

## PACEMAKER IMPULSES DECREASE THE RED BLOOD CELL DEFORMABILITY

MUHTEREM ERCAN\*  
HAYRETTIN AKDENİZ\*\*  
ISMAIL TUNCER\*\*\*  
HASAN IRMAK\*\*

*SUMMARY: The aim of this study is to examine the in vitro effects of pacemaker electrical impulses on red blood cell deformability. The study was performed on 14 venous blood samples. The red cell suspension prepared from blood sample of each volunteer was divided into two parts; first was used to measure control erythrocyte deformability and the second for measuring erythrocyte deformability after pacemaker impulses were applied. In addition, Hct, Hb, MCV, MCH, and MCHC values from the two groups were determined.*

*As a result, it was observed that pacemaker impulses significantly decrease the red cell deformability. Hct, Hb, MCV, MCH, and MCHC values in the filtered red cell suspensions, however, did not change after the exposure.*

*Our data suggested that the pacemaker electrical impulses alter the erythrocyte deformability resulting in microcirculation disorders, which in turn may produce pathological changes.*

*Key Words: Red cell deformability, pacemaker.*

### INTRODUCTION

Numerous complications have been reported in the literature, concerning implanted and functioning cardiac pacemakers. These complications include a variety of entities, some of which are secondary to displacement or pacemaker malfunction. If these events are excluded, the most conspicuous complica-

tions are mainly thromboembolic incidents and infections, some of which remain unexplained.

Taking these complications into account we aimed in this study at investigating the *in vitro* effects of pacemaker impulses on RBC deformability. And we conclude that disturbed RBC deformability in pacemaker implanted patients is leading to microcirculation disorders. Consequently, we consider that in the pathogenesis of thromboembolic events and of unexplained infections, decreased RBC deformability may be playing a significant role besides the other factors uncovered previously.

\*From Department of Physiology, University of Yüzüncü Yil, Van, Türkiye.

\*\*From Department of Bacteriology and Infectious Disease, University of Yüzüncü Yil, Van, Türkiye.

\*\*\*From Department of Internal Medicine, University of Yüzüncü Yil, Van, Türkiye.

**MATERIALS AND METHODS**

**Blood samples preparation**

The study was performed on 14 venous blood samples taken from healthy young volunteers (aged 19-22) following their informed consent. Aliquots were collected into tubes containing EDTA following minimal venous occlusion.

Blood samples were centrifuged (15 min. at 3500 x g), and the buffy coat was carefully removed. Three milliliters of concentrated red cells were aspirated from the middle of the red cell column, removing at least 98% of the white cells and 97% of the platelets and other debris (1). The packed red cells were washed twice and diluted with isotonic PBS (Na-K-phosphate buffered saline: pH 7.4) to prepare the RBC suspensions at haematocrit values of 20%.

The red cell suspension of each volunteer was divided into two parts. One of them was used for measuring control erythrocyte deformability. The other sample was used for measuring the same parameter after exposure to electrical impulses of the pacemaker. Each of the erythrocyte suspensions was filtered within 10 minutes. All measurements were performed within 1 hour of venipuncture at room temperature of 20 °C.

Also, to determine whether there was a true permanent effect on the RBC, a blood sample was exposed to the same electrical impulses in a separate chamber for 30 minutes, then transferred to the filtration apparatus and filtered in the absence of any more applied pulses.

**Measurement of hematologic parameters**

Cell counter analyzer (Coulter MaxM) was used to deter-

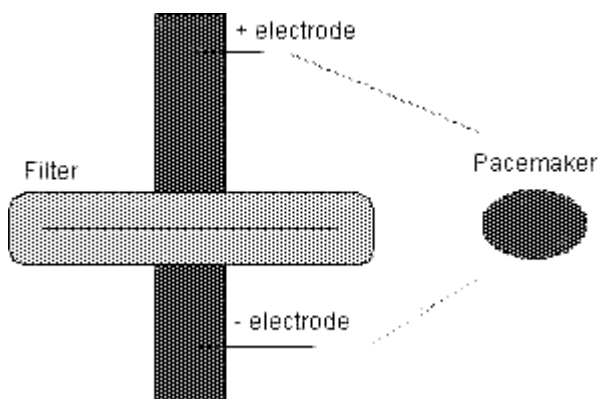


Figure 1: Filtration apparatus. The positive pole of the pacemaker was applied to red cell samples to be filtered from above and the negative pole from below the filter.

mine red blood cells, white blood cells, platelets, Hb, Hct, MCV and MCHC values.

**Filtration apparatus preparation**

Filtration apparatus was developed from systems described in the literature (2,3) (Figure 1). The same apparatus was used in filtration of RBCs for measuring control RBC deformability and RBC deformability with pacemaker impulses. Pacemaker (NEG 5967 Pulse generator DE 3308606K, Met-ronic BU HOLLAND) impulses were applied (75/min and 45 mV) to red cell samples during filtration with electrodes connected to the positive pole pacemaker from above and to the negative pole from below the filter (Figure1).

In order to exclude the possibility that electrical impulses may effect the pores of the filter, and thus reduce the filtration, plasma and physiological serum were filtered with and without impulses and in addition, the filter was examined under a light microscope; and its pores were measured. It was thus confirmed that no change occurred in pore diameters.

**Measurement of RBC deformabilities**

Deformability experiments were performed in accordance with guidelines set by the International Committee for Standardization in Hematology, Expert Panel on Blood Rheology (4).

RBC deformability was measured by filtration of each suspension through 5 µm filters (Nucleopore, Membra-Fil and Filinert, Costar Sci. Corp.) under a constant pressure of 10 cm H<sub>2</sub>O. The passage time of 1 ml RBC suspension was determined opto-electronically. For each measurement, a new filter from the same batch was used.

The filter was filled with suspension before measurements. The tap was opened and suspension began to pass through the filter, the computer's chronometer worked automatically as soon as suspension was seen by a photocell in the exit of the filter and when 1 ml of suspension passed, the chronometer stopped automatically. The tap was closed simultaneously. Hematologic values were evaluated by the cell counter analyzer.

**Interpretation of RBC deformability**

At first, a blank value was obtained by recording the time required for 1 ml of buffered saline to pass through the filter. A qualitative measurement of filtration for control RBC deformability and RBC deformability with pacemaker impulses are expressed as a deformability (filtrability) index, defined as follows: the time required for 1 ml of red cell suspension to filter divided by the time required for an equal volume of buffer to filter.

Figure 2: DI values in control and pacemaker impulses applied groups.

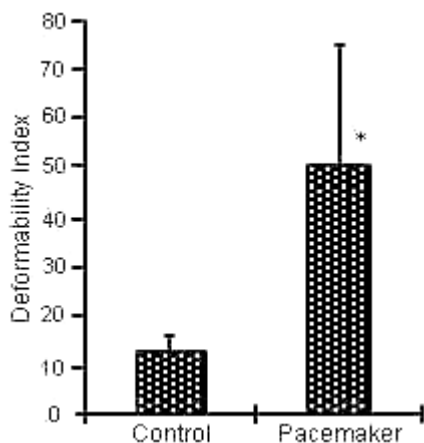


Figure 3: Cell transit values in control and PIA groups.

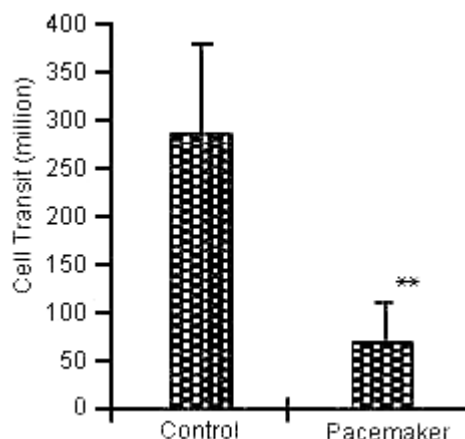


Table 1: Deformability index values and cell transit values in control and pacemaker impulses applied groups.

Groups	Deformability Index	Cell Transit (million)
Control group	11.67 ± 4.05	285.8 ± 99.4*
PIA group	50.56 ± 25.6*	72.9 ± 33.0

\*P=0.000045 (n=14) \*\*P=0.0000029, (n=14)

By this method, the deformability index relates directly to RBC deformability. The deformability index is expressed as the average of two repeated tests.

In addition, RBC counts present in 1 mm<sup>3</sup> filtrate passed through the filter were measured by cell counter analyzer and then RBC counts passing through the filter in 1 second were calculated with mathematical formula. This was expressed as Cell Transit Value (CTV). RBC counts filtering through the filter were compared with each other.

After the filtration, hematologic profiles of the filtered erythrocyte suspensions were determined as mentioned above, and examined by light microscopy to detect evidence of hemolysis or crenation of red cells, neither of which was noted.

**Statistical analysis**

Statistical analysis was carried out using the Student's paired two tailed t-test. Statistical significance was accepted at P <0.05. The results are expressed as means ± SD.

**RESULTS**

The measured deformability index (DI) of RBC for control group was found as 11.67 ± 4.05, and for pacer-

maker impulses applied (PIA) group was found as 50.56 ± 25.6. Their statistical difference is very significant (t = 5.98, P = 0.000045) (Figure 2 and Table 1).

The number of RBC's passing through the filter per second is also interesting: this figure is 285.8 ± 99.4 for the control group and 72.9 ± 33.0 million cells for the pacemaker implanted group and their difference is very highly significant (t = 7.80, p = 0.0000029) (Figure 3 and Table 1).

In measurements of Hct, Hb, MCV, and MCHC, there is no statistically significant difference (Table 2).

**DISCUSSION**

Deformability is a relatively general term that describes the ability of a particle (RBCs, WBCs etc.) to change its shape in response to a deforming force. The stress is usually applied from outside the cell, e.g. by fluid shear stress or local membrane aspiration but it can also originate from within the cell, e.g. RBCs

Table 1: Deformability Index values and cell transit values in control and pacemaker impulses applied groups.

Groups	Hct	Hb	MCV	MCH	MCHC
Control group	20.82 ± 4.3	7.13 ± 1.6	81.2 ± 7.9	27.85 ± 4.2	34.19 ± 2.6
PIA group	21.58 ± 2.6	7.43 ± 1.0	79.14 ± 7.8	27.07 ± 3.6	33.9 ± 1.3

swelling in hypotonic medium or fiber formation in deoxygenated sickle cells. The extent, rate, and mode of deformation depend on the magnitude, rate and direction of the stress. Therefore, the stress-strain relationships are complex and can not be described by a single deformability parameter. Furthermore, the ability of RBCs to deform involves several factors including the geometric features of the cell, and the rheological properties of the intracellular fluid as well as of the cell membrane, and the interactions of these components (6).

In this study, we investigated the *in vitro* effect of electrical impulses produced by a pacemaker apparatus on RBC deformability. Measurements were made for control of RBC deformability under control conditions and during pacemaker impulses application. There were statistically important differences between the deformability of the two groups. In addition, Hct, Hb, MCV, MCH and MCHC values were measured in RBC groups, which revealed insignificant differences between the two groups indicating that electrical impulses had no significant effect on these parameters (Table 2). It was formerly claimed that, an increase in MCHC and Hb, and a decrease in MCV and Hct values result in a reduction in RBC deformability (7). This was not however confirmed by our measurements.

In addition to the factors mentioned above, temperature may also be effective on RBC deformability. Therefore, the temperature was monitored during these experiments. All measurements were made at 20°C constant temperature. Pacemaker impulses led to no local heating in filtered suspensions during experiment, consequently, the differences obtained could not be attributed to the changes in temperature.

The nucleopore filter is not electrically *conducting*, therefore, it can be considered to be loaded with static

electricity. Hence, the apparatus was filled with RBC suspension and it was exposed to electrical impulses for 10 min. Thus any electrical charge developing on the filter could be identified and recorded.

To determine whether there is a true permanent effect on the RBC, a new sample was exposed to the electrical impulses in a separate chamber for 30 minutes. The cell suspension was then transferred to the filtration apparatus and filtered without further exposure. The results of this measurement were identical with that of the control recordings.

Based on this observation abnormal RBC deformability which we uncovered in this study, can only be attributed to the electrical impulses of the pacemaker apparatus.

An *in vitro* experiment, however, does not reflect an *in vivo* event completely, it gives us an opinion about the pathogenesis of events seen in patients with permanently implanted pacemakers. The positive electrode of this apparatus is implanted to the patients *in vivo* into the subclavian vein, in contact with the blood directly, similar to the way in the tubes in *in vitro* experiment, while the negative electrode is inserted subcutaneously.

The above referred deformability may be caused by a slight overall loss of red cell fluidity together with the existence of a subpopulation of more markedly rigid erythrocytes. Recent data confirm that rheological impairments exist in the course of bacterial infection. Although there is uncertainty about several aspects of the pathogenesis of this event, haemorheological disturbances can play a role in the impairment of microvascular flow. Abnormalities in blood flow are known to be precipitating factors for ischaemic events. Therefore, the rheological behaviour of the main leuko-

cyte subpopulations (granulocytes and mononuclear cells etc.) should be studied. It is uncertain which mechanism is involved in altering the white cell rheology in infections (8).

In the literature, numerous complications have been described concerning cardiac pacemakers. Complications associated with permanent pacemaker implantation includes lead dislodgement, infection, hematoma formation, skeletal muscle stimulation, ventricular arrhythmia, migration of the pulse generator, pneumothorax, venous thrombosis or stenosis, pulmonary embolism, and skin erosion (8-10).

The incidence of symptomatic venous thrombosis have been rarely reported, despite the fact that contrast venography is abnormal in 30 to 45 percent of patients, total subclavian vein obstruction occurs in 8 to 20 percent (11,12). Superior vena cava occlusion is another well-recognized and potentially serious complication, (3,14,15). Cases of pulmonary, cardiac and cerebral embolism have been reported in patients with permanent pacemaker apparatus (16,17). It has been considered that although the etiology of these thromboembolic events was probably multifactorial in some patients, permanent cardiac pacemaker implantation has probably a predisposing role. Pacemaker implantation should therefore be considered as an embolic risk factor as suggested by previous epidemiological studies (14,16,18). In our experience, we propose another adverse effect of pacemaker apparatus, acting on RBC deformability by resulting in microcirculation disorders in addition to reasons mentioned previously.

Pacemaker pocket infection is a potentially serious problem after permanent pacemaker implantation. All infections encountered have been confirmed by the same organisms (*Staphylococcus aureus* and *Staphylococcus epidermidis*) being recovered from blood cultures (19-21). Infections are reported in ratios reaching 10% among the late complications of pacemakers (22,23). The most common complication of this consists of septicaemia and endocarditis (15,24,25). They require removal of the entire pacing equipment in addition to appropriate antimicrobial treatment (20,26,27).

Besides these infections, some unexplained infections such as pericarditis and a case of cholestatic hepatitis have been reported in the literature in these patients. Pericarditis have been considered to be related to hypersensitivity or autoimmunity (28). In cholestatic hepatitis, neither a biliary obstruction nor an infection of the liver could be found to explain the liver injury (29). Based on this knowledge, there may be a number of factors in these complications to be considered. Perhaps a decrease in RBC deformability has probably played a role in these events beside the other facilitating reasons.

In conclusion, with this preliminary study, we present an important influence of pacemaker implantation, which we believe to contribute in explaining these complications. We suggest that other prospective studies on pacemaker patients should be made to further elucidate the complications of the pacemaker implantation.

#### REFERENCES

1. Hanss M : Erythrocyte filtrability measurement by the initial flow rate method. *Biorheology*, 20:199-211, 1983.
2. Reid HL, Barnes AJ, Lock PJ, Dormandy JA and Dormandy TL : Technical Methods: A Simple method for measuring erythrocyte deformability. *J Clin P-Pathol*, 29:855-858, 1976.
3. Ernest E and Matrai A : Altered Red and White Blood Cell Rheology in Type II Diabetes. *Diabetes*, 35:1412-1415, 1986.
4. International Committee for Standardization in Haematology : Guidelines for measurement of blood viscosity and erythrocyte deformability. *Clin Hemorheo*, 6:439-453, 1986.
5. Chien S : Red cell deformability and its relevance to blood flow. *Ann Rev Physiol*, 49:177-192, 1987.
6. Turner S, Williams AR and Rees JMH : The role of mean corpuscular haemoglobin concentration in limiting the storage life of human blood. *Vox Sang*, 52:177-181, 1987.
7. Ciuffetti G, Ott C, Mercuri M, Lombardini R and Neri C : Infection and tissue ischemia: What role does the rheologic behavior of blood cells play? *Z-Gesamte-Inn-Med*, 44:555-557, 1989.
8. Chauhan A, Grace AA, Newell SA, Stone DL, Shapiro LM, Schofield PM and Petch MC : Early complications after dual chamber versus single chamber pacemaker implantation. *Pacing and Clinical Electrophysiology*. 17:2012-2015, 1994.
9. Krein A, Eigel P, Buchwald J and Elert O : Current intra- and early postoperative results of transvenous pacemaker implantation. *Helv-Chir-Acta*, 58:485-488, 1992.

10. Sisson D, Thomas WP, Woodfield J, Pion PD, Luethy M and Delellis LA : Permanent transvenous pacemaker implantation in forty dogs. *J-Vet-Intern-Med*, 5:322-331, 1991.
11. Ferguson R, Mccaughan B, May J and Waugh R : Venous occlusion: a rare complication of transvenous cardiac pacing. *Aust-N-Z-J-Surg*, 62:977-980, 1992.
12. Barold SS, Zypes DP : Cardiac pacemakers and antiarrhythmic devices. In *Heart Disease a textbook of cardiovascular medicine*. Ed by Eugene Braunwald. WB Saunders Company, pp 726-755, Philadelphia, 1992.
13. Bestetti RB, Teno LA and Greguolo C : Superior vena cava syndrome as a complication of permanent cardiac pacemaker in a patient with Chagas' heart disease. *Acta-Cardiol*, 48:579-582, 1993.
14. Chytil V and Svejda J : Thrombosis of the superior vena cava as a complication of permanent cardiac pacing. *Vnitř Lek*, 37:496-500, 1991.
15. Murakami Y, Matsuno Y, Izumi S, Murakami R, Tanaka K, Morioka S and Moriyama K : Superior vena cava syndrome as a complication of DDD pacemaker implantation. *Clin-Cardiol*, 3:298-300, 1990.
16. Ahdout DJ, Damani PM, Ultan LB, Damani PM and Ultan LB : Recurrent acute pulmonary emboli in association with acute myocardial infarction. *Chest*, 96:682-684, 1989.
17. Lascault G, Durr A, Juillard JB, Thomas D, Frank R, Gauthier JC and Grosgeat Y : VVI mode cardiac pacing: cause or risk factor of cerebral embolism? *Arch-Mal-Coeur-Vaiss*. 85:1527-1531, 1992.
18. May KJ, JR, Cardone JT, Stroebel PP and Riba AL : Streptokinase dissolution of a right atrial thrombus associated with a temporary pacemaker. *Arch-Intern-Med*. 148:903-904, 1988.
19. Fry DE, Fry RV and Borzotta AP : Nosocomial blood-borne infection secondary to intravascular devices. *Am-J-Surg*, 167:268-272, 1994.
20. Kirito K, Shindo H, Chiba N, Koyama T and Yokoyama H : Sick sinus syndrome caused by amyloidosis associated with multiple myeloma. *Rinsho-Ketsueki*, 33:532-536, 1992.
21. Mounsey JP, Griffith MJ, Tynan M, Gould FK, Macdermott AF, Gold RG and Bexton RS : Antibiotic prophylaxis in permanent pacemaker implantation: a prospective randomised trial. *British Heart Journal*, 72:339-343, 1994.
22. Gutierrez A, Iturralde P, Gutierrez FE, Martinez RN, Martinez RMA, Romero L, Hernandez D and Gonzalez HJA : Permanent pacemaker implantation. Indications and results. *Arch-Inst-Cardiol-Mex*, 60:59-64, 1990.
23. Yener A, Ayrancioglu K, Aslamaci S, Ekici E and Ikizler C : An unusual late complication following surgical repair of tetralogy of Fallot. *J-Cardiovasc-Surg-Torino*, 31:587-588, 1990.
24. El-Kohen M, Millaire A, Leroy O, Beuscart C, Vanjak D, Sivery B, Senneville E and Ducloux G : Infectious endocarditis of the tricuspid valve in patients with endocardial pacemakers. *Arch-Mal-Coeur-Vaiss*, 83:1855-1857, 1990.
25. Teno LA, Bestetti RB, Oliveira WM, Zanardi MC, Freitas OC, Cozac EP, Deus DM and Castilho OT : Tricuspid endocarditis: a rare infectious complication of cardiac pacemaker in a patient with Chagas' disease--a case report. *Angiology*, 44:580-583, 1993.
26. Barold SS and Zypes DP : Cardiac pacemakers and antiarrhythmic devices. In *Heart Disease: A textbook of cardiovascular medicine*. Ed by Eugene Braunwald, WB Saunders Company, pp 726-755, Philadelphia, 1992.
27. Byrd CL, Schwartz SJ and Hedin N : Lead extraction. Indications and techniques. *Cardiol-Clin*, 10:735-748, 1992.
28. Kawecka JK : Pericarditis: classification, etiology, pathogenesis. *Folia-Med-Cracov*, 32:15-22, 1991.
29. Schuppel R and Probst M : Acute cholestatic hepatitis and the sudden rise of the pacing threshold 5 weeks after VVI-pacemaker implantation. *J Inter Med*, 236:353-356, 1994.

Correspondence:

Muhterem Ercan

Gümüşköy Yapı Koop.

Buhara Sokak, No: 9

Pursaklar, Ankara,

TÜRKİYE.

e-mail: ercanm.@netbilgisayar.com.tr.