## Development of an ELISA strip for the detection of α thalassemias

α thalassemia is probably the most common of all single-gene disorders throughout the world. Most incidences of α thalassemia arise from the deletion of one (-α) or both (--) of the α globin genes, which are known as $\alpha^+$ thalassemia (-α/αα) or $\alpha^0$ thalassemia (--/αα), respectively.[1] The deletion of one α globin gene from one chromosome and two α globin genes from the other chromosome leads to HbH disease (-α/--), which is a severe form of α thalassemia. The most severe form of α halassemia is Hb Bart's hydrops fetalis syndrome, where both α globin genes are deleted (--/--). Previous diagnoses of various types of α thalassemia have been based on clinical pictures, hematologic parameters, and percent of Hb Bart's. DNA technology enabled the diagnosis of all different forms of α thalassemia; however, this technology is only available in relatively sophisticated laboratories. More commonly, the differential diagnosis of various types of α thalassemia is determined by measurement of the percentage of Hb Bart's, which is only semi-quantitative.[1]

In previous studies, we produced a highly specific mAb against Hb Bart's, and we developed a corresponding ELISA assay that was able to determine quantitatively the level of Hb Bart's (ng/mL) in hemoglobin solutions of various types of α thalassemia.[2,3] In this report, we describe the development of a simple ELISA strip (Figure 1) for the detection of Hb Bart's, with the goal of producing a convenient, inexpensive, and dependable assay for the screening of α thalassemia.

The participants in this study included 105 subjects from 39 families with HbH disease, 131 pregnant women, and 58 PCR-genotyping negative for α thalassemia. The study was approved by the Institutional Ethics Committee (file no. 275/2549). Analysis by PCR genotyping revealed that for the 105 subjects from the families with HbH disease, 41 had HbH disease, 33 had $\alpha^0$ thalassemia, 12 had $\alpha^+$ thalassemia (-3.7 kb), and 19 were heterozygous HbCS; for the 131 pregnant women, 8 had HbH disease, 18 had $\alpha^0$ thalassemia, 27 had $\alpha^+$ thalassemia (-3.7 kb), 2 had $\alpha^+$ thalassemia (-4.2 kb), and 7 were heterozygous HbCS.[4-7] In contrast, PCR genotyping of all 58 normal subjects were negative for α thalassemia. When the ELISA strip was used to detect Hb Bart's in the 105 subjects from the families with HbH disease, all were positive. There were 56 out of 131 pregnant women positive to the ELISA strip, while 62 out of 131 subjects were positive by PCR genotyping. Among all 294 subjects, the 49 with HbH disease and the 51 with $\alpha^0$ thalassemia were all positive to the ELISA strip (i.e., 100% detection). Of 39 having $\alpha^+$ thalassemia (-3.7 kb), 30 (76.9%) were positive to the ELISA strip. Of 2 having $\alpha^+$ thalassemia (-4.2 kb), both were positive to the ELISA strip. Of 26 having heterozygous HbCS, 24 (92.3%) were positive to the ELISA strip. The ELISA strip was evaluated for its sensitivity, specificity, predictive value of a positive test, and predictive value of a negative test by the 2x2 table,[8] which gave 93.4, 93.7, 95.1 and 91.5%, respectively, as shown in Table 1. There were 8 samples positive by the ELISA strip that could not be genotyped by PCR. These samples might actually be negative for α thalassemia, or they might be positive for some types of α thalassemia that cannot be detected by the PCR genotyping used in this study because there were at least 29 deletions that involve both α globin genes.[1]

The erythrocyte osmotic fragility test (OFT) has been suggested as a simple screening method for $\alpha^0$ thalassemia and β thalassemia.[9,10] When the OFT was compared with PCR genotyping, the sensitivity, specificity, positive predictive value, and negative predictive value of the OFT were 97.7, 74.9, 29.4, and 99.7%, respectively.[10]

In parts of the world where the incidence of thalassemia is high, the disease causes a tremendous economic loss for the society as a whole. Therefore, the development of an easily performed and efficient screening program can be readily justified.

The results presented here, which compare our ELISA strip method to standard PCR genotyping of α thalassemia, demonstrate that the ELISA strip is highly sensitive and highly specific, with excellent predictive values of both positive and negative tests. The ELISA strip test is simple, inexpensive, and requires no sophisticated equipment or expertise. Consequently, it can be readily conducted in small diagnostic laboratories. Given these considerations in their entirety, we wish to promote the ELISA strip as a screening test for the detection of α thalassemia in the general population.

**Table 1.** Comparison of the ELISA strip and PCR genotyping of α thalassemia.

| Number of subjects* | Types of α thalassemia | PCR Genotyping | ELISA Strip +ve** | -ve*** | %positive |
|---|---|---|---|---|---|
| 49 | HbH disease | --/-α | 49 | 0 | 100.0 |
| 51 | $\alpha^0$ thalassemia | --/αα | 51 | 0 | 100.0 |
| 39 | $\alpha^+$ thal (-3.7 kb) | $-\alpha^{3.7}/\alpha\alpha$ | 30 | 9 | 76.9 |
| 2 | $\alpha^+$ thal (-4.2 kb) | $-\alpha^{4.2}/\alpha\alpha$ | 2 | 0 | 100.0 |
| 26 | Heteroz. HbCS | $\alpha\alpha^{cs}/\alpha\alpha$ | 24 | 2 | 92.3 |
| 127**** | Normal | αα/αα | 8 | 119 | 6.3 |

**Number of subjects positive by PCR genotyping=167 (156 positive but 11 negative by ELISA strip). **Number of subjects positive by ELISA strip = 164 (156 positive but 8 negative by PCR genotyping). ***Number of subjects negative by ELISA strip=130 (119 negative but 11 positive by PCR genotyping). ****Number of subjects negative by PCR genotyping = 127 (119 negative but 8 positive by ELISA strip). Sensitivity =156×100/156+11=93.4%; specificity=119×100/119+8=93.7%; predictive value of a positive test ($PV^+$)=156×100/164=95.1%; predictive value of a negative test ($PV^-$)=119×100/130=91.5%.*

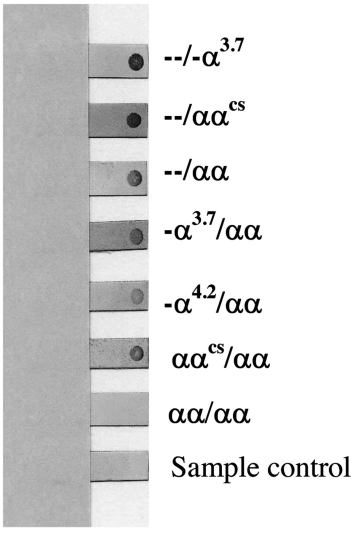


**Figure 1.** Examples of the ELISA strip for the detection of Hb Bart's in hemoglobin solution of various types of α thalassemia. The ELISA strip was positive when a purple circle was observed on the membrane, and negative when no purple circle was observed on the membrane. Sample control means the ELISA strip being treated with all reagents except the hemoglobin solution.

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## Treatment with mycophenolate mofetil followed by recombinant human erythropoietin in patients with low-risk myelodysplastic syndromes resistant to erythropoietin treatment

Anemia is present in most patients with low-risk MDS (LR-MDS).[1] Supportive treatment with transfusions was the final outcome of most of them, and transfusion-dependency is a prognostic factor for poor survival.[2] A number of studies have demonstrated that, overall, one third of LR-MDS responded to growth factor treatment. A score system was developed[3] which, according to transfusion requirements and serum erythropoietin levels, identifies cases with high or low probability of response.[3,4] One important aspect is that the response to growth factors is not maintained, with a median duration of 24 months. However, only 20% of patients had a response which lasted more than four years.[5]

One important aspect, frequently forgotten, is that many LR-MDS are elderly handicapped patients. In fact, the number of patients lost during treatments was as high as 20% in studies using Epo+/-G-CSF-G, which only required subcutaneous injections.[3,4]

Immunological disturbances have been proposed to be among the mechanisms involved in MDS pathogenesis. As in aplastic anemias, in MDS abnormal SMD stem cells trigger an immunological cellular response which in turn attacks abnormal stem cells causing more damage to the remaining stem cells. This finding is probably the physiological basis for anti-lymphocytic gammaglobulin +/- cyclosporine responses.[6] In MDS patients with trisomy 8, an expansion of a number of CD8 T-lymphocyte repertoires have been demonstrated, suggesting their involvement in the pathogenesis of MDS. In patients with refractory anemia, HLA-DR 15 and trisomy 8, the rate of response was 70%.[7,8] However, the immunosuppressive treatment is not well tolerated and requires hospital admission.[9]

The purpose of this work was to rescue LR-MDS patients who had lost their response to Epo, or to treat patients with low probability of response to Epo using the Scandinavian Score.[3] A therapeutical approach using sequentially immunosuppresion and growth factors was investigated. Mycophenolate mofetil and prednisone were used as immunosuppression because mycophenolate mofetil is given orally and is very well tolerated.[10]

The trial scheme (www.clinicaltrials.gov. Identifier: ML20559) used mycophenolate mofetil (Cell Cept) 1 g twice a day orally and oral prednisone 0.5 mg/Kg/d tapering prednisone to 10 mg/d. Mycophenolate mofetil and prednisone 10 mg were maintained to the end of the study. Response was evaluated at 12 weeks. In patients without major erythroid response, subcutaneous 30,000 U/week of recombinant human erythropoietin beta (Neorecormon) was added during six weeks. This was increased to 60,000 U/week in case of no major response following the IWG criteria.[11]

A total of 10 patients were treated (Table 1), including 8 cases that had received erythroid stimulating agents and had lost their response and 2 with high levels of serum erythropoietin. Seven of them were under transfusions. In one case the treatment was stopped because of pneumonia at two weeks. In 5 out of the 9 remaining cases, a response was observed; in 3 this was a major response (in one case under transfusions at the end of the study Hb was 96 g/L without transfusions, in one case with initial Hb of 81 g/L, Hb was 109 g/L after treatment, and in a case under transfusion final Hb was 97 g/L without transfusions). A minor response was seen in 2 patients (in one case the amount of transfusions was reduced from 6 units/month to 2 units, and in one case without transfusions Hb increased from 85 g/L to 101 g/L). Treatment was well-tolerated; 3 cases showed grade 1-2 diarrhea, in one case with diabetes mellitus an increase in diabetes treatment was required, and one case was admitted to hospital due to pneumonia.

In spite of the low number of cases included, this treatment with oral immunosuppresion followed by addition of growth factors, as antiapoptotic agents, obtained a good rate of response. It is worth remembering that the cases included were patients with few alternative treatments.