Disorders of Erythropoiesis

The use of capillary blood samples in a large scale screening approach for the detection of β-thalassemia and hemoglobin variants

Hemoglobinopathies are priority genetic diseases for prevention programs in at-risk populations. We implemented an accurate and simple methodology to identify hemoglobin (Hb) variants and to quantify HbA2 and HbF in capillary blood samples stored at room temperature for up to 7 days after collection. This methodology is particularly indicated for screening for carriers in primary care medical centers in which facilities for collecting venous blood are not available.

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Hemoglobinopathies, the most common autosomal recessive hereditary diseases worldwide, include the α- and β-thalassemia syndromes and structural hemoglobin (Hb) variants.[1] The severity of clinical manifestations in homozygotes or compound heterozygotes - thalassemia intermedia, thalassemia major and sickle cell disease - makes prevention programs for these genetic diseases a priority in at-risk populations.[2-5] Carriers are healthy and their identification relies on the accurate measurement of mean corpuscular volume (MCV), mean cell hemoglobin (MCH), HbA_2, HbF and the identification of Hb variants. β-thalassemia carriers have an elevated HbA2 level (>3.5%) and Hb variant carriers are identified by Hb electrophoresis or high performance liquid chromatography (HPLC). α-thalassemia carriers can be suspected, after exclusion of iron deficiency, by a low MCV and MCH with normal HbA_2; the diagnosis is made through DNA studies. The prevalence of β-thalassemia and Hb variant carriers among the native population of Portuguese is low, being higher in the central and southern regions of the country[6,7] however, as a result of immigrants from Africa, Brazil, East Europe and Asia, hemoglobinopathies are now much more common and there is a wider genetic variability. Concerned about the risk of an increased rate of patients born with severe forms of hemoglobinopathies, we designed a program to screen for carriers, after personal informed consent, among pregnant women until 18 weeks of gestation and young adults attending all the primary care services in the central part of Portugal. In these medical centers, in which the vast majority of the population are cared for by their family doctors, there are no facilities to collect venous blood samples and we had to establish a methodology to identify Hb variants and to quantify HbA_2 and HbF accurately in capillary blood samples within a few days after their collection. This new procedure exploits a kit designed for studying HbA1c, the *HbA1c Capillary Collection System* from BioRad, followed by HPLC in the Variant II Hemoglobin Testing System with the β thal Short Program - BioRad.

In order to test the stability of the capillary blood in the kit's solution, the study was performed on each of 200 random samples on days 3, 5 and 7 after collection: quantification of the HbA_2 and HbF remained stable and we did not observe extra peaks due to the presence of degradation products. Based on these data we consider that samples stored at room temperature are stable for at least 7 days after collection. To validate the method we tested more than 800 capillary and venous blood samples in parallel, including samples from normal individuals, thalassemia and Hb variants carriers and patients with thalassemia intermedia and sickle cell disease. There were no significant differences between results from the same patients using capillary blood and EDTA peripheral blood samples ($p \leq 0.123$). The capillary blood sample methodology can be used for direct detection of β-thalassemia, δβ-thalassemia, the common Hb variants such as HbS, HbE, HbC, HbD, HbLepore and most of the uncommon Hb variants. The HPLC data must always be correlated, by the family doctor, with the red blood cell (RBC) parameters that are available in the individual files, to alert the physician to the possibility of silent β-thalassemia, α-thalassemia carriership and δ- and β-thalassemia compound heterozygosity. Cases with abnormal RBC parameters, namely hypochromia and/or microcytosis, and normal Hb studies are referred to our out-patients clinic for investigation.[1,8,9] In the first year of our study, 12280 unselected pregnant women at their first prenatal visit and young adults were screened; of these 217 were carriers of a hemoglobinopathy (142 β-thalassemia minor, 47 HbS, 11 HbLepore, 13 HbD, 2 HbC, 1 Hb Cocody and 1 Hb Banbury). All the carriers were informed within a few days and, when available, their siblings and partners were invited to undergo Hb studies and measurements of RBC parameters. Four couples at risk of having an affected baby (two for β-thal major and two for sickle cell disease) were found and three of these required pre-natal diagnosis; one of the fetuses was affected. Since the great majority of β-thalassemia and Hb variants (including HbS) can be diagnosed with this methodology, it could be a solution for antenatal screening to detect carriers in primary care medical centers and in schools where there are no facilities to collect venous blood. Samples can be collected anywhere and sent to the reference laboratory by normal mail, allowing widespread screening for hemoglobinopathies. This methodology could also be very useful in sickle-cell neonatal screening programs. Furthermore, it is indicated for use in populations with high frequencies of human immunodeficiency virus infection and hepatitis since there is no direct contact with the sample and a bar code enables the HPLC apparatus to identify the tubes directly.

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