Detection of Stages of Autoimmune Hemolytic Anemia by Evaluating Erythrocyte Deformability and Density

E. S. Shurkhina, V. M. Nesterenko, I. L. Lisovskaya, N. V. Tsvetaeva, S. V. Kolodei, O. F. Nikulina, and F. I. Ataullakhanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 9, pp. 316-319, September, 2004
Original article submitted December 26, 2003

Study of erythrocyte density and deformability in patients with hemolytic anemia, including long-term monitoring of 5 patients, helped us to characterize the pathological processes leading to changes in the erythrocyte population at different terms of the disease and to detect its main stages (agglutination, pathological dehydration, combination of pathological dehydration and microvesiculation, hemolytic crisis, and remission).

Key Words: *erythrocyte deformability; erythrocyte density; AIHA*

Autoimmune hemolytic anemia (AIHA) is characterized by the presence of antibodies to patient's own erythrocytes provoking assembly of complement component complexes [7]. The intricate interactions of erythrocytes with antibodies and complement components in AIHA lead to various changes in the erythrocyte population provoked by agglutination, dehydration [4], and microvesiculation [8] of red blood cells, as well as sequestration of damaged erythrocytes and release of reticulocytes of different degree of maturity into the blood flow.

We analyzed the type and degree of erythrocyte damage during different periods of AIHA, examined their density and deformability.

MATERIALS AND METHODS

AIHA was diagnosed on the basis of results of routine tests (Coombs' test, polybrene test). Blood for the analysis was collected from the ulnar vein and stabilized with heparin. Resuspending solution contained 10 mM HEPES, 3.5 mM KCl, and NaCl in amount needed for preparing solutions with 290-170 mOsm osmolarity, pH 7.4.

Hematological Research Center, Russian Academy of Medical Sciences, Moscow

Erythrocyte distribution by their density (EDD) was evaluated by the Danon—Marikovskii phthalate method, approximated by Bolzman's function, and the following parameters were found: mean density of erythrocytes; EDD width (density interval covering 60% erythrocytes, excerpt 20% lightest and 20% heaviest cells); I_{max} (maximum on EDD curve) — the maximum percentage of erythrocytes in the 0.004 g/liter density interval; percentage of abnormal cells; light fraction consisting of cells with a density <1.086 g/ml (reticulocytes); heavy fraction (cells with density >1.112 g/ml (dehydrated cells, sphero- and microspherocytes) [3]. Control group consisted of 10 healthy volunteers aged 23-42 years (3 women and 7 men).

Erythrocyte deformability was evaluated by the filtration osmotic method [1] based on modulation of erythrocyte filtration at different osmolarity of the medium. The measurements were carried out using Ida1 hemorheometer and Cip1M kinetic filtrometer. Both devices were developed and made at Laboratory of Physical Biochemistry of Hematological Research Center. Using Ida1, it is possible to measure the time of filtration of a fixed volume of fluid and the Ucr parameter depending on the ratio between erythrocyte surface area and its volume (osmolarity at which erythrocyte suspension is no longer filtered because all pores of the filter are blocked). Under selected conditions (3-μ pores, 7.3 cm^{-2} pore density, 10 mm filter

0007-4888/04/1383-0280

diameter, 7 μ filter thickness, 250 μl measured volume; 60 mm H_2O pressure, 0.1% hematocrit; pH 7.4; temperature 24°C), all pores of the filter are blocked, if the studied sample contains at least 30±10% unfilterable erythrocytes [1]. In donors Ucr=195±5 mOsm [1,3].

The kinetic filtrometer recorded dynamic changes in the velocity of filtration of diluted blood. The percentage of unfiltered cells at different osmolarity was determined after mathematical processing of the kinetic curve [2].

RESULTS

The study of erythrocyte density and deformability in 29 patients with AIHA, including long-term (3-5 years) monitoring of 5 patients, helped us to characterize the pathological processes leading to modification of the erythrocyte population during different periods of the disease and to detect its main stages.

Agglutination was detected not once in 7 patients with incomplete thermal antibodies (subgroup 1, n=8) and in 9 patients with cold antibodies (subgroup 2, n=12) and manifested by decreased width of EDD and increase of I_{max} (Table 1, Fig. 1, *a*). The width of whole blood EDD was decreased because of agglutination of erythrocytes with different density and formation of aggregations with averaged density. The mean density of erythrocytes was close to normal (Table 1). Washout led to destruction of aggregations and increased EDD width (Fig. 1, *a*). Erythrocyte deformability changed greatly during the agglutination stage.

Pathological dehydration (PDH) was detected in 9 patients examined 1-17 times (n=25). This condition can persist for many months [3].

The most pronounced differences from the normal were increased mean density of erythrocytes, increased heavy fraction of erythrocytes, and decreased Ucr (Table 1, Fig. 1, *b*). Decrease in Ucr indicates increased ratio of erythrocyte surface area to its volume [1]. These erythrocytes can be filtered at lower osmolarity compared to normal cells.

In 12 patients examined 1-10 times (n=24) erythrocyte PDH was combined with microvesiculation. This combination is characterized by increased mean density of erythrocytes, EDD width, and heavy fraction (Table 1, Fig. 1, *c*). Morphological analysis of the blood showed sphero- and microspherocytes (up to 40%). EDD at this stage was similar to EDD in hereditary microspherocytosis (Fig. 1, *c*). Erythrocyte deformability was deteriorated (Table 1), because of lowered ratio of cell surface area to volume in spherocytes and microspherocytes [9,10].

Hemolytic crisis was observed in 20 patients examined 1-24 times (n=70). The light fraction was in-

TABLE 1. Parameters Characterizing Erythrocyte Density and Deformability at Different Stages of AIHA

Stage	Number of patients	Number of analyses (n)	I_{max}, %	EDD width, g/ml	Mean density of erythrocytes, g/ml	Light fraction, %	Heavy fraction, %	Ucr, mOsm	Hematocrit, %
Control group	10	10	42.9±4.5	0.006±0.001	1.099±0.001	0.6±0.3	0.3±0.3	195±5	35-50
Agglutination									
subgroup 1	7	8	62.1±9.2	0.004±0.001	1.099±0.002	1.1±1.2	0	185±10	40.4±4.5
subgroup 2	9	12	73.5±15.7	0.002±0.001	1.098±0.003	2.4±2.3	0	203±24	34.0±8.0
PDH	9	25	36.9±6.9	0.008±0.002	1.104±0.003	0.5±0.6	8.6±7.7	177±4.7	42.5±3.8
PDH+microvesiculation	12	24	27.2±8.2	0.013±0.005	1.105±0.003	1.1±0.9	18.8±15.0	205±14	36.1±5.2
Hematological crisis	20	70							
subgroup 1		55	19.4±5.7	0.023±0.008	1.098±0.014	19.0±11.4	21.2±11.8	248±27	30.6±6.4
subgroup 2		15	38.3±11.5	0.010±0.005	1.094±0.004	22.9±19.1	0	214±12	28.9±5.9
Remission	5	11	40.8±6.1	0.007±0.001	1.097±0.001	3.5± 0.8	0.1±0.1	194± 2	39.0±5.3

creased in all of these patients (Fig. 1, *d*), as well as Ucr. Heavy fraction was increased in 55 cases (subgroup 1; Table 1, Fig. 1, *d*) and was absent in 15 cases (subgroup 2; Table 1). Deterioration of erythrocyte deformability at this stage was due to the appearance of immature reticulocytes, and in subgroup 1 also due to the presence of spherocytes and microspherocytes. The presence of these cells was confirmed by morphological analysis of blood smears.

Remission was observed in 5 patients examined 1-5 times (n=11). Moderate reticulocytosis was still observed during this stage; due to it there were negligible changes in EDD (the light fraction was slightly increased and the mean density decreased). Other parameters were normal (Table 1).

The number of unfiltered cells changed with decreasing medium osmolarity at different stages of AIHA (Fig. 2). During hemolytic crisis numerous unfiltered cells appeared even when osmolarity was close to normal.

Hence, 5 stages of AIHA were detected by the filtration osmotic method for evaluating the erythrocyte deformability and phthalate method for measuring erythrocyte distribution by density: agglutination, PDH, PDH+microvesiculation, hemolytic crisis, and remission. As erythrocytes are different, they differently bind antibodies and complement components. Therefore different types of pathologically changed cells can be present at any stage, but cells characteristic of this or that stage predominate. The light fraction (reticulocytes) predominates in the blood during the hemolytic crisis, but cells with normal and increased density are also present (dehydrated cells, sphero- and microspherocytes) (Fig. 1, *d*).

The pathogenesis of AIHA depends on the disease stage. The pathogenetic factor of agglutination is dete-

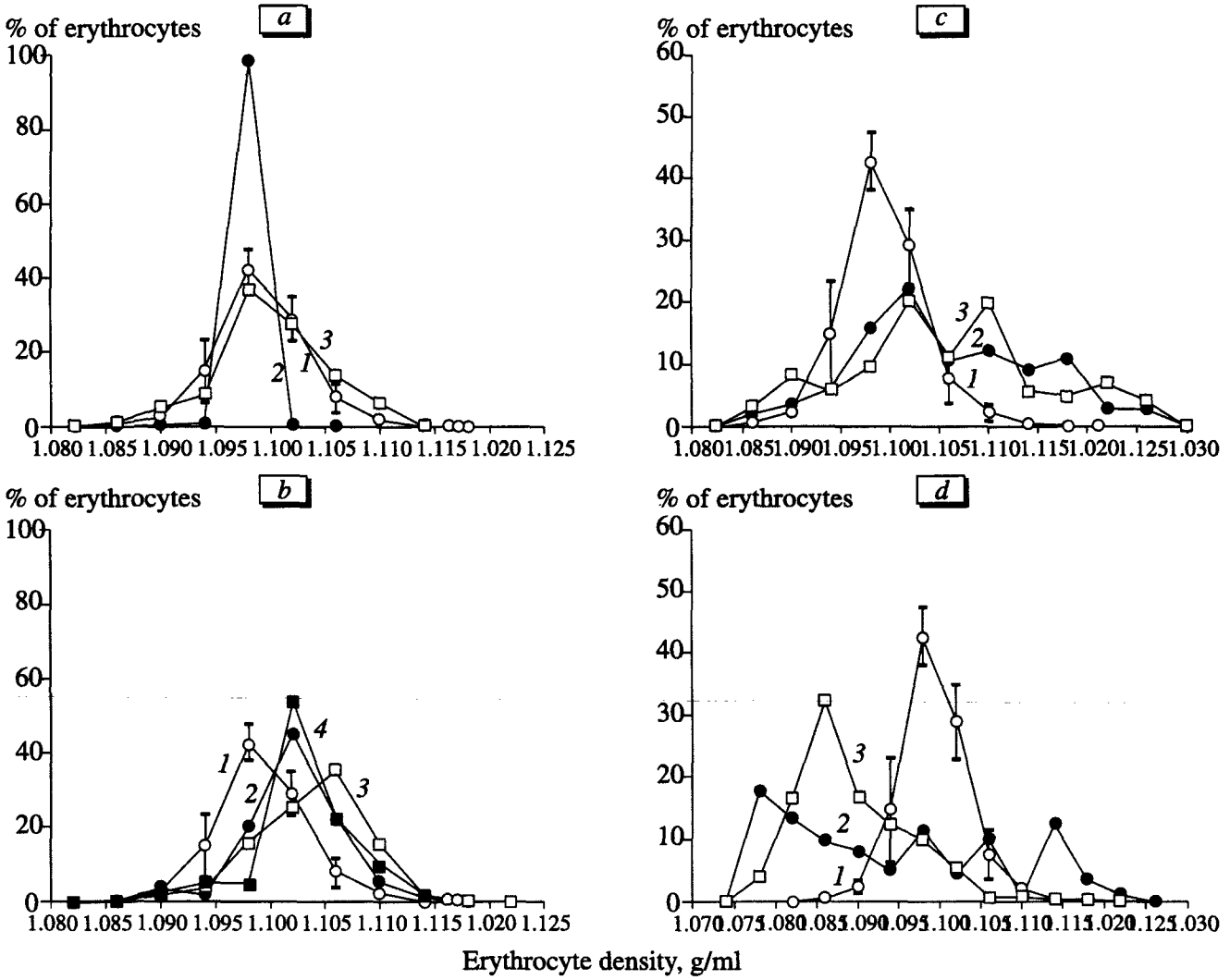

Fig. 1. Distribution of erythrocytes by density at different stages of autoimmune hemolytic anemia. *a*) agglutination: *1*) control, *2*) blood, *3*) washed erythrocytes; *b*) pathological dehydration: *1*) control, *2-4*) different patients; *c*) pathological dehydration in combination with microvesiculation: *1*) control, *2*) autoimmune hemolytic anemia, *3*) hereditary microspherocytosis; *d*) hemolytic crisis: *1*) control, *2*) increased light and heavy fractions, *3*) increased light fraction.

rioration of microcirculation and occlusion of small vessels, and in many cases decreased number of circulating erythrocytes (decrease of hematocrit). In PDH increase of intracellular viscosity results in slower modification of the erythrocyte shape than is needed for microcirculation, which results in worse delivery of oxygen to organs and tissues [9]. Moreover, erythrocyte dehydration promotes their adhesion to the endothelium [10]. The presence of erythrocytes with poor deformability (Ucr>N) in the blood of patients with PDH concomitant with microvesiculation leads to deterioration of microcirculation. Erythrocytes with poor deformability are sequestrated by the spleen [6], which determines low hematocrit and splenomegalia at this stage of disease. After splenectomy the changes in EDD and deformability characteristic of this stage can persist for many months. PDH and microvesiculation are provoked by the increase in intracellular Ca^{2+} content, and hence, drugs modulating Ca homeostasis should be used with care during these stages. A drop of hematocrit is the most dramatic episode in hemolytic crisis; one more hazardous factor is the presence of numerous poorly deformed stress reticulocytes, deteriorating blood rheology because of their poor deformability.

The study of erythrocyte density and deformability will help to correct timely the treatment protocols and to monitor their efficiency. Since the results of the study are computer-processed, they can be easily added to the program of computer diagnosis.

The study was supported by the Russian Foundation for Basic Research (grant No. 02-04-48155).

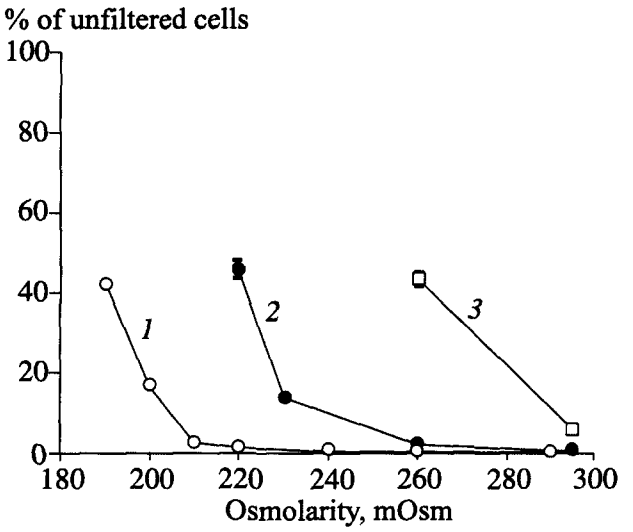

Fig. 2. Relationship between the number of unfiltered cells and medium osmolarity at different stages of autoimmune hemolytic anemia (results obtained on a Cip 1M kinetic filtrometer). *1*) normal level; *2*) combination of pathological dehydration and microvesiculation; *3*) hemolytic crisis.

REFERENCES

1. I. L. Lisovskaya, E. S. Shurkhina, V. M. Nesterenko, *et al.*, *Biol. Memb.*, **15**, 300-307 (1998).
2. Yu. M. Rozenberg, E. S. Shurkhina, I. L. Lisovskaya, *et al.*, *Tromboz, Gemostaz, i Reologiya*, No. 3, 33-39 (2001).
3. E. S. Shurkhina, N. V. Tsvetaeva, S. V. Kolodei, *et al.*, *Gematol. Transfuziol.*, **48**, 19-22 (2003).
4. J. A. Halperin, C. Brugnara, and A. Nicholson-Weller, *J. Clin. Invest.*, **83**, 1466-1471 (1989).
5. S. Hasegawa, T. Nomura, M. Imo, *et al.*, *Clin. Hemorheol.*, **14**, 571-584 (1994).
6. N. Mohandas, W. M. Phillips, and M. Bessis, *Seminars Hematol.*, **16**, 95-115 (1979).
7. H. J. Muller-Eberhand, *Annu. Rev. Immunol.*, No. 4, 503-528 (1986).
8. M. Pascual, H. U. Lutz, G. Steiger, *et al.*, *J. Immunol.*, **51**, 397-404 (1993).
9. W. H. Reinhart and S. Chien, *Am. J. Physiol.*, **248**, 473-479 (1985).
10. A. Rivera, P. Jarolim, and C. Brugnara, *Blood*, **99**, 357-363 (2002).