ASSESSMENT OF ERYTHROCYTE DEFORMABILITY WITH THE LASER-ASSISTED OPTICAL ROTATIONAL CELL ANALYZER (LORCA)

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INTRODUCTION

Deformability is an essential feature of the red cell that allows it to move in the bloodstream and pass unharmed into the microcirculation through openings smaller than its usual dimensions (1). Energy derived from its internal glycolysis is used to both retain and change its shape and in the performance of all its rheological functions during the 120 days of its existence.

The deformability of an erythrocyte is determined by its area/volume ratio, cytoskeleton and membrane characteristics, qualitative and quantitative hemoglobin content, and enzyme activities (2).

Extension and folding are usually employed by a red cell to pass through tiny ostia at low and high pressures respectively (3).

Teitel was the first to investigate deformability in 1967 by passing red cells through $\varnothing$ 5 μ porous membranes (4). In 1976, Evans & Hochmuth (5) aspirated cells with a micropipette-and calculated two parameters: the modulus of elasticity (μ), which expresses the membrane's resistance to extension, and viscosity (η), which expresses its resistance to folding. Several workers have since used this method to determine these parameters in sickle cell anemia, spherocytosis, ellipsocytosis and thalassemia (6-10).

Erythrocyte deformability has been studied in several erythrocyte

disorders by the use of Laser-Assisted Optical Rotational Cell Analyzer (LORCA).

MATERIALS AND METHODS

Erythrocyte deformability was automatically evaluated with a Laser-Assisted Optical Rotational Cell Analyzer (LORCA).

Red cells suspended at 29.7 mPa*s in highly viscous polyvinylpyrrolidone are placed in a transparent rotating cylinder, exposed to torsional stresses and deformed into an ellipse. A laser beam passing through the suspension at right angles is diffracted. The diffracted light pattern is measured by an image analyzer, projected on a screen and processed by a computer, which converts the diffraction ratio into a numerical value. The curve built up for each sample at different torsional stresses represents a cell elongation index (EI), i.e. its deformability. The torsional stresses ranged from 0.30 to 30 Pascals.

Blood samples from fifty subjects were studied:

- 22 normal
- 6 with severe hypochromic hyposideremic anemia
- 2 hemizygotes and 1 heterozygote for G-6PD deficiency
- 10 thalassemics: 3 intermediate (1 HbC/β°, 1 homozygote β for Orkin's haplotype VI, 1 $\beta^{\circ}/\beta\delta$ Sicilian type) and 7 heterozygotes for β Th
- 6 with hereditary spherocytosis (including 2 with structural alteration of the spectrin β chain)
- 3 with type II congenital dyserythropoietic anemia (CDA II).

RESULTS

The general and hematological data of the 28 patients (sex, age, splenectomy, Hb, RBl, Hct, MCV, MCH, PTL, WBC), EI and diagnoses are listed in table 1. The EI values of the patients are compared with the mean for the normal subjects in table 2.

Tab. 1 - Hematological findings of patients.

Patients	Age aa	Sex	Hb g/dl	RBC ($\times 10^{12}$/l)	Hct %	MCV fl	MCH pg	WBC $\times 10^{9}$/l	PTL $\times 10^{9}$/l	E.I.	Diagnosis
n.1 (C.A.)	30	F	11.8	4.07	35.8	87.9	29.0	6	411	0.581	βTh-hetero
n.2 (D.V.)	66	M	13.1	6.37	40.3	63.1	20.6	4.56	160	0.575	βTh-hetero
n.3 (B.S.)	30	F	12.4	5.58	38	68.1	22.2	7.7	210	0.574	βTh-hetero
n.4 (B.E.)	30	M	14.0	6.27	42.1	67.2	22.3	6.5	196	0.567	βTh-hetero
n.5 (M.A.)	27	F	10.2	4.03	35.7	88.5	25.3	6.5	240	0.563	βTh-hetero
n.6 (G.F.)	57	F	13.1	5.71	40.3	70.6	23.0	8.9	210	0.541	βTh-hetero
n.7 (B.G.)	28	M	13.8	6.29	41.1	65.3	22.0	7.7	164	0.528	βTh-hetero
n.8 (M.E.)	45	M	7.1	3.05	23.3	71.1	21.7	5.99	129	0,547	Th. $\beta^{\circ}/\beta\delta$
n.9 (T.V.)	58	M	9.5	4.91	33.1	65.6	19.4	12.45	645	0.351	β^{+}/β^{+} haplotype VI Splenect.
n.10(T.M.)	63	M	9.8	5.25	29.6	59.8	18.9	8.55	163	0.122	HbC-β°Th
n.11(P.M.)	56	F	9.3	2.93	31.7	87.7	31.8	6.84	192	0.561	Spherocytosis/βSpectrine
n.12 (P.U.)	25	M	11.9	3.83	38	99.1	31	12.45	170	0.544	Spherocytosis/βSpectrine
n.13 (P.L.)	12	M	8.1	3.4	32	94.1	23.8	7.9	190	0.365	H.S.
n.14 (P.V.)	14	F	7.0	3.5	30	85.7	20	6	160	0.256	H.S.
n.15 (F.N.)	25	F	12.8	4.71	35	74.3	27.2	12.3	272	0.558	H.S.
n.16 (F.V.)	28	M	15.3	4.82	42	87.1	31.7	10	544	0.542	H.S.
n.17 (T.M.)	51	M	12.8	4.33	38.3	88.9	29.8	9.54	591	0.531	CDA II/Splenect.
n.18 (C.M)	48	M	12.5	4.23	37	87.4	29.5	8.2	290	0.519	CDA II
n.19 (C.G.)	55	M	12.8	4.13	40	99.7	31.2	10.4	568	0.498	CDA II/Splenect.
n.20 (P.E.)	35	M	15.9	4.79	46.8	97.8	33.2	8.39	261	0.599	G6PD deficiency
n.21 (L.F.)	17	M	14.8	5.32	45	84	27	5.5	234	0.612	G6PD deficiency
n.22 (P.V.)	55	F	13.5	4.5	42	93.3	30	6.8	300	0.587	G6PD deficiency
n.23 (C.A.)	40	M	8.4	4.12	30	72.8	20.3	6.7	370	0.607	A.Hypochrom. Hyposid.
n.24 (S.L.)	42	M	7.5	3.91	28	71.6	19.1	5.8	390	0.611	A.Hypochrom. Hyposid.
n.25(C.M.)	72	F	7.2	3.47	25	71.4	20.1	4.4	352	0.594	A.Hypochrom. Hyposid.
n.26 (M.R.)	51	F	7.7	3.71	27	72.9	20.1	5.9	372	0.585	A.Hypochrom. Hyposid.
n.27 (V.L.)	35	M	12.2	4.86	38	78	25	7.3	334	0.566	Ovalcytosis Hyposid.
n.28 (D.V.)	65	F	8.0	3.95	32	81	20.2	4.05	472	0.605	A.Hypochrom.Hyposid.
Normal subjects	47+/ -9,5*	M-F	13,9 +/0,9	4.561 +/-0.450	43,3 +/-48	91,3 +/2,3	30,5 +/-1,4	6.3 +/-1.3	227 +/-26.8	0.59 +/-0.01	

* mean +/- 1 SD

Tab. 2 - Elongation Index in 28 anemic patients.

G6PD deficiency	**0.612**
A.Hypochrom.Hypos.	**0.611**
A.Hypochrom.Hypos.	**0.607**
A.Hypochrom.Hypos.	**0.605**
G6PD deficiency	**0.599**
A.Hypochrom.Hypos.	**0.594**
Elongation Index (normal index)	**> 0.590 +/-0.01**
G6PD deficiency	**0.587**
A.Hypochrom.Hypos.	**0.585**
βTh hetero	**0.581**
βTh hetero	**0.575**
βTh hetero	**0.574**
Ovalcytosis Hiposid	**0.566**
βTh hetero	**0.567**
βTh hetero	**0.563**
Spherocytosis/βSpctrine	**0.561**
HS	**0.558**
β°/βδ Th	**0.547**
Spherocytosis/βSpectrine	**0.544**
HS	**0.542**
βTh hetero	**0.541**
CDA II	**0.531**
βTh hetero	**0.528**
CDA II	**0.519**
CDA II	**0.498**
H.S.	**0.365**
β^+/β^+haplotypeVI Splenec	**0.351**
H.S.	**0.256**
HbC/ β°Th	**0.122**

DISCUSSION

The above-normal EIs in patients with hypochromic hyposideremic anemia show that their red cells are more plastic. The increase in their surface/ volume ratio makes them more deformable. This may serve as a sort of compensation for the depressed oxygen transfer caused by their reduced Hb content.

The normal EIs of the G-6PFD-deficient patients reflect the absence of both membrane structure changes and Heinz bodies in the intercritical stage, though there was an enzyme alteration in the pentose pathway impairing the ability to produce reduced compounds such as NADPH.

During or immediately before a hemolytic crisis, the deformability of these red cells is presumably altered due to the presence of Heinz bodies. The EIs of the βTh heterozygotes were normal or slightly reduced, whereas those of the intermediate thalassemics were frankly pathological. The low EI of the homozygote for Orkin's haplotype VI (No.9) was also pathological (0.351). β chain synthesis was reduced, whereas that of the α chains, which precipitate in the erythroblasts to form Fessas bodies (11), was normal. In cases of this kind, red cells are usually destroyed in the spleen and these bodies are removed via the splenic endothelial reticulum. In unsplenectomised subjects, in fact, evidence of this removal can be seen in the presence of a large number of dacryocytes. Moreover, their deformed cells do not regain their shape because the elasticity of their membrane is reduced.

Our patient had been splenectomised and his peripheral blood picture included numerous target cells and orthochromatic erythroblasts, but no dacryocytes. The circulating erythrocytes contained the α-chain precipitates known to be the prime cause of reduced membrane deformability (9).

The mixed HbC/β° Th heterozygote (No.10) possessed only HbC and a small amount of HbF. HbC is a variant with substitution of lysine for glutamic acid at the sixth position of the β chain. This structural abnormality reduces the solubility of the entire molecule and promotes its precipitation in the form of crystals (12). Loss of red cell deformabilty is the result of increased viscosity and rigidity (13). Despite his very low EI (0.122), this patient displayed no evidence of reduced peripheral perfusion, presumably because HbC crystallisation takes place when the molecule is oxygenated, and not in the microcirculation when the red cells contain deoxygenated Hb.

The patient with Sicilian-type β°/βδ intermediate thalassemia (No.8) presented bilateral malleolar trophic ulcers and reduced peripheral oxygen perfusion (demonstrated by percutaneous oximetry). His EI was reduced (0.547), but much higher than in case 9.

The reduced EIs of the six patients with hereditary spherocytosis illustrate the close relationship between erythrocyte deformability and the

morphology, surface/volume ratio and intrinsic properties of the red cell membrane.

The EIs of the three CDA type II (HEMPAS) patients were also reduced. This disease is caused by changes in the structure of membrane glycoproteins (14) due to either N-acetylglucosaminyl transferase (GnT II) or α-mannosidase II deficiency that alter the erythroblast membrane (typically doubled in the electron microscope) and reduce deformability, in addition to being the cause of a characteristic binuclearity.

In conclusion, this LORCA study of erythrocyte deformability in 28 anemia patients showed that the EI was:

1) increased in hypochromic hyposideremic anemia;

2) normal in G-6PD deficiency;

3) markedly reduced in intermediate thalassemia to varying degrees in function of the molecular diversity of the Hb defect. Imbalanced polypeptide chain synthesis impairs the red cell membrane, changes its shape and reduces its deformability;

4) reduced (as in the literature) in hereditary spherocytosis, including two cases with a structural spectrin β-chain anomaly;

5) reduced in three non-correlated cases of CDA II, in full agreement with the membrane structure abnormalities underlying the erythroblast and red cell alterations associated with this disease.

The erythrocyte deformability of 28 patients with anemia was evaluated with the laser-assisted optical rotational cell analyzer (LORCA), an image analyzer that converts into numerical form the degree of refraction of a laser beam induced by red cells subjected to a range of torsional stresses. The patients were 10 thalassemics, including three with intermediate forms (1 HbC/β°, 1 homozygote β for Orkin's haplotype VI, 1 β°/βδ Sicilian type) and seven heteroygotes for β Th; six with hereditary spherocytosis (including 2 with structural alteration of the spectrin β chain); three with type II congenital dyserythropoietic anemia (HEMPAS), two hemizygotes and one heterozygote for G-6PD deficiency, and six with severe hypochromic hyposideremic anemia.

Red cell deformability was reduced in intermediate thalassemia, hereditary spherocytosis and HEMPAS, normal in heterozygous β thalassemia and G-6PD deficiency, and increased in hypochromic hyposideremic anemia. These results show that erythrocyte deformability can be impaired by an Hb chain imbalance, membrane and cyto skeleton structure anomalies and changes in the red cell area/volume ratio.

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