LETTER TO EDITOR

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Hereditary Spherocytosis Characterized by Decreased Spectrin/Band-3 Ratio*

Dziedziczna sferocytoza związana z obniżonym stosunkiem spektryny/białka pasma 3

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Hereditary spherocytosis (HS) is a relatively common hemolytic disorder characterized by wide heterogeneity at the clinical, biochemical, and genetic levels. Various abnormalities in the skeletal proteins of the red cell membrane are responsible for HS. The most common biochemical finding is spectrin deficiency, usually expressed as a decrease in the spectrin/band-3 ratio, which leads to decreased mechanical stability of the red cell membrane and loss of surface area.

Reported here is HS associated with a decreased spectrin/band-3 ratio in a 50-year-old woman after splenectomy (Tab. 1, Fig. 1, patient 2). In one of her daughters (patient 4), who had also had a splenectomy, spherocytes were seen only single in her blood smear and no decreased spectrin/band-3 ratio was detected. In the second daughter (patient 3), the spectrin/band-3 ratio was at the lower end of the normal range. A patient from another family, a 48-year-old woman (patient 5) with HS, was tested. She had also had a splenectomy. Spherocytes were not seen in her blood smear and no decrease in the spectrin/band-3 ratio was detected.

Table 1. Protein ratios

Tabela 1. Stosunki poszczególnych białek

Protein ratios normal values	HS Patients (Pacjenci z dzieczizną sferocytozą)			
(Stosunki białek)	p2	p3	p4	p5
Spectrin/band 3	0.90	1.03	1.10	1.15
n.v. = 1.08 ± 0.06	0.88	1.00	1.07	1.03
Ankyrin/band 3	0.20	0.24	0.20	0.23
n.v. = 0.26 ± 0.02	0.27	0.18	0.22	0.19
Protein 4.1/band 3	0.23	0.24	0.23	0.26
n.v. = 0.21 ± 0.05	0.18	0.18	0.30	0.26
Protein 4.2/band 3	0.23	0.17	0.19	0.19
n.v. = 0.18 ± 0.02	0.24	0.24	0.19	0.19

The cytoskeleton was studied after membrane preparation. Membrane proteins were analyzed by 4–10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gradient gel [1–3] and then stained with Coomassie blue. The density of spectrin in the membrane was calculated from the ratio between the integrated area of spectrin (bands 1 and 2) and that of band 3 (spectrin/band-3). The densities of ankyrin, protein 4.1, and protein 4.2 were also calculated from the ratio between the area of each protein and that of band 3.

In patient 2 (the mother) the ratios of ankyrin, protein 4.1, and protein 4.2 to band 3 were within the normal range, whereas a decreased spectrin/band-3 ratio was found (Tab. 1, Fig 1). In patients 3 and 4 (her daughters), all these protein ratios were within the normal range. Table 1 summarizes the membrane protein ratios for the patients and controls (six normal subjects). In patient 4 (a daughter) who had had splenectomy, only single spherocytes were seen in her blood smear and no reduction in spectrin was found. In patient 5 (from another family) who had had splenectomy, spherocytes were not seen in her

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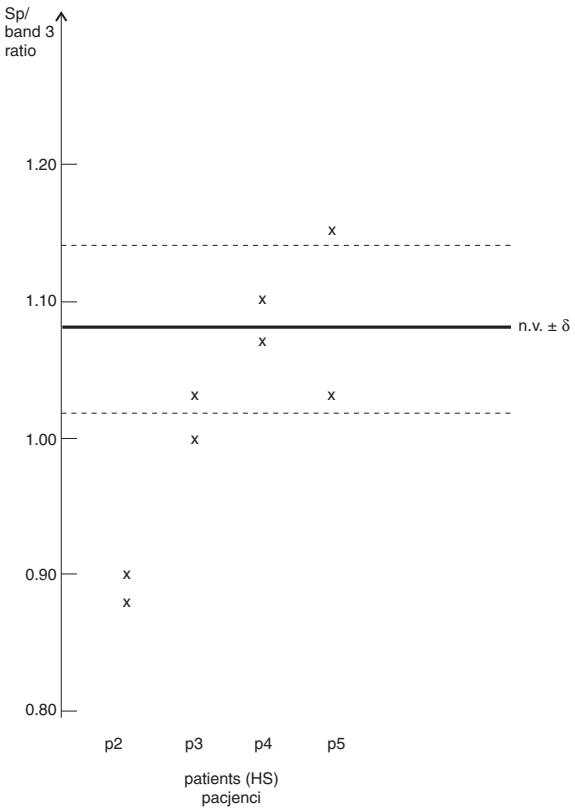

Fig. 1. Spectrin/band-3 ratio in patients with HS (patients 2, 4, and 5 were splenectomized) and normal values ±δ from 6 normal subjects

Ryc. 1. Stosunek spektryny do białka pasma 3 u pacjentów z dziedziczną sferocytozą (pacjent 2, 4, 5 po splenektomii) i prawidłowe wartości ±δ od 6 zdrowych osób z grupy kontrolnej

blood smear and no reduction in spectrin was found. The ratios of akyrin, protein 4.1, and protein 4.2 to band 3 were within the normal ranges.

Spectrin deficiency is the main feature of HS patients. Erythrocytes from most patients with typical HS show some degree of spectrin deficiency, which is thought to play a principal role in destabilization of the lipid bilayer. The extent of spectrin deficiency is usually mild and correlates well with clinical severity, the degree of spherocytosis, and response to splenectomy [4]. About one third of patients have a mild form of HS, with slight reticulocytosis. They have compensated hemolysis. A recent observation showed that serum erythropoietin is inappropriately high in patients with mild HS, maintaining bone marrow hyperplasia. The diagnosis may be difficult because spherocytes are evident in only two third of cases [5].

Spectrin deficiency is the main feature of the majority of HS patients, and in cases of decreased spectrin/band-3 ratio it can be a diagnostic tool. It has been demonstrated that the loss of cell surface area and decreased mechanical stability are directly related to the decrease in membrane spectrin density expressed as the spectrin/band-3 ratio. The results show that evaluation of membrane spectrin permits a diagnosis of HS. Patients with HS have been defined based on specific protein deficiencies in the red blood cell membrane involving spectrin, ankyrin, band 3, and protein 4.2 [6]. Mutations of the genes encoding these proteins are still being studied. To determine the fine molecular nature of spectrin deficiency, which is the main feature of the majority of HS patients, genetic studies are necessary.

References

[1] **Cutillo S, Pinto L, Nobili B, Miraglia del Giudice E, Iolascon A:** Spectrin/band 3 ratio as diagnostic tool in hereditary spherocytosis. Eur J Pediatr 1992, 151, 35–37.

[2] **Laemmli UK:** Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature. 1970, 227, 680–685.

[3] **Pacuszka T, Panasiewicz M:** Photochemical labeling of human erythrocyte membranes with radioiodinatable azidosalicylic acid derivative of globoside. Biochim Biophys Acta 1995, 1257, 256–273.

[4] **Gallager PG, Forget BG:** Sperctrin genes in health and disease. Semin Hematol 1993, 30, 1, 4–21.

[5] **Eber S, Lux SE:** Hereditary spherocytosis – defects in protein that connect the membrane skeleton to the lipid bilayer. Semin Hematol 2004, 41, 2, 118–141.

[6] **Hassoun JN, Vassiliadis JN, Murray J, Najstad PR, Rogus JJ, Ballas SK, Schaffer F, Jarolim P:** Characterization of the underlying molecular defect in hereditary spherocytosis associated with spectrin deficiency. Blood 1997, 90, 1, 398–406.

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