

# THE ERYTHROCYTE ADHESIVENESS/AGGREGATION TEST (EAAT) IN THE PERIPHERAL BLOOD OF PATIENTS WITH ISCHEMIC HEART AND BRAIN DISEASE WITH NORMAL FIBRINOGEN CONCENTRATIONS

D. Zeltser<sup>1</sup>, R. Rotstein<sup>1</sup>, O. Rogowski<sup>1</sup>, R. Fusman<sup>1</sup>, I. Shapira<sup>1</sup>, V. Prochorov<sup>1</sup>, N. M. Bornstein<sup>2</sup>, A. Roth<sup>3</sup>, G. Keren<sup>3</sup>, D. Avitzour<sup>4</sup>, N. Arber<sup>5</sup>, A. Eldor<sup>6</sup>, S. Berliner<sup>1\*</sup>

Departments of Internal Medicine "D"<sup>1</sup>, Neurology<sup>2</sup>, Cardiology<sup>3</sup>, Gastroenterology<sup>5</sup> and Hematology<sup>6</sup>, Tel Aviv Sourasky Medical Center, Tel Aviv, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel,  
and Timorim<sup>4</sup> Technologies, Jerusalem, Israel

\* Department of Internal Medicine "D", Tel-Aviv Sourasky Medical Center, 6 Weizman Street  
Tel Aviv 64239, Israel  
Phone: x972.3.6973313  
Fax: x972.3.6974961  
E-mail: ronace@inter.net.il

Received: 8.5.2000, Final version: 20.9.2000

## ABSTRACT

Previous studies have documented the presence of increased red blood cell aggregability in patients with ischemic heart and brain diseases. We adapted a simple slide technique and image analysis to reveal the state of erythrocyte adhesiveness/aggregation (EAA) in the peripheral blood of 206 such patients and in 174 controls. A significant ( $P < 0.0001$ ) correlation was noted between the EAA state and both fibrinogen concentrations and erythrocyte sedimentation rate in the whole cohort. Noteworthy was a highly significant increment in the EAA state in a subgroup of 49 patients who had normal fibrinogen concentrations when compared to matched healthy controls. This was also the case in 82 patients who had a normal erythrocyte sedimentation rate. Thus, the EAA assay appears to have the advantage of revealing the presence of acute phase response sticky proteins not detected by either the erythrocyte sedimentation rate or clottable fibrinogen in some patients with a documented ischemic vascular disease.

## ZUSAMMENFASSUNG

Vorangegangene Untersuchungen haben ein erhöhtes Aggregationsvermögen roter Blutkörperchen bei Patienten mit kardialen und cerebralen Ischämien zeigen können. Mit einer einfachen rheologischen Analysetechnik wurde in dieser Studie die Erythrozytenadhäsion und -aggregation (EAA) im peripheren Blut von 206 solcher Patienten untersucht und mit 174 Kontrollpersonen verglichen. Es wurde eine signifikante ( $p < 0.0001$ ) Korrelation zwischen der EAA und der Fibrinogenkonzentration sowie der Blutkörperchensenkungsgeschwindigkeit (BKS) bei den untersuchten Personen gefunden. Erwähnenswert war ein hochsignifikanter Anstieg der EAA in einer Untergruppe von 49 Patienten, welche im Vergleich zugesunden Kontrollpersonen normale Fibrinogenkonzentrationen hatten. Das war auch bei 82 Patienten der Fall, die eine normale BKS aufwiesen. Folglich scheint die Untersuchung der EAA den Vorteil zu bieten, die Präsenz von adhärenenten Akute-Phase-Proteinen anzuzeigen, welche weder mittels BKS noch anhand einer Fibrinogenbestimmung bei einigen Patienten mit ischämischen Gefäßerkrankungen festgestellt werden kann.

## RÉSUMÉ

Des études précédentes ont certifié la présence d'une aggrégabilité accrue des cellules sanguines chez des patients avec un coeur ischémique et des maladies du cerveau. Nous avons adapté une technique simple de glissement et d'analyse d'image afin de révéler l'état d'aggrégation/non aggrégation de l'érythrocyte (EAA) dans le sang périphérique de 206 patients et pour 174 tests. Une corrélation significative ( $P < 0.0001$ ) a été remarquée entre l'état de l'EAA et les concentrations en fibrinogènes et le taux de sédimentation de l'érythrocyte dans tous les tests effectués. Plus particulièrement, dans un sous groupe de 49 patients, qui possèdent des concentrations normales en fibrinogènes, nous avons remarqué une augmentation très significative de l'état de l'EAA par rapport à des contrôles saints équivalents. Ce fut également le cas pour 82 patients présentant un taux normal de sédimentation de l'érythrocyte. Ainsi, le test de l'EAA semble avoir l'avantage de pouvoir détecter la présence de protéines collantes, qui ne peuvent être révélées par le taux de sédimentation de l'érythrocyte ou par la présence de fibrinogène coagulant, chez des patients atteints de maladies vasculaires de nature ischémique.

**KEY WORDS:** Erythrocyte aggregation, ischemic vascular disease

© Appl. Rheol. 10, 5, 231-237 (2000)

This is an extract of the complete reprint-pdf, available at the Applied Rheology website  
<http://www.appliedrheology.org>

This is an extract of the complete reprint-pdf, available at the Applied Rheology website  
<http://www.appliedrheology.org> Applied Rheology  
September/October 2000

231

many) [34]. However, we introduced our slide test due to its simplicity, low cost and a potential of becoming a real time (almost bed-side) methodology. The question was therefore, is it superior to the indirect determination that one can obtain by using the westergen sedimentation rate [35].

In conclusion, we have demonstrated that a simple slide test and image analysis enables the detection of the presence of aggregated red blood cells in the peripheral blood of patients with ischemic heart and brain diseases. Of interest was the discovery that the test remained positive even when fibrinogen concentrations or the erythrocyte sedimentation rate were comparable to those obtained in a control group of healthy volunteers. Thus, the EAAT might be useful for unmasking relevant information that relates to the acute phase response that is not always given by either determining the concentration of clottable fibrinogen or the erythrocyte sedimentation rate.

#### ACKNOWLEDGEMENT

We are indebted to Ms. Esther Eshkol for editorial assistance.

#### REFERENCES

- [1] Chien S: Blood Rheology in Myocardial Infarction and Hypertension, *Biorheology* 23 (1986) 633-653.
- [2] Boss N, Wietelmann H et al.: Red Blood Cell Aggregation in Men With Coronary Artery Disease, *Eur. J Cardiol.* 12 (1980) 47-54.
- [3] Rainer C, Kawanishi DT et al.: Changes in Blood Rheology in Patients With Stable Angina Pectoris As a Result of Coronary Artery Disease, *Circulation* 76 (1987) 15-20.
- [4] Arntz HR, Perchalla G et al.: Blood Rheology in Acute Myocardial Infarction: Effects of High-Dose I.V. Streptokinase Compared to Placebo, *Eur. Heart J.* 13 (1992) 275-280.
- [5] Dintenfass L, Forbes CD: Effect of Fibrinogen on Aggregation of Red Cells and on Apparent Viscosity of Artificial Thrombi in Haemophilia, Myocardial Infarction, Thyroid Disease, Cancer and Control Systems: Effect of ABO Blood Groups, *Microvasc. Res.* 9 (1975) 107-118.
- [6] Grottemeyer KH: Abnormal Hemorheological Parameters in Vertebrobasilar-Insufficiency, *Acta Neurol. Scand.* 81 (1990) 529-532.
- [7] Fisher M, Meiselman HJ: Hemorheological Factors in Cerebral Ischemia, *Stroke* 22 (1991) 1164-1169.
- [8] Tanahashi N, Gotoh F et al.: Enhanced Erythrocyte Aggregability in Occlusive Cerebrovascular Disease, *Stroke* 20 (1989) 1202-1207.
- [9] Kee DB Jr, Wood JH: Rheology of the Cerebral Circulation, *Neurosurgery* 15 (1984) 125-131.
- [10] Atkinson RP: Ancrod in the Treatment of Acute Ischemic Stroke. A Review of Clinical Data, *Cerebrovasc. Dis* 8 Suppl 1 (1998) 23-28.
- [11] Ehrly AM: Improvement of the Flow Properties of Blood: a New Therapeutical Approach in Occlusive Arterial Disease, *Angiology* 27 (1976) 188-196.
- [12] Walzl M, Lechner H et al.: Improved Neurological Recovery of Cerebral Infarctions After Plasmapheretic Reduction of Lipids and Fibrinogen, *Stroke* 24 (1993) 1447-1451.
- [13] Tanahashi N, Fukuuchi Y et al.: Ticlopidine Improves the Enhanced Erythrocyte Aggregability in Patients With Cerebral Infarction, *Stroke* 24 (1993) 1083-1086.
- [14] Beigel Y, Fuchs J et al.: Lovastatin Therapy in Hypercholesterolemia: Effect on Fibrinogen, Hemorheologic Parameters, Platelet Activity, and Red Blood Cell Morphology, *J Clin. Pharmacol.* 31 (1991) 512-517.
- [15] Branchi A, Rovellini A et al.: Effect of Three Fibrate Derivatives and of Two HMG-CoA Reductase Inhibitors on Plasma Fibrinogen Level in Patients With Primary Hypercholesterolemia, *Thromb. Haemost.* 70 (1993) 241-243.
- [16] Dujovne CA, Harris WS et al.: Effect of Atorvastatin on Hemorheologic-Hemostatic Parameters and Serum Fibrinogen Levels in Hyperlipidemic Patients, *Am. J. Cardiol.* 85 (2000) 350-353.
- [17] Weng X, Cloutier G et al.: Influence of Acute-Phase Proteins on Erythrocyte Aggregation, *Am J Physiol.* 271 (1996) H2346-H2352.
- [18] Weng X, Roederer GO et al.: Contribution of Acute-Phase Proteins and Cardiovascular Risk Factors to Erythrocyte Aggregation in Normolipidemic and Hyperlipidemic Individuals, *Thromb. Haemost.* 80 (1998) 903-908.
- [19] Martinez M, Vaya A et al.: Alterations in Erythrocyte Aggregability in Diabetics: the Influence of Plasmatic Fibrinogen and Phospholipids of the Red Blood Cell Membrane, *Clin. Hemorheol. Microcirc.* 18 (1998) 253-258.
- [20] Berliner S, Shapira I et al.: Combined Leukocyte and Erythrocyte Aggregation in the Peripheral Venous Blood During Sepsis. A Clue to the Presence of a Commonly Shared Adhesive Protein(s), *Int. J. Clin. Lab. Res.* 30 (2000) 27-31.
- [21] Rotstein R, Zeltser D et al.: The Inflammation Meter (INFLAMET). A New Diagnostic Approach to Reveal the Presence of an Inflammatory Response and for the Assessment of Its Intensity, *Isr. Med. Assoc. J.* 2 (2000) 476-477.
- [22] Fusman R, Zeltser D et al.: INFLAMET: an Image

- Analyzer to Display Erythrocyte Adhesiveness/Aggregation, *Eur. J. Int. Med.* (in press 2000).
- [23] Maharshak N, Kassirer M et al.: The Inflammation Meter: Novel Technique to Detect the Presence of Infection/Inflammation in Patients Without Leukocytosis but With an Increased Leukocyte Adhesiveness/Aggregation, *Acta Haematol.* (in press).
- [24] Rotstein R, Zeltser D et al.: An Inflammation Meter to Reveal the Presence and Extent of Inflammation in Elderly Patients, *J. Am. Ger. Soc.* (letter to editor, in press).
- [25] Rogowski O, Zeltser D et al.: Correlated Expression of Adhesive Properties for Both White and Red Blood Cells During Inflammation. A Clue to the Presence of Denominating Proteins, *Biorheology* (in press).
- [26] International Committee for Standardization in Hematology. Recommendation of Measurement of Erythrocyte Sedimentation Rate of Human Blood., *Immunochemistry* 2 (1965) 235-254.
- [27] Clauss A: Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens, *Acta Haematol. Basel* 17 (1957) 237-246.
- [28] Berliner S, Fishelson Z et al.: The Phenomenon of Leukergy: Induction and Detection of Leukocyte Aggregation in Whole Human Blood., *J. Lab. Clin. Med.* 109 (1987) 575-582.
- [29] Fabry TL: Mechanism of Erythrocyte Aggregation and Sedimentation, *Blood* 70 (1987) 1572-1576.
- [30] Vaya A, Martinez M et al.: Red Blood Cell Aggregation and Primary Hyperlipoproteinemia, *Thromb. Res.* 72 (1993) 119-126.
- [31] Hadengue A, Razavian SM et al.: Influence of Sialic Acid on Erythrocyte Aggregation in Hypercholesterolemia, *Thromb. Haemost.* 76 (1996) 944-949.
- [32] Weng X, Cloutier G et al.: Contribution of the -455G/A Polymorphism at the Beta-Fibrinogen Gene to Erythrocyte Aggregation in Patients with Coronary Artery Disease, *Throm. Haemost.* 82 (1999) 1406-1411.
- [33] Chen S, Eldor A et al.: Enhanced Aggregability of Red Blood Cells of Beta-Thalassemia Major Patients, *Am. J. Physiol.* 270 (1996) H1951-H1956.
- [34] Zhao H, Wang X et al.: Comparison of Three Optical Methods to Study Erythrocyte Aggregation, *Clin. Hemorheol. Microcirc.* 21 (1999) 297-302.
- [35] Danesh J, Collins R et al.: Haematocrit, Viscosity, Erythrocyte Sedimentation Rate: Meta-Analyses of Prospective Studies of Coronary Heart Disease., *Eur. Heart. J.* 21 (2000) 515-520.

