLC in the measurement of Hb A_2 compared to the other methods,[8,9] which is why this has become the method of choice. On the other hand, in automated HPLC, the measurement of Hb A_2 is inaccurate when Hb S is present.[2,3,10] As the amount of Hb S is related to the degree of inaccuracy, levels are higher in patients with sickle cell anemia (SCA) or Hb S/β-thalassemia (S-βThal) than in sickle cell trait.[2] Thus, the amount of Hb A_2 does not indicate BTT when Hb A and beta gene variants are found together.[11] Furthermore, when beta gene variants are present without Hb A, the diagnosis of concomitant BTT is not necessarily associated to the elevation of Hb A_2 and so further investigations using family studies or DNA analysis are necessary.[10]

As mentioned above, α-Thal is capable of interfering in the determination of Hb A_2.[4] Individuals with the α^0-thalassemia trait or homozygous for α^+-thalassemia have lower levels of Hb A_2, but the influence of the coinheritance of α-Thal and BTT on Hb A_2 levels is uncertain.[6]

In Brazil, the incidence of α-Thal varies from 0.11 to 0.22% depending on the geographical region studied.[12–15] It is well known that the association of α-Thal and SCA is common in

☆ See paper by Fonseca et al. on pages 296–301.

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Table 1 – Causes of variation in the percentage of hemoglobin A_2.

Hb A_2	Inherited	Acquired
Increased (>3.4%)	β-Thalassemia heterozygosity Deletional HPFH from Vietnamese/South East Asian Hereditary high Hb A_2 Unstable hemoglobin Sickle cell trait Sickle cell anemia (particularly coexisting α-thalassemia) Hb S/β^0-thalassemia Congenital dyserythropoietic anemia (some cases) Heterozygosity for other β-chain variants	Thyrotoxicosis HIV infection Zidovudine therapy Megaloblastic anemia (some cases)
Decreased (<2.2%)	α-thalassemia: α^+ homozygosity, a^0 heterozygosity, and HbH disease Deletional HPFH (except Vietnamese/South East Asian type) δβ and $^A\gamma\delta\beta$-thalassemia heterozygosity (some cases) δ-Thalassemia Hemoglobin Lepore Hemoglobin Kenya	Severe iron deficiency Anemia of chronic disease Sideroblastic anemia Lead poisoning Juvenile myelomonocytic leukemia Acquired Hb H disease Acute myeloid leukemia (some cases, particularly erythroleukemia) Aplastic anemia (some cases) Hypothyroidism Chemotherapy-induced increased Hb F synthesis

Source: Modified from Bain et al.[4]

Hb A_2 – hemoglobin A_2; HPFH: hereditary persistence of fetal hemoglobin; HIV: human immunodeficiency virus; HbH: hemoglobin H; Hb F: hemoglobin F or fetal hemoglobin.

Brazil.[16–18] Since SCA is considered a public health problem in Brazil and due to the clinical significance of α-Thal in respect to this anemia, diagnosis is important.[19–21] However, diagnosis is mainly achieved by molecular techniques that are expensive and not easily accessible. It is also important to remember that the co-inheritance of α-Thal and SCA results in increased levels of Hb A_2 as measured by automated HPLC, and could result in a misdiagnosis of S-βThal.[4]

There lies the importance of the paper entitled "Hemoglobin A_2 values quantified by high performance liquid chromatography in patients with sickle cell disease, and the influence of the presence of alpha-thalassemia" written by Fonseca et al. and published in this edition of the Revista Brasileira de Hematologia e Hemoterapia.[22] The authors demonstrate that Hb A_2 was overestimated not only in individuals with Hb S but also in patients with Hb C, and that the Hb A_2 level was influenced by the genotype of α-Thal.

In conclusion, in a country with a high degree of miscegenation such as Brazil, not only the diagnosis of double heterozygous states, such as S-βThal, but also the diagnosis of co-inheritance of SCA with α-Thal should be carried out carefully, taking into consideration the limitations of the available laboratory techniques. Family studies or DNA analysis, when possible, are desirable to confirm the correct diagnosis.

Conflicts of interest

The authors declare no conflicts of interest.

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