Amidolytic activity of plasma euglobulins

Jasielczuk J¹, Dąbrowka M², Gacko M³, Jankiewicz M¹

¹Department of Instrumental Analysis, ²Department of Hematological Diagnostics, ³Clinic of Vascular Surgery and Transplantation, Medical University of Bialystok, Poland

Abstract

Amidolytic activity of plasmin, produced in euglobulin fraction, does not correlate with the time of euglobulin fibrinolysis. It does not depend on fibrinogen concentration.

Key words: Euglobulin fibrinolysis time, fibrinogen concentration, plasmin amidolytic activity.

Introduction

Blood fibrinolytic activity is evaluated by the time of lysis of fibrin clot produced in the plasma euglobulin fraction [1, 2]. The time depends on two variables: different fibrinolytic activity and different fibrinogen concentration. It hinders a diagnostic interpretation of plasma euglobulin fibrinolysis time measurements.

Material and methods

The blood was taken from 74 patients and citrate plasma was obtained. Fibrinogen concentrations [3], fibrinolysis time [4], and amidolytic activity of the euglobulin fraction [5] were assessed. In order to determine amidolytic activity, 0.5ml 3 mmol/l of H-D-Val-Leu-Lys-pNA was added to 0.5ml of euglobulins dissolved in 0.5ml of borate buffer, containing 0.01mol/l of sodium citrate, and incubated for 30 min in 37°C. The reaction was interrupted by adding 0.1ml of 50% acetic

acid. The absorbance was measured in a cuvette with a 0.5 cm thick layer at 405 nm, as referred to the controls, in which acetic acid was added before the substrate was introduced. The results were read from a calibrated diagram, made with the use of model solutions of p-nitroaniline (pNa).

Results

The time of euglobulin fibrinolysis was lengthened, together with an increase in fibrinogen concentrations (Table 1). However, there were no relationships observed between plasmin amidolytic activity and both the time of euglobulin fibrinolysis and fibrinogen concentrations.

Discussion

The results confirm the dependence of euglobulin fibrinolysis time on fibrinogen concentration [4, 6]. It debases the diagnostic value of euglobulin fibrinolysis time determination as a test, estimating plasma fibrinolytic activity. A reliable way of evaluation of plasmin activity, which is produced in plasma euglobulin fraction, is the measurement of its amidolytic activity. The activity depends on plasminogen activator tissue concentration, its inhibitor, and plasminogen [7, 8]. The measurement of amidolytic activity is thus an actual insight into the present status of plasma fibrinolytic activity. An additional advantage of amidolytic test is short determination time and measurement objectivism.

References

- 1. Chakrabarti R, Bielawiec M, Evans J F, Fearnley G R. Methological study and recomended technique for determining the euglobulin lysis time. J Clin Pathol, 1968; 21: 698-701.
 - 2. Kowarzyk H, Buluk K. Advances in blood coagula-

ADDRESS FOR CORRESPONDENCE:

Doanna Jasielczuk
Department of Instrumental Analysis
Medical University of Białystok
Mickiewicza 2 c, 15-089 Białystok, Poland
e-mail: analist @amb.edu.pl

Fibrinogen concentration, mg/dl	Euglobulin lysis time, min	Amidolytic activity, pNA, nmol/ml
195 ± 10,8 (n=22)	169 ± 17,9	47,1 ± 5,3
252 ± 34,0 (n=22)	286 ± 37,0	55,3 ± 4,1
364 ± 28,4 (n=18)	437 ± 54,6	41,3 ± 2,6
793 ± 82,9 (n=12)	936 ±128,0	50.2 ± 3.7

Table 1. Fibrinogen concentration, euglobulin lysis time and amidolytic activity of plasma euglobulin.

tion. Post Hig Med Dośw, 1950; 2: 1-13.

- 3. Quick A J. The physiology and pathology of haemostasis. Kimpton London, 1951; 156.
- 4. Kowalski E, Kopeć M, Niewiarowski S. An evaluation of the euglobulin method for the determination of fibrinolysis. J Clin Pathol, 1959; 12: 215-8.
- 5. Friberg P. Methods for the determination of plasmin, antiplasmin and plasminogen by mens of the substrate S-2251. Haemostasis, 1975; 7: 138-45.
 - 6. Borawski J, Myśliwiec M. Plasma fibrinogen level is
- an important determinant of prolonged euglobulin clot lysis time in hemodialysis patints. Clin Appl Thrombos Hemostas, 2001; 7: 296-9.
- 7. Smith A A, Jacobson L J, Miller B I, Hathaway W E, Manco-Johnson M J. A new euglobulin clot lysis assay for global fibrynolysis. Thrombos Res, 2003; 112: 329-37.
- 8. Urano T, Nishikawa T, Nagai N, Takada Y, Takada A. Amounts of tPA and PAI-1 in the euglobulin fraction obtained at different pH: their relation to the euglobulin clot lysis time. Thrombos Res, 1997; 88: 75-80.