## Iron deficiency does not compromise the diagnosis of high $HbA_2$ β thalassemia trait

Hemoglobinopathies are the only genetic disease in which it is possible to detect carriers using hematologic findings rather than DNA analysis. Complete screening is based on the detection of red cell indices, $HbA_2$, HbF and hemoglobin variant values. The classical phenotype of heterozygous β-thalassemia includes an elevated $HbA_2$ 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] $HbA_2$ determination plays a key role in screening programs for β-thalassemia because a small increase in this fraction is the most important marker of β-thalassemia heterozygous carriers.[2] Measurement of $HbA_2$ is undertaken in many laboratories worldwide, often with a lack of agreement in the obtained result. This is probably because there is no international standardization of $HbA_2$ determination. Reduced production of β-globin, with a relative excess of α-globin chains, and also a "compensatory" increase in δ-globin synthesis, favor the formation of αδ dimmers and the assembly of $HbA_2$ tetramers. Low $HbA_2$ values are in most instances the result of either reduced synthesis of the δ-globin chain, or posttranslational modifications in the assembly of the $HbA_2$ tetramer due to a reduction in the synthesis of α-globin chains.[3]

Some authors[4] have reported that iron deficiency (ID) is a potential source of diagnostic interference in tests for $HbA_2$ determination that may give false-positive or negative results. In fact, intracellurar lack of iron reduces a-globin chain synthesis relative to that of non-α globin chains; when the supply of β-globin chains is limited, β-globin chains compete more effectively for α-globin chains than δ-globin chains, resulting in reduced levels of Hb $A_2$.[3] Studies from India reported that the β-thalassemic trait does not confer an advantage in maintaining iron balance, and that $HbA_2$ 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 $\beta^{+}$- or $\beta^{++}$-thalassemia,[2,6] so that a reliable $HbA_2$ assessment is essential for accurate diagnosis and genetic counseling.

The purpose of the present study was to quantify the effect of iron deficiency on $HbA_2$ levels in order to improve the detection of β thalassemia trait with and without iron deficiency.

This study was approved by the Ethical Committee of the Villa Sofia-Cervello Hospital, Palermo, and informed consent was obtained from all subjects. A retrospective analysis was carried out on 9,625 samples, without Hb variants, obtained during a program for β-thalassemia carrier screening in the Sicilian population in the last two years. We selected 1,133 samples with estimated serum ferritin and 253 samples with estimated serum ferritin and molecular analysis result.

Blood samples from all patients were collected and analyzed as previously described.[6]

For statistical analysis, we divided these samples into two groups, A and B, using serum ferritin value of 30 μg/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, Texas, 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 the test in group A

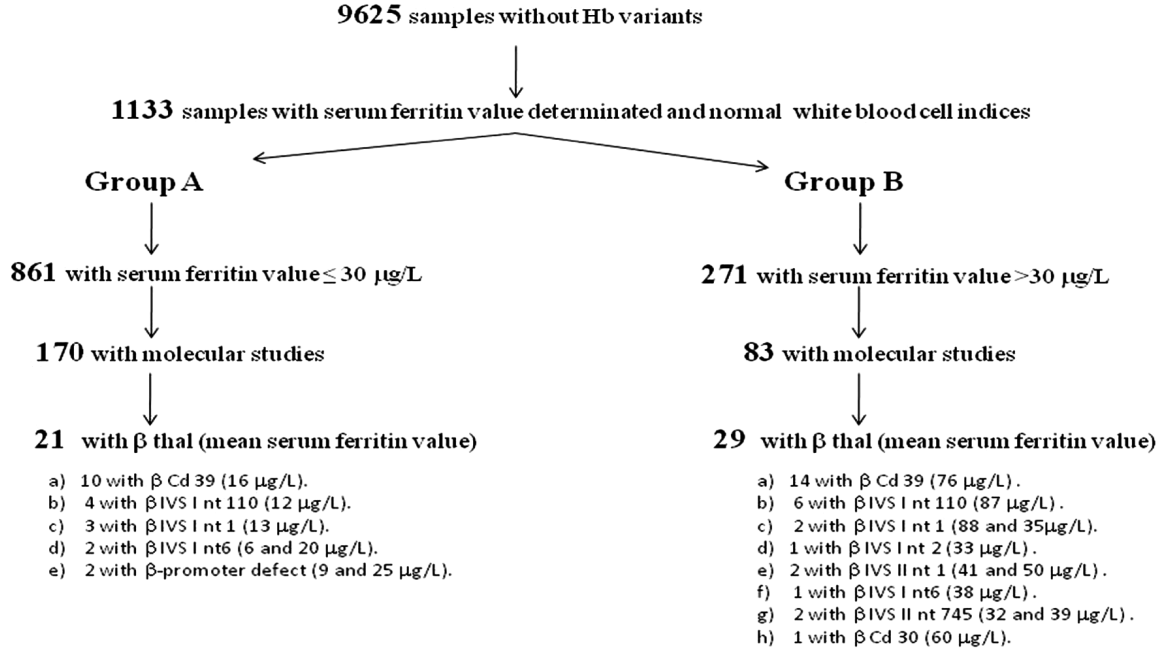


Figure 1. Profile of study used to evaluate the effect of iron deficiency on $HbA_2$ levels.

using the $HbA_2$ value of 3.4% as cut off.[8]

Using the value of 30 μg/L for serum ferritin as cut off, 861 samples showed iron deficiency (ID) (group A) and 271 were without ID (group B) (Figure 1). The mean $HbA_2$ value was 2.8%±0.79 in group A and 3.50%±1.23 in group B, with a significant difference ($P$=0.00001) among between the two groups.

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

From 170 samples of group A, 21 resulted positive for a β-globin gene mutation with a mean of $HbA_2$ value of 4.90%±1.29. From 83 samples of group B, 29 resulted positive for a β-mutation (mean of $HbA_2$ value: 5.37%±0.87).

The comparison between the $HbA_2$ mean value of β-thalassemia carriers with (group A) or without (group B) ID, using the value of 30 μg/L as serum ferritin cut off, does not show a significant difference ($P$=0.060) and in both groups $HbA_2$ levels are more than 3.4%. Reduction of $HbA_2$ has been reported to be linked to the severity of anemia[9] so that it is possible that the value of 30 μg/L for serum ferritin defines an ID not sufficiently severe or not sufficiently prolonged to significantly reduce the level of $HbA_2$.

The ROC analysis, performed with samples of group A, at the 3.4% $HbA_2$ cut-off value, showed sensitivity and specificity of 74.19% and 95.8%, respectively. The false negative samples were 8 of 30 (26,6%): 3 presented Hb β variants, co-eluting with HbA, (Hb Valletta, Hb Ernz and Hb City of Hope) that do not require prenatal diagnosis, one showed an undefined single nucleotide polymorphism (SNP) in the β-globin gene of ambiguous diagnostic significance,[8] 2 samples presented, respectively, the α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$), and the β-globin gene promoter mutation, -101 (HBB c.-151C→T), and finally, 2 samples showed co-heredity of $\beta^+$ and $\delta^+$ mutation.

The samples with $\alpha\alpha\alpha^{anti3.7}$ and the -101 beta mutation showed, respectively, $HbA_2$ values of 3.0% and 3.2% with a lower value of Hb (<12 g/dL) and MCV (<75 fL). The contemporary analysis of hematologic and hemoglobin data enable us to identify these subjects as samples that must be submitted to molecular analysis if their partners are carriers of β thalassemia.[6] While, in other cases, it is recommended to remeasure $HbA_2$ after ID treatment.

In the 2 samples showing co-heredity of $\beta^+$ and $\delta^+$ mutation, the large 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 β carrier in our population. There could be some problems in the presence of silent β mutation or α gene triplication with ID, because $HbA_2$ shows almost normal levels. However, the reduction in total Hb and MCV, and possible persistently low MCV and MCH after iron suppletion, should suggest greater attention is needed and molecular analysis exploring both the α and the β genes should be carried out, especially if the subject is a partner of a classical β thalassemia carrier.

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*Key words: iron deficiency, serum ferritin, HbA2 levels, β-thalassemia.*

*Acknowledgments: the authors would like to thank the Foundation Franca and Piera Cutino, Palermo-Italy, for the supporting to this work.*

*Citation: Cristina Passarello, Antonino Giambona, Monica Cannata, Margherita Vinciguerra, Disma Renda, and Aurelio Maggio. Iron deficiency does not compromise the diagnosis of high HbA2 beta thalassemia trait. Haematologica 2012;97(3):472-473. doi:10.3324/haematol.2011.052357*

*The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.*

*Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.*

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## Efficacy of combining dasatinib and FLAG-IDA for patients with chronic myeloid leukemia in blastic transformation

The prognosis of patients with chronic myeloid leukemia (CML) has improved considerably over the last ten years with the introduction of ABL tyrosine kinase inhibitors (TKI) into clinical practice. TKIs induce complete cytogenetic remissions (CCyR) in 10-45% of patients who are treated with these drugs in advanced phase with minimal toxicity; unfortunately, these remissions are typically short lasting.[1,2] Dasatinib alone induces CCyR in 20-40% of patients.[2,3] However, the majority relapse within one year and the median survival is eight months.[2] Conventional chemotherapy regimens, such as FLAG-IDA, can induce CCyR in 30-40% of patients who have progressed to blastic phase, but again most patients relapse within six months and survival is poor.[4] A logical approach might be to combine both strategies in order to improve the outcome. The proposed schedule of combination TKI and chemotherapy is supported by two clinical