Iron deficiency do not compromise the diagnosis of high HbA2 beta thalassemia trait

by Cristina Passarello, Antonino Giambona, Monica Cannata, Margherita Vinciguerra, Disma Renda, and Aurelio Maggio

Haematologica 2011 [Epub ahead of print]

Citation: Passarello C, Giambona A, Cannata M, Vinciguerra M, Renda D, and Maggio A. Iron deficiency do not compromise the diagnosis of high HbA2 beta thalassemia trait. Haematologica. 2011; 96:xxx

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors’ final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature.
Iron deficiency do not compromise the diagnosis of high HbA₂ beta thalassemia trait

Cristina Passarello, Antonino Giambona, Monica Cannata, Margherita Vinciguerra, Disma Renda, and Aurelio Maggio

U.O. C. Ematologia e Malattie rare del Sangue e Organi Ematopoietici, Azienda Ospedaliera Ospedali Riuniti “Villa Sofia-Cervello”, Palermo, Italy

Key words: Iron deficiency, serum ferritin, HbA₂ levels, b-thalassemia

Correspondence
Antonino Giambona, lab. diagnosi prenatale e molecolare-talassemia, Azienda Ospedaliera Ospedali Riuniti “Villa Sofia-Cervello”, via Trabucco 180, 90146 Palermo, Italy. E-mail: giambic@libero.it

Acknowledgments
The authors would like to thank Foundation Franca and Piera Cutino, Palermo-Italy, for the support to this work.
Hemoglobinopathies are the only genetic disease where it is possible to detect carriers using hematological findings rather than DNA analysis. Complete screening is based on the detection of red cell indices, HbA2, HbF and hemoglobin variant values. The classical phenotype of heterozygous β-thalassemia includes an elevated HbA2 level (3.4-6.0%), a relatively high red cell count, a markedly reduced mean corpuscular volume (MCV 60-75 fl) and reduced mean corpuscular hemoglobin levels (MCH 18-24 pg).1 HbA2 determination plays a key role in screening programs for β-thalassemia because a small increase in this fraction is the most important markers of β-thalassemia heterozygous carriers.2 Measurement of HbA2 is undertaken in many laboratories worldwide, often with a lack of agreement in the obtained results probably because there is no international standardization of HbA2 determination. Reduced production of b-globin, with a relative excess of a-globin chains, and also a "compensatory" increase in d-globin synthesis, favor the formation of ad dimmers and the assembly of HbA2 tetramers. Low HbA2 values are in most instances the result of either reduced synthesis of the d-globin chain, or posttranslational modifications in the assembly of the HbA2 tetramer due to a reduction in the synthesis of a-globin chains.3 Some author4 reported that iron deficiency (ID) is a potential source of diagnostic interference in tests for HbA2 determination, that may give false-positive or negative results. In fact, intracellular lack of iron reduces a-globin chain synthesis relative to that of non-a-globin chains; when the supply of b-globin chains is limited, b-globin chains compete more effectively for a-globin chains than d-globin chains, resulting in reduced levels of Hb A2.3 Studies from India reported that the trait beta-thalassemic do not confer an advantage in maintaining iron balance, and that HbA2 is not significantly lowered in the presence of ID.5 In Sicily there is a high heterogeneity of molecular defects and a prevalence of mutations causing b*- or b**- thalassemia,2,6 so that a reliable HbA2 assessment is essential for accurate diagnosis and genetic counselling.

The purpose of the present study was to quantify the effect of iron deficiency on HbA2 levels in order to improve the detection of beta thalassemia trait with and without iron deficiency.

This study was approved by the Ethical Committee of A.O.O.R. “Villa Sofia-Cervello” Hospital, and informed consent was obtained from all subjects. A retrospective analysis was carried out on 9625 samples, without Hb variants, obtained during a program for b-thalassemia carrier screening in the Sicilian population in the last two years. We selected 1133 samples with serum ferritin estimated and 253 samples with serum ferritin estimated and molecular analysis result.

Blood sample, from all patients, were collected and analyzed as previously described.6 For statistical analysis we divided these samples into two group, A and B, using serum Ferritin value of 30 mg/L as cut-off.7 Figure 1 shows the profile of study performed in this work. Given that Ferritin is an acute-phase protein, samples with altered white blood cell indices were excluded from analysis to avoid a potential bias.

All statistical analyses were performed with STATA 9 (StataCorp, 4905 Lakeway Drive, College Station, Texas 77845 USA). Means are reported with standard deviation (SD); proportions and differences are reported with 95% confidence intervals (CI). A Receiver Operating Characteristic (ROC) analysis was performed to determine sensitivity and specificity of test in the group A using the HbA2 value of 3.4% as cut-off.8
Using the value of 30 mg/L for serum Ferritin as cut-off, 861 samples showed iron deficiency (ID) (group A) and 271 resulted without ID (group B) (Figure 1). The mean of HbA2 value was 2.8%±0.79 in the group A and 3.50%±1.23 in the group B with a significative difference (p=0.00001) among the two groups. 

The distribution of the 253 samples with molecular analysis among group A and B showed that 170 samples were part of the group A and 83 of the group B (Figure 1).

From 170 samples of the group A, 21 resulted positive for a b-globin gene mutation with a mean of HbA2 value of 4.90%±1.29. From 83 samples of the group B, 29 resulted positive for a b-mutation (mean of HbA2 value: 5.37%±0.87).

The comparison between the HbA2 mean value of beta carriers with (group A) or without ID (group B), using the value of 30 mg/L as serum ferritin cut-off, do not show a significative difference (p=0.060) and in both group the HbA2’s levels are upper the 3,4% value. Reduction of HbA2 has been reported linked to the severity of anemia so that is possible that the value of 30 mg/L for serum ferritin define an ID not sufficiently severe or not sufficiently prolonged to significantly reduced the level of HbA2.

The ROC analysis, performed with samples of group A, at the 3.4% HbA2 cut-off value, showed sensitivity and specificity 74.19% and 95.8% respectively. The false negative samples were 8/30 (26.6%): three presented Hb b Variants, co-eluting with HbA, (Hb Valletta, Hb Ernz and Hb City of Hope), that don’t require prenatal diagnosis, one showed an undefined Single Nucleotide Polymorphism (SNP) in the b-globin gene of ambiguous diagnostic significance, two samples presented, respectively, the a-globin gene triplication (aaa in $\text{anti}^{3.7}$), and the b-globin gene promoter mutation, -101 (HBB c.-151C→T), and finally 2 sample showing co-heredity of beta* and delta* mutation.

The samples with aaa in $\text{anti}^{3.7}$ and the -101 beta mutation showed, respectively, HbA2 value of 3,0% and 3,2% with a lower value of Hb (<12 g/dL) and MCV (<75 fl), so that the contemporary analysis of hematologic and hemoglobin data enable to identify these subjects as samples that must be submitted to molecular analysis if their partners are carriers of beta thalassemia; instead, in other cases, remeasuring HbA2 after ID treatment is recommended.

In case of the 2 sample showing co-heredity of beta+ and delta+ mutation, the deep reduction is principally associated with the presence of delta mutation rather than serum ferritin value.

Our results show that the presence of iron deficiency did not preclude the detection of classical beta carrier in our population. Some problems could be in presence of silent beta mutation or alpha gene triplication with ID, because HbA2 shows levels almost normal, but the reduction of total Hb and MCV, eventually persistent low MCV and MCH after iron suppletion, should advise more attention and molecular analysis, exploring both the alpha and the beta genes, should be done, especially if the subject is partner of a classical beta thalassemia carrier.

Authorship and Disclosures
CP: a molecular biologist, planned, developed and performed most experiments, designed the tables and figure and wrote the manuscript. AG: is in charge of the molecular laboratory, supervised the experimental work and co-wrote the manuscript. MC, MV: molecular biologists, performed measure of serum ferritin levels and molecular analysis. DR: hematologist, was consultant in this study; AM, head of U.O. C. Hematology and rare diseases of blood and blood forming organs and of the laboratory, coordinated the study and supervised the writing. The authors reported no potential conflicts of interest.
References


**Figure 1.** Profile of study used to evaluate the effect of iron deficiency on HbA$_2$ levels.

9625 samples without Hb variants

1133 samples with serum ferritin value determined and normal white blood cell indices

**Group A**

861 with serum ferritin value $\leq$ 30 $\mu$g/L

170 with molecular studies

21 with $\beta$ thal (mean serum ferritin value)
  a) 10 with $\beta$ Cd 39 (16 $\mu$g/L).
  b) 4 with $\beta$ IVS I nt 110 (12 $\mu$g/L).
  c) 3 with $\beta$ IVS I nt 1 (13 $\mu$g/L).
  d) 2 with $\beta$ IVS I nt6 (6 and 20 $\mu$g/L).
  e) 2 with $\beta$-promoter defect (9 and 25 $\mu$g/L).

**Group B**

271 with serum ferritin value $>$ 30 $\mu$g/L

83 with molecular studies

29 with $\beta$ thal (mean serum ferritin value)
  a) 14 with $\beta$ Cd 39 (76 $\mu$g/L).
  b) 6 with $\beta$ IVS I nt 110 (87 $\mu$g/L).
  c) 2 with $\beta$ IVS I nt 1 (88 and 35 $\mu$g/L).
  d) 1 with $\beta$ IVS I nt 2 (33 $\mu$g/L).
  e) 2 with $\beta$ IVS II nt 1 (41 and 50 $\mu$g/L).
  f) 1 with $\beta$ IVS I nt6 (38 $\mu$g/L).
  g) 2 with $\beta$ IVS II nt 745 (32 and 39 $\mu$g/L).
  h) 1 with $\beta$ Cd 30 (60 $\mu$g/L).

*Passarello et al., Figure 1*