Laboratory Indices in Patients with Positive and Borderline Flow-cytometry EMA-screening Test Results for Hereditary Spherocytosis

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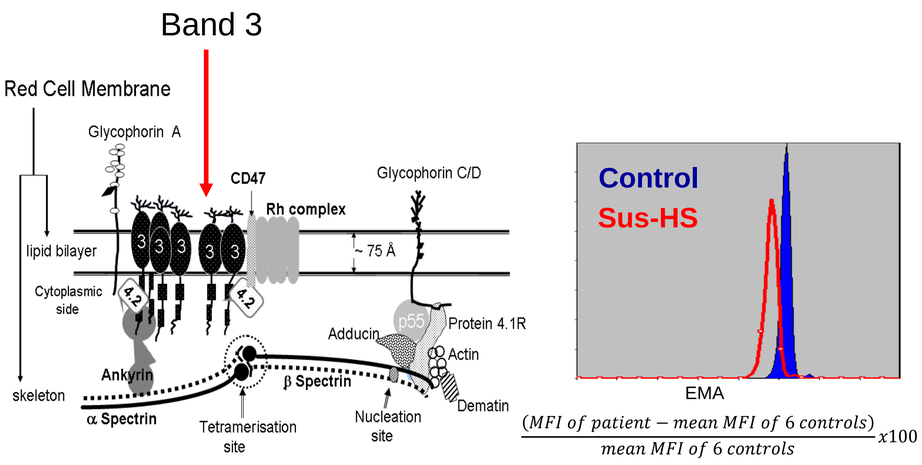
Background & Objective

- Hereditary spherocytosis (HS) is the most common hereditary hemolytic anemia (1:5000 births).
- Eosin-5-maleimide (EMA) is a fluorescent dye that binds to the ε-NH_2 group of lysine on the anion exchange protein band 3, CD47 and Rh-related proteins on the red blood cell (RBC) membrane.
- Hence, measurement of the degree of reduced EMA signal in RBCs is the basis of a validated and highly specific and sensitive flow cytometry screening test developed for detection of HS.



- Patients with HS usually show >21% lower EMA signals as compared to control specimens.
- Hence, measurement of the degree of reduced EMA signal in RBCs is the basis of a validated and highly specific and sensitive flow cytometry screening test developed for detection of HS
- However, some HS patients present with borderline staining (16-21% reduced EMA staining)

Objective

- To evaluate laboratory Indices in patients with hereditary spherocytosis (HS), with positive and borderline flow-cytometry eosin-5-melamide (EMA)-bound red blood cells screening test.

Methods

- We compared laboratory indices of 151 samples obtained from 139 different individual patients with negative, borderline, or positive EMA-test results.
- We also compared the clinical data of the patients in each EMA test results group

Conclusions

- Based on laboratory data, borderline EMA-test results may be an indication of a more severe form of HS.
- Using MCHC as a cutoff may help predict and reduce negative EMA tests without compromising sensitivity.
- This finding needs to be further validated in other FC laboratories with a large EMA test sample pool.

Results

Borderline EMA-test results were obtained for 13 patients and were associated with more severe anemia, and lower reticulocyte count and reticulocyte production index, as compared with samples with positive EMA-test results.

Table I. Patients and samples characterization

Total patients	139 (100)
Sex male (%):female (%)	70(50.3):69(49.7)
Age (y) (median + range)	0 (0-75)
Age (d) (median + range)	79.5 (0-27 333)
Total sample N (%)	151 (100)
EMA negative	115 (76.16)
EMA borderline	13 (8.6)
EMA positive	23 (15.2)

Table II. Comparison of laboratory indices between samples with negative borderline and positive EMA test

Indices	EMA Negative	EMA Borderline	EMA Positive	*P* value EMA Negative vs Positive	*P* value EMA Borderline vs Positive
Age (years) (Average ± SD)	10.25 ± 18.91 (0-74)	8.09 ± 8.89 (0-26)	7.05 ± 10.7 (0-30)	0.5	0.7
Age (years) (Median)	0	4	0		
RBCs X103/μL (range)	3.27 ± 1.13 (0.9-5.99)	3.14 ± 0.69 (2.09-3.68)	3.99 ± 0.92 (2.18-5.47)	0.0051	0.0072
Hb g/dL	9.9 ± 3.4 (3.1-20.8)	9.2 ± 2.2 (6.3-12)	12.2 ± 3.9 (6.6-20.7)	0.0057	0.015
HCT %	29.7 ± 10.2 (9.2-61.1)	26.5 ± 6.0 (18.5-34.9)	34.6 ± 11.1 (19.1-59)	0.04	0.02
Mean cell volume fL	91.8 ± 12.6 (56.2-119.6)	84.3 ± 5.7 (75.3-97.2)	83.3 ± 17.2 (28.6-109.3)	0.0064	0.84
Mean cell Hb pg	30.8 ± 4.5 (15.6-41.1)	29.2 ± 2.1 (25.1-33.4)	30.3 ± 4.4 (22.2-39.2)	0.62	0.40
MCHC g/dL	33.5 ± 1.8 (27.8-42)	34.7 ± 1.5 (32.9-37.9)	35.4 ± 1.4 (32.6-38.6)	<0.0001	0.16
Red cell distribution Width %	17.0 ± 3.2 (11.9-28.2)	19.5 ± 3.8 (11.7-25)	19.9 ± 3.5 (13.3-26.9)	0.0001	0.70
Absolute reticulocytes X109/L	164.2 ± 132.4 (1.6-713.2)	160.7 ± 147.9 (0.1-426.9)	293.7 ± 168.1 (0.2-539)	0.0002	0.03
Reticulocytes %	6.3 ± 6.1 (0.16-35.8)	5.8 ± 5.3 (0.3-16.6)	7.4 ± 3.5 (1.9-14.4)	0.43	0.30
Reticulocyte Index	2.0 ± 2.4 (0-13.3)	1.1 ± 1.3 (0-3.5)	4.1 ± 3.6 (0-11.4)	0.0013	0.01
Bilirubin total mg/dL	5.9 ± 5.0 (0-19.3)	1.5 ± 1.1 (0.5-3.6)	6.4 ± 4.6 (1.2-15.1)	0.73	0.003
Bilirubin direct mg/dL	0.9 ± 1.1 (0.2-5.7)	0.5 ± 0.2 (0.4-0.6)	0.7 ± 0.4 (0.3-1.7)	0.56	0.20
Lactate Dehydrogenase IU/L	583.2 ± 623.9 (107-3201.8)	579.6 ± 305.1 (189-623)	361.7 ± 161.8 (239-598)	0.32	0.34
Chloride mmol/L	104.3 ± 2.9 (96.6-116.4)	103.2 ± 2.2 (99.5-106)	106.8 ± 2.1 (103-110)	0.0063	0.002
B12 pg/ml	598.4 ± 406.7 (195-1846)	457.7 ± 77.1 (400.4-444.5)	777.2 ± 844.9 (180-1744)	0.54	0.47

A higher prevalence of clinical markers typical of HS were found in patients with borderline or positive as compared to negative EMA test samples

Table III. Clinical markers of hereditary spherocytosis

EMA status	Jaundice	Transfusions	Cholecystectomy	Splenomegaly	Splenectomy
EMA positive (n = 19)	37% (n = 9)	42% (n = 8)	16% (n = 3)	52.5% (N = 10)	16% (N = 3)
EMA borderline (n = 9)	44% (n = 4)	55% (n = 5)	11% (n = 1)	77% (N = 7)	11% (N = 1)
EMA negative (n = 92)	56.5% (n = 52)	33.5% (n = 31)	4.5% (n = 4)	25% (N = 23)	3% (N = 3)

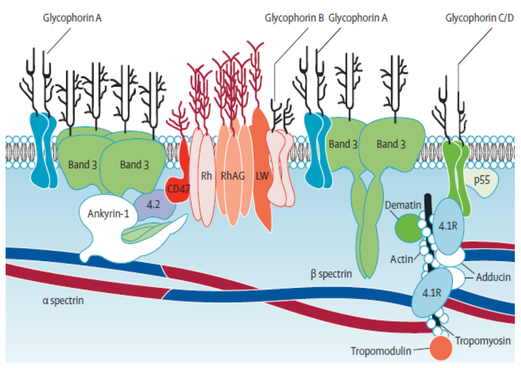
A receiving operator characteristic analysis identified MCHC < 32.5 g/dL as a cutoff, between positive/borderline and negative test results with 100% sensitivity.

MCHC ROC table

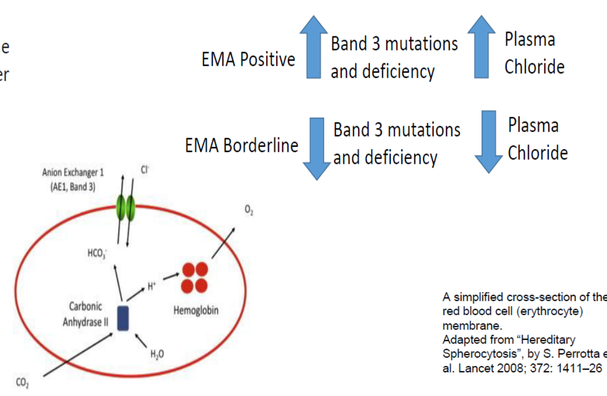
X-MCHC	Specificity (%)	Sensitivity (%)	True Pos	True Neg	False Pos	False Neg
33.81*	57.4	80.5	29	66	49	7
32.60*	28	100	36	32	83	0

MCHC= mean corpuscular hemoglobin concentration

Patients with Borderline EMA-test results may show low Band 3 mutations?



- Band 3 or anion exchanger 1 (AE1), the founding member of the Solute Carrier 4 family of bicarbonate transporters (SLC4A1), is the predominant glycoprotein of red blood cell membrane where it carries out chloride/bicarbonate anion exchange across the plasma membrane—a process necessary for efficient respiration.
- The bicarbonate is transported out of the cell in exchange for chloride



A simplified cross-section of the red blood cell (erythrocyte) membrane.
Adapted from "Hereditary Spherocytosis", by S. Perrotta et al. Lancet 2008; 372: 1411–26