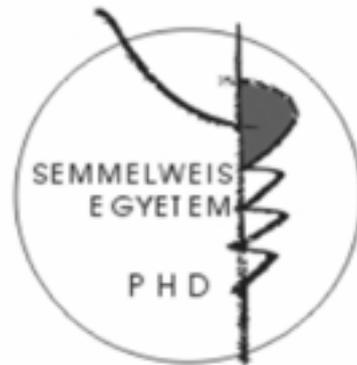


Genetic and Haemorheological Blood Clotting Factors Influencing the Risk and Outcome of Ischaemic Stroke

PhD thesis

Endre Pongrácz MD

Semmelweis University
PhD School of Clinical Medicine



Tutor: Prof. Zoltán Nagy MD, PhD, DsC

Official reviewers: Prof. Péter Sándor MD, PhD, DsC
Prof. István Fekete MD PhD

President of the PhD Theoretical Exam: Prof. István Karádi PhD, DsC
Members of the PhD Theoretical Exam: Prof. László Csiba PhD, DsC
Prof. Tibor Kerényi PhD, DsC

Budapest
2008

Introduction

Epidemiological data confirms that strokes are a prominent public health concern. Statistically stroke is the third most common cause of mortality after the circulatory and malignant diseases and it is the first among the ones which lead to permanent disability. In Hungary, around 40.000 new stroke patients appear each year, of whom 71 percent are disabled and there are many bed-ridden patients among them.

Around 80 percent of strokes are of an ischemic origin. In 85 percent of cases, atherothrombosis can be detected in the pathogenesis of ischemic strokes. Pathological blood clotting and pathologically diverted haemorheological parameters could be responsible for the development of this process. It is important to identify the prothrombotic blood markers before the development of thrombus formation. The prothrombotic state can be prevented either by treatment which generates favourable influence on these markers or by lifestyle changes.

Genetic polymorphisms as the risk factors of strokes

Strokes are a multi-causal disorder; their form relating to only one certain gene is rare. Twin studies on the one hand and familial incidence of stroke on the other revealed that the genetic disposition is a risk factor of strokes. Traditional risk factors are responsible only in 60 percent for the triggering and incidence of strokes; so genetic risk factors play a role as well in the in the pathogenesis of strokes. However, some of the conventional risk factors themselves are also under genetic control; for instance, hypertension, diabetes mellitus and the disorders of the lipoprotein metabolism. Adverse alleles which are responsible for thrombosis could increase the risk of stroke. Both the primary (platelet function) and secondary (blood protein) clotting processes are under genetic control, but this state could be moderated by adverse lifestyle habits and external risk factors. The pathological degree of blood clotting factors can also influence the outcome of strokes.

The allele frequencies of different genetic polymorphisms are generally between 0.01-0.47. The rarer is its prevalence in the population, the greater it is its significance for the individual patient. At the same time, the prothrombotic effect of the genetic polymorphisms with higher allele frequency can be more frequent in certain ethnical group which means an increased risk.

Polymorphisms can also combine with each other increasing the individual risk profile. It could be stated that: 1) the rare point mutations can cause a permanent prothrombotic state 2) the genetic polymorphisms can modify the classification of traditional risk factors 3) if necessary, they can influence our therapeutic decisions (E.g. the introduction of long-lasting anticoagulation).

Haemorheological parameters as risk factors

The objective of haemorheology is to assess the blood fluidity in normal and pathological circumstances. Clinical haemorheology studies the connection between pathological blood fluidity and vascular diseases in order to prevent or improve their outcome. In physiological circumstances the determining factors of blood fluidity are: the *perfusion pressure* and the *blood vessel diameter*. In pathological circumstances, when the hemodynamic reserve is depleted, and the vessel diameter cannot be changed either then the haemorheological factors are able to worsen the haemoperfusion of organs. Macro and micro (cell) rheological parameters can be distinguished. The former represent the property of large vessels while the latter that of the capillary-postcapillary flow, the microcirculation. The relevant macrorheological parameters are: 1) the *whole blood viscosity* (WBV), which could be defined as a dependable variable, which depends on a relative sheargradient of laminar blood fluidity and relates to the suspension character of the blood (non-Newtonian liquid). The WBV is measurable *ex vivo* with capillary (Hevimet 40, Hemorex Ltd., Budapest) and rotational viscometers (Wells-Brookfield USA, Contraves UK, Carry-Med UK) 2) *haematocrit*: above a value of 0.45 by its linear increase the WBV increases exponentially. If the haematocrit increases from 0,40 to 0,50 the entire cerebral blood flow will decrease by 50 percent. 3) *plasma viscosity* (PV): the plasma is a so-called Newton liquid, its viscosity is independent from its sheargradient. A number of epidemiological studies have confirmed the connection between pathological macro-rheological parameters and stroke, the Framingham study is the most widely known. The cardinal determining factor of WBV is the haematocrit (htc). Higher htc increases the risk of stroke and mortality. At the same time, the haemoglobin concentration at the highest normal level increases the extent of cerebral infarct as well. It is also true that pathological haemorheological parameters have been found in more than half of stroke patients. The WBV, PV and erythrocyte aggregation have been found higher in stroke patients as compared with the control groups. Furthermore, it can be established that the level of haemorheological pathological factors are proportional to the severity of the cerebrovascular disorders and it influences adversely the outcome of the disease.

The role of platelet aggregation inhibition in the secondary prevention of stroke

Large multi-centred trials support the efficacy of platelet aggregation inhibition in the secondary prevention of ischemic stroke. The treatment with anti-aggregating drugs is suggested by international recommendations (AHA, EUSI). A 20-25 percent relative risk reduction can be achieved by this treatment. However, in clinical practice the measurement of platelet aggregation in the laboratory is not yet recommended. In order to obtain relevant data it was required to create a multi-centred prospective study to judge the efficacy of anti-aggregating drugs. The previous findings confirm the necessity for new studies since earlier studies

- 1) applied only clinical end points,
- 2) put mainly dose-searching,
- 3) any laboratory measurement has not taken place to clarify the patient group from the perspective of the clinical ends. The phenomenon of laboratory measurable non-response is important when this result is in connection with a new clinical event or with vascular mortality. This possible connection was important during the new study.

Objectives

Genetic studies

The investigated genetic polymorphisms were chosen to represent the detailed process of blood clotting. PLA2 variant was examined on platelet GP IIb/IIIa receptor (primary haemostasis), as well as the Leiden mutation, the prothrombin gene G20210A variant and the FIB -455 G/A polymorphism representing the secondary haemostasis. We studied the endothel system participating in the thrombotic process as well i.e. the ACE gene I/D and also the MTHFR variants. In the recent years our attention was drawn to the antiatherogenic genetic polymorphism (ABCA1) of lipid metabolism (HDL cholesterol) in cardiovascular and stroke patients. Our further goals were to study the function of platelets having the PLA2 variant of the GP IIb/IIIa receptor and compare them to the wild types by aggregation and flow cytometric methods in the in vitro system.

In each study of genetic polymorphism the following issues were expressed:

- 1) Is there any difference in the prevalence of different polymorphisms between Hungarian (the central-Hungarian population has been studied) and other European countries in healthy

persons, 2) We studied the prevalence of genetic polymorphisms in stroke cohorts compared with similar stroke groups abroad.

3) The allele frequencies were studied separately in patient populations both under 50 years (i.e. young group) and above.

4) Furthermore, we studied the incidence of different genotypes in stroke groups (the ones with wild types and the others with heterozygous or homozygous types) according to the occurrence of stroke or vascular diseases in patients and in their family anamnesis.

5) We also studied the patients' CT/MRI and laboratory data (haemostaseological and haemorheological) and examined their correlation with the occurrence of genotypes in different groups.

6) We compared the clustering of hetero or homozygosity between controls and stroke cohorts (gene dose effect).

Haemorheological measuring in chronic stroke patients with brainstem stroke

(BAEP examinations in brainstem stroke patients with hyperviscosity)

The connection between pathological haemorheological factors and (sub)clinical symptoms is more pronounced in the posterior circulation than in the supratentorial area due to the fact that the arterial circulation has an end-arterial character.

Our aim was to study:

1) Whether BAEP patterns are involved in blood hyperviscosity in patients having posterior circulatory disturbances,

2) Whether the BAEP patterns in posterior circulatory arterial diseases show features for clustering in the presence of blood hyperviscosity,

3) Whether there is any specific BAEP pattern in the group of healthy, neurological symptoms free persons with hyperviscosity blood parameters.

Connections between genetic polymorphisms and haemorheological parameters

In this area, little data has been published in the literature; therefore, we measured both the haemorheological factors and genetic polymorphisms in the stroke cohort.

Our aim was to study which polymorphisms show a connection with macro-rheological parameters such plasma FIB concentration, haematocrit, whole blood and plasma viscosity.

Platelet aggregation examinations in cerebrovascular patients

In recent years we measured the platelet aggregation in cooperation with several research centres by using different agonists. Thousand of patients participated in it.

Our aim was to study:

- 1) the standardisation of the laboratory method by using healthy people's blood samples,
- 2) the efficacy of platelet aggregation in blood samples of patients taking anti-aggregating drugs with different agonists,
- 3) to assess the possible connection between non-responder state and mortality of stroke patients in a 28 month-long follow-up study.

Methods

Control participants recruited in the examinations

A total of 273 voluntary healthy people were recruited as controls in this *genetic* examination. The control group consisted of healthy blood donors from the Institute of Haematology Budapest. Blood was separated for PCR with the written consent of the ethical committee.

The number of controls / the mean age in case of *genetic polymorphisms* was: Factor II: 273 (30±11), ACE I/D: 173 (41±11), GP IIb/IIIa: 132 (46±15), Factor V: 171 (41±11), Fibrinogen -455 G/A: 173 (49±12) MTHFR: 173 (41±11) ABCA1: 193 (35±11).

Controls in BAEP and blood viscosity study: 45 persons (46±10).

The samples of 150 (31±15) healthy blood donors were collected for the standardisation of the aggregometer examinations. In the *in vitro* platelet GPIIb/IIIa receptor investigation controls consisted of patients having PLA1 wild types 51 (56±12).

Patients

Patients who participated in the genetic study according to genetic polymorphisms are the following: Factor II:101 (40±10), ACE I/D:252 (57±12), GP IIb/IIIa: 253 (56±16), Leiden mutation: 254 (57±14), FIB -455G/A: 278 (56±13), ABCA1: 244 (53±14) stroke patients, 150 (61±9) with coronariasclerosis, MTHFR: 273 (51±11). Family anamnesis of vascular diseases

was registered in each patient by a routine questionnaire. In addition, the traditional risk factor profile, CT or MRI and laboratory data was registered in each cohort of patients during our prospective study.

Ex vivo GP IIb/IIIa examinations: The blood samples (whole blood and PRP) of 105 ischemic stroke patients were examined. 51 patients had PL^{A1/A1} wild types (controls) and 54 had PL^{A1/A2} genotypes of platelet GP IIb/IIIa receptors. *BAEP studies:* 47 patients with brainstem stroke, 26 patients without stroke symptoms but with blood hyperviscosity.

The involved patients in the platelet aggregation (clinical) studies: 2425 vascular patients in the resistance measurements and 921 ischemic stroke patients in the mortality study were followed for 28 months.

Methods

Genetic polymorphisms were detected by PCR methods.

Whole blood viscosity: measured at 10, 40 and 90 sec⁻¹ sheargradient, with thermostabilized (37 °C) HEVIMET - 40 (Hemorex Ltd. Budapest) capillar viscometer. Also the plasma viscosity at 90 sec⁻¹ sheargradient (37 °C) was measured by the same viscometer. The concentration of plasma fibrinogen was measured according to Clauss turbidimety (Fibrinogen assay kit, Reanal, Budapest, Hungary).

Method of platelet aggregation: The level of platelet aggregation was detected by CARAT TX 4, four channel, computerised aggregometer. The basis of the measurement was the method used and described by Born in 1962. As reagent the THERACONT TA-3 inductor (CARAT Diagnostics Ltd.) was used which contained 5 µM and 10 µM ADP, 2 µg/ml collagen and 10 µM epinephrine concentration. The reference range of the aggregometer was calibrated and measured by the platelet aggregation levels (in PRP) of 150 healthy persons who did not take any drugs.

The ex vivo examination of platelet receptor GP IIb/IIIa PLA2 polymorphism:

Platelet aggregation: method of Ingerman-Wojenski was used in whole blood induced by ristocetin, ADP and collagen in the presence of luciferin luciferase with the aggregometer Chrono-Par (Chrono-Log). In the other part of our study the platelet aggregation was measured in PRP by Born (Chrono-Log Corp., USA). *Flow cytometry measuring:* platelet activation has been detected in whole blood and PRP with CD41a (GP IIb/IIIa complex), CD

61 (GP IIIa), CD 62P (P selectin) CD 42b (GP Ib) markers and with bound fibrinogen. In PRP double labelled antibodies (CD 41/CD42b, CD 41/CD61) and CD 62P have been measured.

BAEP: was recorded by the following sound clicks 75 dB above the hearing threshold determined previously. Clicks were generated by a stimulator and delivered through headphones. The amplifier's band pass was between 150 and 3000 Hz. Waves were recorded by gold cup electrodes with the active electrode placed at the vertex (Cz); different electrodes were placed at the mastoid ipsilateral for ear stimulation and the ground at the contra lateral mastoid (inter-electrode impedance <5 kOhm). Each test consisted of a minimum of 2000 stimulus presentations, and at least 2 tests demonstrating reproducibility were obtained for each ear. (Instrument: Sierra Cadwell).

Detection of risk profile in the patients' family anamnesis: The following data was registered using a routine questionnaire: stroke, hypertension, diabetes mellitus, myocardial infarction, POAD, migraine. In patients following previous vascular events we registered: hypertension, diabetes mellitus, nicotine habit, TIA or stroke, myocardial infarction, migraine, alcohol use habits, hyperuricaemia and hyperlipidaemia.

Results

1) *Prothrombin G20210A*: The prevalence of 2010A allele among our healthy participants was 0.9 %, which corresponds to the European average. This polymorphism prevalence is significantly higher in the young ischemic stroke group compared with the young controls and it is more frequent in the subgroup of patients having 'low traditional risk' factors as compared to the ones with the „high risks". The Factor II polymorphism prevalence coupled with PLA2 variant has been found to be significantly higher in our young cohort compared with the controls which increases the thrombosis risk profile in the blood.

2) *ACE gene I/D*: The prevalence of D allele in our controls did not differ from the published data by different European authors. In the subgroup of patients (age <50 years) the prevalence of the D allele was found significantly higher in contrast with the controls. The prevalence of D allele was found significantly higher in the group of patients in whose family stroke, POAD or hypertension occurred. The D/D genotype is also significantly more frequent in patients who previously had myocardial infarction. So the D/D genotype of ACE gene is a possible risk factor in the development of coronary endothel dysfunction or in the atherosclerotic process of heart supplied vasculature. We found significantly more hyperlipidemia in the young stroke group both with D/D and in I/D genotype in contrast with the ones with the wild type.

3) A GP IIb/IIIa (Leu33Pro) polymorphisms: (clinical observation) the PLA2 variant is more frequent both in healthy controls and stroke patients in Hungary compared with the data published by other European authors. In stroke patients the PLA2 alleles represent only a moderate risk increase both in the young and in older patient subgroups compared with the controls. The PLA2 variant coupled with moderate ACI stenosis was more prevalent than in the patient group with the wild types. A synergistic effect is generated by the presence of PLA2 allele coupled with prothrombin gene heterozygous variant.

(Experimental observation): Our new findings in our ex vivo PLA2 heterozygous platelet aggregation study is that only the ristocetin inductor evoked hyper aggregation among traditional inductors (collagen, ADP) compared to the wild type. This phenomenon points out the connection with VWF. In another setting, i.e. in the flow cytometric study, ristocetin agonist increased the expression and FIB binding capability of the platelet GP IIb/IIIa receptors having the heterozygous type compared with the wild type. Thus that polymorphism can activate this important cell surface receptor in several ways. In addition, this feature can explain the possible mechanism of platelet non-response for different drugs.

4) Leiden mutation: We revealed that the prevalence of Leiden mutation in Hungarian healthy people and in ischemic stroke patients is higher than the data published by other European authors. Significantly higher stroke prevalence was found in the family anamneses of patients with Leiden mutation compared with the ones with the wild types. Furthermore, we were able to assess that patients with Leiden mutation coupled significantly more with hyper-lipidemie compared with patients with the wild types, supposing a synergistic adverse connection between the genetic and metabolic risk factors.

5) ABCA1 polymorphism: The ABCA1 R219K and V771M polymorphisms can play a protective role in coronariasclerosis and ischaemic stroke. We assessed that the protective effect is more pronounced in young patients than in older ones, and in patients who carry on their ABCA1 gene both the R219K and V771M polymorphism as well.

6) Totalised genetic assessments: In the group of young patients, the prothrombin gene 20210A and the D allele of ACE gene represent an increasing significant risk increasing in comparison with the healthy controls. Based on the results of the examination of two polymorphisms, the most important from the prothrombotic point of view is that the *protrombin gene A* allele coupled with other polymorphisms; therefore, its role is prominent among the assessed genetic polymorphisms.

We have not found any connection between CT/MRI assessed subgroups of ischaemic stroke patients and the prevalence of different genetic polymorphisms. Similarly, we were not able

to detect any connection between genetic variants and the prevalence of POAD or migraine either.

7) BAEP and blood viscosity: It was revealed that the BAEP patterns of the damaged ischaemic brain stem can be influenced by hyperviscosity of the circulatory blood. We found the following results: - when symmetric pathological BAEP patterns could be detected– we observed bilateral failure of wave III., - significant latency elongation of wave III. was registered, - significant bilateral elongation of IV-V complex detected and we registered many cases in which all waveforms exhibited major simultaneous bilateral deformation. In the patient group without neurological symptoms, but with blood hyperviscosity, both the wave morphological BAEP pattern aberration and the elongation of latency were similar to the patterns of patients with ischemic stroke. That phenomenon supports the theory that the blood hyperviscosity could be responsible for the symmetrical aberration of the BAEP pattern.

8) Fibrinogen gene -455 G/A polymorphism, Leiden mutation and haemorheology If the patient has H2/H2 genotype of FIB -455 G/A as opposed to H1/H1, not only significantly higher FIB concentration but also increased whole blood viscosity can be measured in the plasma. In non-smoker patients as against smokers the favourable low level FIB concentration has been equalised when they had H2/H2 genotypes. Both in the whole group of patients and in young ones with Leiden mutation higher plasma viscosity has been found compared with the ones with wild types.

9) Platelet aggregation study We assessed the reference range of CARAT TX aggregometer. The results of aggregation levels of healthy persons measured in different centres were found to be in good agreement. The collagen and adrenalin inductors were revealed to be sensitive to aspirin intake, while the ADP inductor to the thienopyridine intake. In patients taking aspirin, the laboratory resistance was found to be 17 percent, for ticlopidine 4 per cent and for clopidogrel 18 percent, respectively. We detected that in patients taking ASA significantly higher mortality occurs when the drug therapy is continued despite of the laboratory resistance compared with changing the therapy to thienopyridine.

Conclusion

In the Central-Hungarian healthy cohort, the prevalence of assessed blood clotting-genetic polymorphisms is similar in prevalence to the ones detected in other European healthy groups. The platelet GP IIb/IIIa PLA2 variant represents an exception since it is about 40 percent more frequent in our cohort. This figure was confirmed by other Hungarian research groups as well. Although the relative risk increasing effect of this polymorphism is moderate in patients, its high prevalence in the population represents a high risk. Therefore, we support

its detection within the thrombophilia panel of vascular genetic examinations. Further data supports the importance of our observations revealed by our in vitro examination of the heterozygosity state of the Gp IIb/IIIa variant. We detected that the materialisation of platelet hyper activation with PLA2 variant occurs in several ways so it could be responsible for the attenuated response against antithrombotic drugs (ASA and thienopyridine).

We clarified that - among the examined genetic polymorphisms - the prothrombin gene and ACE D allele are the most prevalent in young ischemic stroke patients in contrast to the controls. Their detection in the genetic PCR panel is important because they increase synergistically the thrombophilic ability of traditional vascular risk factors.

Our new result is that the prevalence of dual genetic polymorphisms in the young stroke cohort is significantly higher compared with the healthy controls. The dual polymorphisms proved to have a gene – dose increasing effect in the stroke cohort, i.e. the prothrombin gene linkage to other polymorphisms is the most important. Consequently, not only the G20210A variant itself is a genetic risk factor, but also its coupling with others increases the genetic risk.

It can be considered as a further result that the examined important genetic polymorphisms which facilitate the pro-thrombotic condition participate not only in the biochemical blood clotting process but also in that of blood fluidity and modify the haemorheological profile of the blood flow. We detected that among these polymorphisms the FIB variant and Leiden mutation take part in this process which increase both the blood and plasma viscosity. The consequently produced circulatory disturbances further increase the thrombotic risk in stroke patients.

Furthermore, we revealed as new a result that the long-lasting blood hyperviscosity can lead to subclinical lesions also in healthy persons. These thus generated circulatory disturbances can be detected by electrophysiological methods (BAEP) and are characterised by symmetrical appearance. Normalisation of the blood hyperviscosity by haemorheological treatment (isovolemic haemodilution) can prevent the development of neurological lesions (primer prevention) and in patients having primer or secunder polyglobulie the therapy can prevent new clinical events (secunder prevention).

The results of our examinations of platelet aggregation pointed out that in 10-30 percent cases laboratory measurable non-response can be detected in the background of new clinical events despite drug therapy. The method is suitable for the detection of platelet inhibition. The statements above have been confirmed by our examinations covering a large number of cases. We revealed that with regard to the laboratory measurable resistance of

platelets the treatment changes (from ASA to thienopyridine) and can save the life of stroke patients.

List of own publications

Pongrácz E., Tordai A., Andrikovics H., Szén L., Nagy Z.: A prothrombin gén G20210A polimorfizmusának vizsgálata fiatalkori ischaemiás stroke-os betegeknel.

Agyérbetegségek 2001; 2: 9-13.

Pongrácz E., Tordai A., Csornai M., Nagy Z. Stroke betegek insertios-deletios ACE- gén-polimorfizmusának vizsgálata. *Clin Neurosci/Ideggyógyászati Szemle* 2002; 55 (5-6): 156-163.

Pongrácz E., Tordai A., Csornai M., Nagy Z. Stroke betegek thrombocytá glikoprotein IIb/IIIa (Leu Pro 33) polimorfizmusának vizsgálata *Orvosi Hetilap* 2001; 142 (15): 781-785.

E. Pongrácz, K. Schweitzer, J. Fűrész, J. Fent, A. Tordai, Z. Nagy. The effects of platelet receptor GP IIb/IIIa polymorphism (Leu Pro33) on the receptor expression and platelet aggregation in patients with ischaemic stroke. *Turk J Hematol.* 2007; 24: 155-163.

Pongrácz E., Tordai A., Csornai M., Nagy Z.: Van-e jelentősége a factor V gén A506G (Leiden) mutációjának ischaemiás stroke betegekben? *Clin Neurosci/Ideggyógyászati Szemle* 2003; 56 (5-6): 144-154.

H. Andrikovics, E. Pongrácz, Á. Kalina, A.Szilvási, Ch. Aslanidis, G. Schmitz, A. Tordai. Decreased frequencies of ABCA1 polymorphism R219K and V771M in Hungarian patients with cerebrovascular and cardiovascular diseases.

Cerebrovasc Dis 2006; 2: 254-259. **(IF: 2,16)**

Pongrácz E., Andrikovics H., Keresztúri M., Csornai M., Nagy Z. Az agyi ischaemia rizikóját fokozó genetikai polimorfizmusok halmozódásának vizsgálata. *Agyérbetegségek.* 2006; 12(4): 14-21.

Pongrácz E.: Véralvadási genetika fiatalkori stroke-ban. *Clin Neurosci /Ideggyógyászati Szemle* 2002; 55 (3-4): 111-117.

Bernáth, S.I. Bernát, E. Pongrácz, P. Köves, Z. Szakács, A. Horváth: Effects of blood hyperviscosity on functional integrity in the brain stem: a brain stem evoked auditory potential study. *Clin Hemorheology and Microcirculation* 2004; 31:123-128. **(IF.:0.63)**

Pongrácz E., Bernáth I: Az agytörzsi mikrokeringés vizsgálata BAEP és hemorheológiai módszerekkel. *Honvédorvos* 1996; 48: 282-293.

E. Pongrácz, H. Andrikovics, M. Csornai, IS. Bernát, Z. Nagy: Contribution of the -455G/A polymorphism at β -fibrinogen gene and Leiden mutation to haemorheological parameters in ischaemic stroke patients. *Clin Hemorheology and Microcirculation* 2006; 35:75-82. **(IF.: 1.03)**

E. Pongrácz, H. Andrikovics, I.S. Bernát, Z. Nagy. Connection between genetically determined blood coagulation factors and haemorheology. *Clin Hemorheology and Microcirculation* 2008; közlésre elfogadva **(IF.:1,03)**

E. Pongrácz Measurement of platelet aggregation during antiplatelet therapy in ischemic stroke. *Clin. Hemorheology and Microcirculation* 2004; 30 (3-4): 237-42. **(IF.:0.63)**

E. Pongrácz, Z. Káposzta: Antiplatelet therapy in ischemic stroke. *Expert Review of Neurotherapeutics* 2005; Vol. 5 (4): 541-549.

RG Kiss, G. Kerecsen, L. Rudas, D. Apró, Gy. Stef, E. Pongracz, I. Bernat, I. Préda.: Aspirin and ticlopidine resistance in cardiovascular patients. Multicenter database of 2425 patients aimed to standardize the laboratory control of antiplatelet therapy. *Eur Heart J. (Suppl)*. 2003; 24: 371.**(IF.: 6,13)**

E. Pongrácz, SI. Bernát, L. Rudas, GY. Stef, RG. Kiss. Resistance during antiplatelet therapy based on a multicenter database of ischaemic stroke and cardiovascular patients. *Cerebrovasc Dis*. 2004; 17 (suppl.5): 73. **(IF.:2,14)**

E. Pongrácz, G. Stef, G. Kerecsen, F. Poór, E. Seres, L. Németh, I. Préda, R.G. Kiss. Continuation of aspirin therapy despite aspirin resistance is associated with higher mortality. A two year follow up in 921 ischemic stroke and cardiovascular patients. *Cerebrovasc Dis*. 2005; 19 (suppl 2):159. **(IF.:2,16)**

Publications related to the dissertation

Pongrácz E.: A hemorheológia jelentősége a cerebrovasularis betegségekben. *Honvédervos* 1988; 4: 253 – 266.

Pongrácz E.: A hemorheológiai therapia kiindulási alapja és stratégiája. *Honvédervos* 1995; 47: 54 – 65.

Pongrácz E.: Rheológiai szempontok a stroke kezelésében. *Háziorvos Továbbképző Szemle* 1997; 2: 364-366.

Bernát SI, Pongrácz E.: Homocisztein szerepe az ischaemias cardio és cerebrovasularis betegségekben. *Cardiologia Hungarica* 2001; 4: 315-319.

Pongrácz E.: A trombocita aggregáció gátlás jelentősége a stroke szekunder prevenciójában. *Recept* 2001; 12: 19-21.

Pongrácz. E., Bauer E. Bernát S.I. Bálint K: ASA non-responsio vizsgálata, az Ipaton filmtablettával szerzett tapasztalatok. *Novitates* 2002; 1: 15-23.

Bernát S.I, Pongrácz E., Gonda F.: Az aszpirin dózisának változtatása és a trombocita-aggregáció gátlás mértéke a kardio- és iszkémiás cerebrovaszkuláris betegségek szekunder prevenciója során. *Cardiologia Hungarica* 2003; 33: 240-244.

Pongrácz E., Csornai M.: A haemodilutio cerebrovascularis betegségekben. *Érbetegségek* 2005; (suppl.1): 35-37.

Pongrácz E. A trombocitaaggregáció mérés értéke iszkémiás stroke szekunder profilaxisában. *Metabolizmus* 2005; 2: 108-113.

Bernát S.I., Pongrácz E., Sidó Z.: Az iszkémiás szívbetegség és a trombocitaaggregáció-gátlás kapcsolata. *Cardiologia Hungarica* 2005; 35: 59-63.

Bernát S.I, Pongrácz E., Sidó Z. Hematológiai, hemostazeológiai és hemoreológiai eltérések elhízásban, *Metabolizmus* 2005;(4): 246-250.

S.I.Bernat, E. Pongracz, Z. Sidó. Effects of fluvastatin therapy on plasma fibrinogen concentration and plasma viscosity. *Cardiol. Hungarica* 2006; 36: 248-250.

Peer review, in english journal presented abstracts

Pongrácz E., Bernát S.I.: Haemorheological examinations of healthy, middle aged persons. *Clin. Haemorheol.* 1989; 3: 511. **(IF.:1,20)**

Bernát S. I., Pongrácz E.: Abnormal erythrocyte deformability in malignant tumors *Ann Haemathol* 1992, 65: 59-60. **(IF.: 1,29)**

Bernát S.I., Pongrácz E.: Haemorheological examinations in malignant tumors. *Clin. Haemorheol.* 1993; 13: 595. **(IF.: 0,51)**

Pongrácz E., Tordai A., Nagy Z.: Genetic polymorphism of GP IIb/IIIa in young ischemic cerebrovascular patients. *Cerebrovasc. Dis.* 1998, 8 (suppl 4) 50. **(IF.: 1,51)**

Pongrácz E. Schweizer K., Nagy Z.: GP IIb/IIIa polymorphism and platelet function in ischemic stroke patients. *Cerebrovasc. Dis.* 1999; 9. 22. **(IF.:1,78)**

Andrikovics H., Pongrácz E., Kalmár L., Bors A., Szén L., Tordai A. Screening for genetic risk factors among patients with stroke. *Genetics.* 2000; 5: 145. **(IF.: 4,48)**

Pongrácz E., Andrikovics H. Tordai A. Szén L. Csornai M., Nagy Z. Screening for genetic clotting factors (prothrombin gene G20210A and factor V Leiden) among patients with ischemic stroke. *Cerebrovasc. Dis.* 2000; (4): 126. **(IF.:1,78)**

Pongrácz E. Tordai A., Csornai M., Nagy Z. MTHFR polymorphism and stroke. *Cerebrovasc. Dis.* 2001; (9): 127.**(IF.:1,78)**

Impact factor of own publications: 5,48

Impact factors of peer review, in English journal presented abstracts: 24,74