Flow cytometry test for hereditary spherocytosis

We read with interest the recent article of Bianchi *et al.* published in Haematologica that compared laboratory tests for hereditary spherocytosis (HS) in 150 patients.[1] The authors reported the usefulness of the eosine-5-maleimide binding (EMA) test in the diagnosis of HS with high sensitivity and specificity, and confirmed, as we had previously reported, that this test was independent of the type of molecular defect.[2]

Despite a very surprisingly low percentage of ankyrine deficiency compared with band 3 and spectrine deficiencies, the authors reported an uncommonly high sensitivity (143 of 150) of SDS-PAGE analysis, far higher than the sensitivity usually reported.[3]

Interestingly, the authors adopted an original approach using the EMA test, preferring high sensitivity rather than specificity with the aim of distinguishing red cell membrane disorders from other abnormalities. In the second step, the diagnosis of either HS, congenital dyserythropoietic anemia (CDA) or hereditary elliptocytosis (HE) could be made. As a consequence, the cut off for the optimum decrease in fluorescence expressed as the percentage of fluorescence reduction of the patients' samples compared with the mean fluorescence of 6 normal controls was much lower than ours (11% *vs.* 16% in our study). We previously reported a 'gray zone' (16-21% decrease in fluorescence), in which case other tests were necessary to correctly diagnose a membrane abnormality.[2]

In order to validate the strategy and the cut-off value described by Bianchi *et al.* we re-analyzed a large series of data collected in our laboratory over the last ten years concerning the EMA test. Of the 546 tests performed, 98 showed a decrease in fluorescence of over 21%, and patients were subsequently diagnosed with HS, whereas 410 showed a reduction in fluorescence of under 11% making the diagnosis of HS very unlikely. Of the 38 patients with an 11-21% decrease in fluorescence (Table 1), 3 were finally diagnosed with HS (with a band 3 deficiency), 2 with HE, and one with CDA II using ecktacytometry and SDS-PAGE analysis. The diagnosis of HS was excluded on the basis of the ektacytometry test in 2 patients. All the membrane disorders showed a reduction in fluorescence of over 14%. One pyruvate kinase (PK) and one case of G6PD deficiencies were noted, and 2 autoimmune hemolytic anemias (AIHA) were observed. In 12 patients, HS was very unlikely given the small variation in mean sphered corpuscular volume.[4] Finally, 14 patients were lost to follow up, thus preventing further laboratory tests.

Taken together, our data, based on more than 500 EMA tests, confirm the relevance of the diagnostic strategy described by Bianchi *et al.* to diagnose HS in clinical practice. However, in our experience, the best discriminating cut-off value of fluorescence reduction was higher than that reported.

Table 1. Reduction of fluorescence using the EMA binding test, related to the final diagnosis.

Final diagnosis	Decrease in fluorescence
HS band 3 deficiency	14.8%
HS band 3 deficiency	19.5%
HS	14.9%
HE	16.5%
HE	15.7%
CDA II	20.7%
PK deficiency	13.5%
G6PD deficiency	20.1%
AIHA	13.5%
AIHA	11.1%

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References

1. Bianchi P, Fermo E, Vercellati C, Marcello AP, Porretti L, Cortelezzi A, et al. Diagnostic power of laboratory tests for hereditary spherocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics. Haematologica;97(4):516-23.
2. Girodon F, Garcon L, Bergoin E, Largier M, Delaunay J, Feneant-Thibault M, et al. Usefulness of the eosin-5'-maleimide cytometric method as a first-line screening test for the diagnosis of hereditary spherocytosis: comparison with ektacytometry and protein electrophoresis. Br J Haematol. 2008;140(4):468-70.
3. Delaunay J. The molecular basis of hereditary red cell membrane disorders. Blood Rev. 2007;21(1):1-20.
4. Broseus J, Visomblain B, Guy J, Maynadie M, Girodon F. Evaluation of mean sphered corpuscular volume for predicting hereditary spherocytosis. Int J Lab Hematol 2010;32(5):519-23.