Testing for hereditary spherocytosis: a French experience

In a recent article, Bianchi *et al.* have compared several laboratory tests for diagnosis of hereditary spherocytosis (HS) by studying 150 HS patients.[1] They concluded that the association of the eosin-5-maleimide (EMA) binding test and the acidified glycerol lysis test (AGLT) could identify all HS patients and represents an effective diagnostic tool. According to a receiver operating characteristic (ROC) curve analysis, the EMA cut-off value or the optimum fluorescence decrease that discriminates HS patients from controls was 11%. In these conditions, the test specificity was 98% and sensitivity 93%. In 93% of the cases, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) identified the molecular defect.

Osmotic gradient ektacytometry has demonstrated unequalled sensitivity for HS diagnosis, and easily discriminates between distinct red cell membrane disorders. It is not, however, readily available.[2,3] We have used osmotic gradient ektacytometry to diagnose hemolytic diseases with suspected red cell membrane defects and compared these results with those of the EMA binding test. Eighty-two consecutive HS patients from 78 distinct families originating from different parts of France with typical ektacytometry curve and negative direct antiglobulin test were tested in parallel with 134 controls, 35 other red cell membrane disorders (7 congenital dyserythropoietic anemias type II (CDAII), 7 hereditary elliptocytosis, one pyropoikilocytosis, 4 Southeast Asian ovalocytosis (SAO), 16 xerocytosis) and 78 hemolytic syndromes with normal ektacytometric results. Thirty-two samples from non-related HS subjects were submitted to SDS-PAGE for red cell membrane protein analysis. The EMA binding test was performed as previously described and results expressed as proposed by Girodon *et al.*[4,5] The distribution of the results obtained in the HS population compared to hemolytic diseases not related to red cell membrane disorder and controls is shown in Figure 1. Since different cut-off values have been used in previously published series (11% by Bianchi *et al.*, 16% and 21% by Girodon *et al.*) our results were stratified in 4 categories: under 11%, between 11 and 15%, between 16 and 20% and over 21% (Table 1).[1,5] Overall, 85% of the HS cases showed a decrease in EMA binding of over 16% (with 72% over 21%). Only 4% were found in the 11-15% range and, notably, 11% of the HS displayed normal EMA binding. One control was in the 11-15% range. EMA binding test was normal in all 16 tested xerocytosis samples. As previously described, a significant decrease in EMA binding was also observed in other red cell membrane disorders, namely SAO, CDAII, elliptocytosis and pyropoikilocytosis. Careful examination of blood films can identify pyropoikilocytosis, elliptocytosis and SAO but not CDAII, frequently associated with spherocytes and that can be misdiagnosed for HS. Out of the 32 HS samples tested by SDS PAGE, the protein defect was identified in 23 (72%) cases, with 9 (39%) spectrin, 8 band 3 (35%), 5 (22%) ankyrin and one protein 4.2 deficiency. This is in agreement with previous reports, although these proportions can differ in different populations.[1,6-7]

In agreement with Bianchi *et al.*, these data confirmed that the EMA binding test is a sensitive and specific method for HS diagnosis even if the best cut-off value remains a subject of debate. In this series, the ROC curve analysis showed an optimal cut off at 10% in order to discriminate HS from controls (maximal Youden index 0.90, sensitivity 91%, specificity 99%). However, comparing HS to other hemolytic diseases (distinct from HS but including other red cell membrane disorders) indicated an optimal cut off at 14% (maximal Youden index 0.76, sensitivity 89%, specificity 87%). Furthermore, a higher cut-off value may be preferred in order to promote test specificity rather than sensitivity.

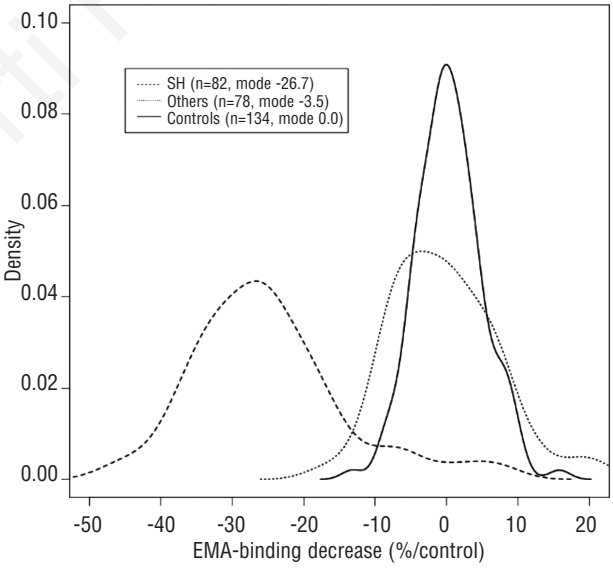

Figure 1. Density of probability of EMA binding decrease in HS (SH) *versus* hemolytic syndromes not related to red cell membrane disorders (others) and controls.

Table 1. Results of the EMA binding test in red cell membrane defect-related or non-related hemolytic syndromes.

% decrease in MFI	HS	CDAII	HE/PPK	SAO	Xerocytosis	Others	Controls
<11	9 (11%)	1	0	0	16	74 (95%)	133
11 to 15	3 (4%)	1	4	0	0	3 (4%)	1
16 to 21	11 (13%)	4	3	0	0	1 (1%)	0
>21	59 (72%)	1	1 (PPK)	4	0	0	0
Total	82	7	8	4	16	78	134

MFI: mean fluorescence intensity: HS: hereditary spherocytosis; CDAII: congenital dyserythropoietic anemias type II; HE: hereditary elliptocytosis; PPK: pyropoikilocytosis; SAO: Southeast Asian ovalocytosis; others: hemolytic syndromes with normal ektacytometry.

We conclude that the EMA binding test can be used as a first screening test for HS, provided that red cell morphology and family history are also taken into account. When family history is absent and reticulocyte count is lower than expected in hemolytic anemia, or when the EMA result is normal but HS diagnosis still suspected, a second line of investigation is required. Although not readily available, ektacytometry associated with SDS-PAGE red cell membrane protein analysis can be recommended as a second line of investigation since diagnosis of very mild HS can be challenging.

*Caroline Mayeur-Rousse,[1] Mélanie Gentil,[2] Jérémie Botton,[3,4] Madeleine Fénéant Thibaut,[5] Corinne Guitton,[6] and Véronique Picard[2,4]**

[1]Laboratoire d'Hématologie, CHRU de Hautepierre, Strasbourg; [2]Laboratoire d'Hématologie, AP-HP, CHU Bicêtre, Université Paris Sud, Le Kremlin-Bicêtre; [3]CESP UMR-S 1018, Inserm, Villejuif; [4]UFR Pharmacie, Université Paris Sud, Châtenay-Malabry; [5]Laboratoire de Biochimie, AP-HP CHU Bicêtre, Le Kremlin-Bicêtre; [6]Service de Pédiatrie, AP-HP CHU Bicêtre, Le Kremlin-Bicêtre, France

Correspondence: Véronique Picard, Laboratoire d'Hématologie, AP-HP, CHU Bicêtre, Université Paris Sud, Le Kremlin-Bicêtre, France; E-mail: veronique.picard@u-psud.fr

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