Reply to "Flow cytometry test for hereditary spherocytosis". Haematologica. 2012;97(12):e47.

The letter of Mackiewicz *et al.*[1] raises the important question concerning the definition of the cut off in flow cytometric eosin-5-maleimide (EMA) binding test. The authors use a cut off of 21% with a 'gray-zone' between 21% and 16%.[2] The cut off of 11% applied by our group was determined on the basis of the results of the Receiver Operating characteristic (ROC)curve, and subsequently validated over the years in our laboratory on approximately 800 patients with hemolytic anemia. We re-evaluated the 150 HS patients included in our study[3] considering both the 16% and 21% cut-off values proposed by Girodon *et al.*[2] The results of the analysis are reported in Table 1. In these conditions, the sensitivity of EMA binding test drastically decreases to 84% and 75%, respectively, with an increase in specificity from 98% to 100%. With regard to disease specificity, the new cut offs exclude the 2 HE patients which previously tested positive (14% decrease in fluorescence) but did not significantly improve the discrimination between HS and congenital dyserythropoietic anemia type II (CDAII) (EMA binding was still positive in 10 of 14 CDAII with 16% cut off and 6 of 14 with 21% cut off).

We analyzed in detail the 14 HS patients with a decrease in fluorescence of between 11% and 15%: 6 had spectrin deficiency, 4 had band 3 deficiency and 4 did not show any abnormality at SDS-PAGE (Table 2). Interestingly, 11 of 14 had mild or compensated anemia, and most of them had few spherocytes on peripheral blood smear. Therefore, in our experience, the 11% cut off is associated with much higher sensitivity and minimal loss in specificity compared with the 21% cut off, and is more appropriate for the diagnosis of HS in our patients with Coombs negative-hemolytic anemia. It is possible that differences in the definition of the cut-off point may depend on the clinical phenotype of the patient population examined. With reference to enzyme deficiencies, we have so far used EMA binding to test 20 patients with various defects of glycolysis and nucleotide metabolism, and normal or even increased values were always observed.

As regards SDS-PAGE of red cell membrane proteins, we perform this analysis on all those patients with a confirmed diagnosis of HS to define the biochemical abnormality, and on atypical cases to validate the diagnosis. The SDS-PAGE sensitivity and the distribution of various membrane protein defects varies among authors and the different HS populations studied.[4-9] Furthermore, we observed that ankyrin deficiency is more frequently diagnosed in childhood than in adulthood, and that splenectomy may disclose spectrin or combined ankyrin and spectrin defects that were undetectable before surgery.[9]

In conclusion, taken together, Mackiewicz's data and our observations confirm the relevance of the EMA binding test in the diagnosis of HS and suggest an opportunity for inter-laboratory standardization of this method on the same subset of patients.

Table1. Specificity and sensitivity of EMA binding test using different cut off values.

Cut off	Sensitivity	Specificity	Disease specificity*
>11%	140/150 (93%)	98%	13/14 CDAII; 2/10 HE
>16%	126/150 (84%)	100%	10/14 CDAII
>21%	112/150 (75%)	100%	6/14 CDAII

**Positive EMA binding test in other hemolytic anemias.*

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Table 2. Results of SDS-PAGE analysis, EMA binding test and osmotic fragility tests in the 14 patients with EMA-binding decrease in fluorescence comprised between 11% and 15%.

SDS-PAGE defect	Hb g/dL	Retics. (x10^9/L)	Spherocytes %	EMA-binding %	AGLT	NaCl fresh OF	NaCl Inc. OF	Pink test
Spectrin	13,3	80	11	-15%	+	N	N	+
Undetected	12,9	104	5	-15%	+	N	N	+
Undetected	12,9	144	2	-14%	+	N	N	N
Spectrin	5,5	52	3	-14%	+	N	N	+
Spectrin	6,4	250	1	-13%	+	N	N	+
Band 3	10,6	175	7	-13%	+	N	N	+
Spectrin	12,9	88	3	-13%	+	N	N	+
Spectrin	15,9	82	6	-13%	+	N	+	N
Band 3	14,9	nd	4	-12%	N	N	N	+
Undetected *	13,8	86	2	-12%	+	N	+	N
Band 3	9,1	214	9	-12%	+	N	+	N
Undetected *	12,6	394	8	-11%	+	N	N	+
Spectrin	14,2	124	2	-11%	+	N	+	+
Band 3	12,4	97	6	-11%	+	+	+	+
n.v		24-84						

*+: positive, N: normal, nd not determined. *positive family history of HS. OF: osmotic fragility.*

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