Pathomorphosis of Erythrocytes in Patients with Acquired Valvular Heart Diseases and under Conditions of Their Correction

V. V. Novitskii, N. V. Ryazantseva, Yu. Yu. Vecherskii*, O. I. Lapitskaya, and E. A. Stepovaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 3, pp. 336-340, March, 2004
Original article submitted June 10, 2003

Scanning electron microscopic study of the morphology and surface architectonics of erythrocytes in patients with acquired aortic and mitral valve diseases showed signs of morphological restructuring of the erythrocyte population. Reversibly transformed transitional red blood cells and irreversibly changed prehemolytical and degenerative erythrocytes were much more incident in these patients than in donors. The number of functionally intact biconcave discocytes notably decreased in comparison with donors. Morphological heterogeneity of the erythrocyte pool increased during the immediate period after replacement of heart valves with mechanical disc prostheses. Disorganization of the surface relief of red blood cells persisted 12-24 months after surgery.

Key Words: *erythrocyte; acquired heart valvular disease; scanning electron microscopy*

Acquired cardiac valvular diseases belong to the most severe and prevalent diseases of the cardiovascular system. Apart from hemodynamic disorders and toxic infection syndrome changes in the blood system occupy an important place in the clinical picture of valvular heart disease, mainly of rheumatic origin. Hypoxia progressing under conditions of augmenting circulatory insufficiency stimulates production of red blood cells by the bone marrow compartment of the erythron possessing a high regeneration potential. However, rapid maturing of erythrocytes is fraught with the release into the bloodflow of qualitatively defective cells characterized by high vulnerability and short life span [8,9]. The mechanisms of increased destruction of erythrocytes in rheumatic heart disease can be associated with direct damaging effects of toxins and streptococcal enzymes on red blood cells, immune and autoimmune aggression, and mechanical injury to erythrocytes during blood passage through heart valves involved in rheumatic process [5,6].

Surgical intervention aimed at repair of the function of cardiac valve can also augment the imbalance of erythrocytic homeostasis [10,12,13]. This problem acquires special importance because of routine use of artificial circulation during surgery [11]. If disorders in the peripheral component of the erythron during the immediate postoperative period are quite justified, structural and functional changes in the pool of circulating erythrocytes in delayed postoperative period are obviously underevaluated. In light of this we studied surface architectonics and morphology of red blood cells as the integral parameters reflecting erythrocyte homeostasis [7] at different stages of surgical treatment of patients with acquired heart disease.

MATERIALS AND METHODS

The study was carried out on 22 patients (17 men and 5 women aged 34-48 years) with aortic and 23 patients

Siberian State Medical University, Tomsk; *Institute of Cardiology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. ***Address for correspondence:*** ryazan@mail.tomsknet.ru. Ryazantseva N. V.

0007-4888/04/1373-0298

(16 men and 7 women aged 28-50 years) with mitral valve disease of rheumatic origin. The diagnosis was confirmed by clinical, laboratory, and instrumental (ultrasonic, angiographic) findings. The patients were examined before the intervention and 7 days, 12 and 24 months after replacement of the involved heart valves with mechanical disc prostheses (LIKS and EMIKS). Control group consisted of 30 age- and sex-matched donors.

Surface architectonics of erythrocytes was examined by scanning electron microscopy [4]. To this end, blood samples were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and dehydrated in ascending alcohols. The resultant cell suspension was layered onto an aluminum matrix, dried, and coated with ultrathin layer of silver. The resultant samples were examined under a REM-200 electron microscope at accelerating voltage of 35 kV, 0.63 A current, 35° slope. In order to characterize quantitatively the distribution of morphological forms of erythrocytes, 1000 cells were counted in each sample using classifications [3,4]. The external cell diameter and the size of central concavity were measured in 50 arbitrarily selected discocytes and the percent ratio of these parameters was estimated. The preparations were photographed under a JEM-100 electron microscope.

The significance of differences between the groups was evaluated using Student's *t* test, and nonparametrical Mann—Whitney's test (in case of deviation from normal distribution).

RESULTS

Electron microscopy the peripheral blood erythrocytes in patients with acquired heart disease showed appreciable changes in red blood cell morphology. The percentage of transformed cells at different stages of the ontogeny increased. Ellipsoid erythrocytes, cells shaped as flat disks, discocytes with a crest, cells with a small process in the center of the concavity, discocytes with numerous conical protrusions with round apices, and mulberry-shaped erythrocytes were often seen in the blood of patients with aortic valve defects (Fig. 1, *a*). These transformations were reversible, the total percentage of transitional forms of erythrocytes was 13.72±0.35%, which 1.3-fold surpassed the corresponding parameter in donors. The percentage of irreversibly transformed cells (spherical, cupola-shaped, and loose ball-shaped) in the peripheral blood of patients increased 2-fold (to 4.9±0.2% *vs.* 1.92±0.11% in donors). The content of poorly identified degenerative erythrocytes was 6-fold higher than in donors. The percentage of functionally intact biconcave discocytes was only 80.75±0.46%. The ratio of concavity diameter to external diameter of mature erythrocytes surpassed the normal (52.27±0.23 *vs.* 36.85±0.23%, $p<0.001$), this attested to the development of adaptive planocytosis of red blood cells. Common regularities in changes of the surface architectonics of erythrocytes in patients with mitral valve disease were similar to those described above (Table 1, Fig. 1, *b*).

TABLE 1. Distribution of Morphological Forms of Erythrocytes (in %) with Different Surface Relief in Patients with Acquired Valvular Heart Disease and After Its Surgical Correction (Electron Microscopy Data, $X\pm m$)

Group		Morphological forms of erythrocytes			
		biconcave discocytes	reversibly transformed transitional forms	irreversibly transformed prehemolytical forms	degenerative forms
Donors		87.47±0.88	10.50±0.27	1.92±0.11	0.11±0.01
Patients with aortic valve disease					
before surgery		80.75±0.46*	13.72±0.35*	4.90±0.20*	0.61±0.04*
after surgery:	7 days	76.91±0.39*+	13.74±0.53*	8.52±0.65*+	0.83±0.01*++
	12 months	78.14±0.42*++	14.85±0.74*	6.27±0.17*+	0.72±0.07*
	24 months	82.63±0.23*+++	12.74±0.36*+++	4.10±0.31*+++	0.53±0.05*
Patients with mitral valve disease					
before surgery		79.24±0.84*	15.44±0.78*	4.73±0.38*	0.58±0.05*
after surgery:	7 days	77.10±0.37*+++	15.41±0.33*	6.61±0.18*++	0.87±0.05*+
	12 months	78.41±0.46*	15.53±0.23*	5.37±0.22*	0.67±0.04*
	24 months	82.73±0.51*++	13.34±0.29*+	3.48±0.19*	0.44±0.03*

Note. *$p<0.001$ compared to donors; +$p<0.001$, ++$p<0.01$, +++$p<0.05$ compared to not operated patients.

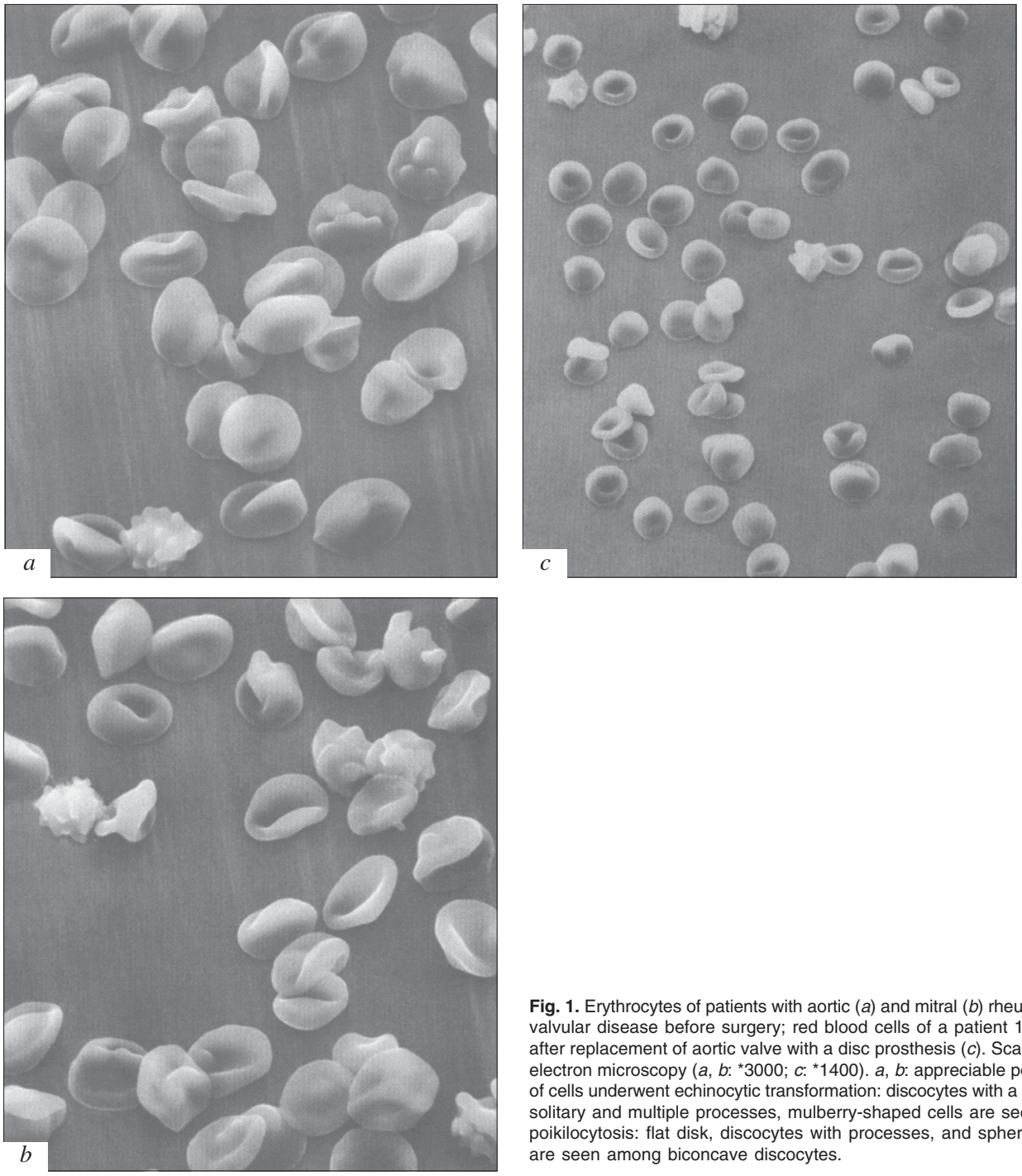

Fig. 1. Erythrocytes of patients with aortic (*a*) and mitral (*b*) rheumatic valvular disease before surgery; red blood cells of a patient 1 year after replacement of aortic valve with a disc prosthesis (*c*). Scanning electron microscopy (*a*, *b*: *3000; *c*: *1400). *a*, *b*: appreciable portion of cells underwent echinocytic transformation: discocytes with a crest, solitary and multiple processes, mulberry-shaped cells are seen; *c*: poikilocytosis: flat disk, discocytes with processes, and spherocyte are seen among biconcave discocytes.

The electron microscopic findings in erythrocytes from patients with acquired aortic and mitral valve diseases of rheumatic origin prove structural and functional deficiency of circulating red blood cells. However, erythrocyte as the object of research attracts special attention when we speak about valve replacement surgery. This interest is explained primarily by the fact that cardiopulmonary bypass surgery is fraught with hazards of mechanical and physicochemical injuries to erythrocytes [2,10]. Mechanical injury to red blood elements can be caused by negative pressure of vacuum pumps, roller pumps, arterial filter, and local turbulent flows in the extracorporeal contour. Physicochemical factors causing damage to erythrocytes are abnormal osmolarity of the primary volume and low surface tension created by antiphosphomilane used for impregnation of filters in oxygenators and reservoirs. In addition, blood contact with synthetic materials of the extracorporeal contour under conditions of cardiopulmonary bypass leads to activation of a cascade

reactions inducing systemic inflammatory response, which can also affect circulating erythrocytes [1,2].

During the early period (day 7) after valve replacement with mechanical disc prosthesis the percentage of biconcave discocytes decreased still more in comparison with the preoperative period, reaching 76.91±0.39% in patients with replaced aortic valve. The number of transitional cell forms remained virtually unchanged, but the content of prehemolytically transformed and degenerative erythrocytes increased. Pronounced transformation of erythrocytes was observed during the early postoperative period in patients with replaced mitral valves (Table 1).

The data of morphological studies of erythrocytes during delayed postoperative period (12 and 24 months after implantation of artificial heart valves) deserve special interest. The available results of electron-microscopic visualization of red blood cells indicate persistent morphological heterogeneity of circulating erythrocyte pool despite stabilization of hemodynamics and normalization of other essential functions of the body and improvement of patients' quality of life after the intervention (Fig. 1, *c*). By the end of the 2nd year after valve replacement the content of transformed erythrocytes (discocytes with a crest, echinocytes, spherocytes, stomatocytes) in the blood remains high (18%). Presumably, increased content of cells with abnormal relief can result from persistent production of qualitatively deficient erythrocytes. This situation is justified under conditions of strained erythropoiesis because of high level of erythrocyte hemolysis. Destruction of erythrocytes in patients with implanted mechanical heart valves can be caused by (along with direct injury to erythrocytes contacting with the prosthesis surface) hemodynamic factors (formation of turbulent blood flow zones, *etc.*) [1,6] and immune disorders persisting under conditions of progressing rheumatic process [13].

As we see, pathogenesis of the involvement of red blood system in the pathological process in patients with acquired valvular diseases and under conditions of surgical correction is very intricate. However, the facts described in this paper persuasively prove the necessity of long-term hematological monitoring of patients after heart valve replacement.

REFERENCES

1. L. S. Barbarash, N. A. Barbarash, and I. Yu. Zhuravleva, *Heart Valve Bioprostheses: Problems and Prospects* [in Russian], Moscow (1995).
2. S. L. Dzemeshkevich and L. W. Stivenson, *Mitral Valve Diseases. Function, Diagnosis, Treatment* [in Russian], Moscow (2000).
3. B. V. Ionov and A. M. Chernukh, *Byull. Eksp. Biol. Med.*, **92**, No. 12, 749-751 (1981).
4. G. I. Kozinets and Yu. A. Simovart, *Surface Architectonics of Peripheral Blood Cells in Health and Blood System Diseases* [in Russian], Tallinn (1984).
5. V. I. Makolkin, *Acquired Heart Diseases* [in Russian], Moscow (1986).
6. V. A. Nasonova, *Rheumatic Diseases* [in Russian], Moscow (1997).
7. V. V. Novitskii, N. V. Ryazantseva, E. A. Stepovaya, *et al.*, *Atlas. Clinical Pathomorphosis of Erythrocyte* [in Russian], Tomsk (2003).
8. S. I. Ryabov and G. D. Shostka, *Molecular Genetic Aspects of Erythropoiesis* [in Russian], Leningrad (1973).
9. F. E. Fainshtein, O. K. Gavrilo, and I. S. Turbina, *Hemopoiesis Depressions* [in Russian], Moscow (1987).
10. R. J. Cerfolio, T. A. Orszulak, R. C. Daly, and H. V. Schaff, *Eur. J. Cardiothorac. Surg.*, **11**, No. 3, 479-484 (1997).
11. R. Koppensteiner, A. Moritz, W. Schlick, *et al.*, *Am. J. Cardiol.*, **67**, No. 1, 79-83 (1991).
12. F. A. Kuypers, *J. Heart Valve Dis.*, **7**, No. 4, 387-395 (1998).
13. K. Takeuchi, S. Suzuki, K. Koyama, *et al.*, *Thorac. Cardiovasc. Surg.*, **41**, No. 2, 104-106 (1993).