

# Pregnancy-associated Plasma Protein-A in Patients with Cerebrovascular Diseases – a Pilot Study

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**Abstract:** Pregnancy-associated plasma protein-A (PAPP-A) was described as a novel marker of acute coronary syndrome. The aim of our study was to investigate how serum pregnancy-associated plasma protein-A (PAPP-A) levels change in patients with ischaemic stroke and intracerebral haemorrhage and to evaluate if PAPP-A might be a marker not only of myocardial infarction but also a useful parameter in cerebrovascular disorders. 43 patients with acute cerebrovascular events were divided into 3 groups – patients with ischaemic stroke (n=16), patients with intracranial haemorrhage (n=10) and patients with both ischaemic stroke and coronary artery disease (n=17). The control group consisted of 12 subjects. PAPP-A was measured by TRACE (Time Resolved Amplified Cryptate Emission) technology. PAPP-A levels in patients with intracranial haemorrhage and those with both ischaemic stroke and coronary artery disease were increased in comparison with the control group ( $p < 0.005$ ,  $p < 0.01$ , respectively) as well as with patients with ischaemic stroke only ( $p < 0.01$ ,  $p < 0.05$ , respectively). A positive correlation between PAPP-A and total cholesterol in patients with both ischaemic stroke and coronary artery disease ( $r = 0.497$ ,  $p < 0.05$ ) was observed. Serum PAPP-A levels in all studied patients correlated positively with serum creatinine ( $r = 0.395$ ,  $p < 0.05$ ). PAPP-A levels are increased in patients with intracranial haemorrhage and in the patients whose ischaemic stroke is associated with coronary artery disease. The atherosclerotic process may contribute to increased serum PAPP-A levels. PAPP-A may be a marker of increased risk of atherothrombotic events in general.

## Introduction

Cerebrovascular diseases represent a common cause of morbidity and mortality in developed societies. Atherosclerosis plays an important role in their pathogenesis. One of the well accepted concepts explains atherosclerosis as a specific form of chronic inflammatory process in which plasma lipoproteins, inflammatory cells (monocytes/macrophages, T lymphocytes), endothelial cells, smooth muscle cells and extracellular matrix interact within the arterial wall. The monocytes recruitment into the intima is a characteristic feature for the initial stages of atherosclerosis. Monocytes mature to macrophages in the subendothelial space and secrete a spectrum of proinflammatory mediators including cytokines, metalloproteinases and active oxygen species [1, 2, 3].

Recently, a novel marker of acute coronary syndrome was described – pregnancy-associated plasma protein-A (PAPP-A) [4]. The immunohistochemical quantitative analysis of atherosclerotic arteries of patients who died of cardiac events showed that PAPP-A expression is more pronounced in fibrous eroded and ruptured plaques than in the stable plaques. The increased production of PAPP-A in atherosclerotic arteries is reflected in elevated serum PAPP-A levels in patients with myocardial infarction or unstable angina [4].

We hypothesised that similar increase of circulating PAPP-A as in patients with acute coronary syndromes may also occur in those with acute cerebrovascular events. The aim of the present study was to investigate how serum PAPP-A levels together with anticardiolipin antibodies (ACA) which are associated with a variety of neurological syndromes change in patients with ischaemic stroke and intracerebral haemorrhage and to assess if PAPP-A might be a marker not only of myocardial infarction and unstable angina pectoris but also a useful parameter in cerebrovascular disorders.

## Materials and methods

### *Patients*

43 patients (21 women and 22 men, mean age  $71 \pm 10$  year) with acute cerebrovascular events admitted to the hospital were enrolled in the study. The patients were divided into 3 groups – group 1 (patients with ischaemic stroke,  $n=16$ ), group 2 (patients with intracranial haemorrhage,  $n=10$ ) and group 3 (patients with both ischaemic stroke and coronary artery disease,  $n=17$ ). Selected patient characteristics (age, sex, blood pressure, serum levels of total cholesterol and creatinine) are shown in Table 1. The assessment of patients included medical history, neurological examination and cranial computed tomography. The majority of blood sampling was performed within 72 hours after hospital admission.

The control group consisted of 12 subjects (mean age  $66 \pm 2$  years) without clinical signs of atherosclerosis (no stroke, coronary artery diseases or peripheral vascular diseases in medical history).

All blood samples were centrifuged for 10 minutes at 1450 g ( $4^\circ\text{C}$ ). The study was approved by local Institutional Ethical Committee.

### *Methods*

**PAPP-A Assay** PAPP-A was measured by TRACE (Time Resolved Amplified Cryptate Emission) technology based on non-radiating energy transfer. Commercial kit KRYPTOR-PAPP-A (Brahms, Berlin, Germany) contains two different monoclonal antibodies – one is conjugated with europium cryptate and the other one with fluorescent agents XL 665. The antigens (PAPP-A) present in serum samples are sandwiched between two conjugates. The fluorescent signal measured during the formation of the antigen-antibody complex by the KRYPTOR analyser (Brahms, Berlin, Germany) is proportional to the antigen concentration. The intra- and inter-assay coefficients of variation were 9.6% ( $n=7$ ) and 8.8% ( $n=7$ ), respectively.

**Total cholesterol and creatinine** Total cholesterol and creatinine concentration were measured by standard clinical chemistry methods recommended by IFCC (International Federation of Clinical Chemistry).

*Anticardiolipin antibodies* Standardized ELISA utilising microplates coated with purified bovine cardiolipin and saturated with human beta<sub>2</sub>-glycoprotein I (ORGENTEC, Mainz, Germany) was used for anticardiolipin antibodies determination. The results are expressed in units (U/ml) assigned as GPL for IgG class and MPL for IgM class.

### Statistical analysis

Standard statistical methods were used for analysis of the data. Results of PAPP-A, ACA IgG and IgM are expressed as median (interquartile range) and the results of other variables as mean  $\pm$  standard deviation (SD). The statistical significance between groups was analysed using Mann-Whitney U test for PAPP-A, ACA IgG and IgM. Associations between analysed parameters were assessed with Spearman's and Pearson's correlation coefficients. All results were considered as statistically significant at  $p < 0.05$ .

### Results

#### *Patients characteristic*

Age, sex, blood pressure, serum levels of total cholesterol and creatinine are shown in Table 1. There was no difference between patients' groups in systolic and diastolic blood pressure and total cholesterol. The patients of group 2 were older than those in group 1 ( $p < 0.05$ ), but no correlation between age and PAPP-A was found. Serum creatinine was significantly higher in group 3 than in group 1 ( $p < 0.05$ ).

#### *Serum PAPP-A, ACA IgG and IgM levels*

Serum PAPP-A levels are shown in Figure 1. PAPP-A levels in patients with both ischaemic stroke and coronary artery disease as well as in those with intracranial

**Table 1 – Characteristic of patients with cerebrovascular diseases**

	Total (n=43)*	Group 1 (n=16) ischaemic stroke*	Group 2 (n=10) intracranial haemorrhage*	Group 3 (n=17) both ischaemic stroke and coronary artery diseases*
Age (years)	71 $\pm$ 10	66 $\pm$ 11	76 $\pm$ 8**	74 $\pm$ 8
Sex (male/female)	22/21	7/9	3/7	12/5
Systolic blood pressure (mm Hg)	159 $\pm$ 20	153 $\pm$ 20	163 $\pm$ 24	159 $\pm$ 17
Diastolic blood pressure (mm Hg)	90 $\pm$ 14	88 $\pm$ 12	90 $\pm$ 11	89 $\pm$ 18
Total cholesterol (mmol/l)	5.52 $\pm$ 1.31	5.56 $\pm$ 1.43	5.33 $\pm$ 1.79	5.50 $\pm$ 1.43
Serum creatinine ( $\mu$ mol/l)	90.12 $\pm$ 24.10	86.21 $\pm$ 11.76	91.20 $\pm$ 25.12	99.75 $\pm$ 20.96 <sup>‡</sup>

\*results are expressed as mean  $\pm$  SD (standard deviation), \*\*group 2 vs group 1  $p < 0.05$ , †group 3 vs group 1  $p < 0.05$ , ‡one missing data

haemorrhage were increased when compared with control group ( $p < 0.01$ ,  $p < 0.005$ , respectively). The levels in patients with ischaemic stroke were slightly but not significantly higher than in controls. When all patients with cerebrovascular disease were taken together, PAPP-A levels [10.57 (8.33–12.75) mU/l] were also significantly higher than in controls [7.48 (6.30–10.50) mU/l] ( $p < 0.05$ ). There was a significant difference between patients with ischaemic stroke (group 1) and those with intracranial haemorrhage (group 2) ( $p < 0.01$ ) and also between group 1 and group 3 ( $p < 0.05$ ). PAPP-A levels above 10 mU/l were found in 31.25 % of group 1 patients and in 70% of group 2 as well as of group 3 patients. PAPP-A level in one patient with ischaemic stroke who suffered simultaneously from myocardial infarction was 37 mU/l. An extremely high PAPP-A level (79 mU/l) was found in a patient with thrombosis of arteria basilaris.

ACA IgG and IgM did not differ between patient groups 1–3 (Table 2). Increased ACA IgG ( $> 10$  GPL) with simultaneously elevated IgM ( $> 10$  MPL) was seen in one patient. Other two patients had increased either ACA IgG or IgM.

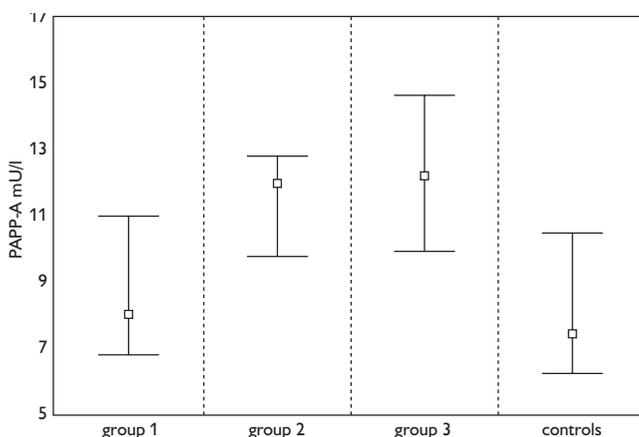


Figure 1 – Serum PAPP-A levels in patients with cerebrovascular stroke – group 1 – ischaemic stroke, group 2 – intracranial haemorrhage, group 3 – both ischaemic stroke and coronary artery diseases (group 2 vs controls  $p < 0.005$ , group 3 vs controls  $p < 0.01$ , group 1 vs group 2  $p < 0.01$ , group 1 vs group 3  $p < 0.05$ ). Results are expressed as median (interquartile range).

**Table 2 – Serum ACA IgG and ACA IgM levels in patients with cerebrovascular stroke**

	Total (n=43)	Group 1 (n=16) ischaemic stroke*	Group 2 (n=10) intracranial haemorrhage*	Group 3 (n=17) both ischaemic stroke and coronary artery diseases*
ACA IgG (GPL)	1.80 (1.00–3.00)	1.65 (1.00–2.35)	1.83 (1.40–2.10)	2.00 (1.20–3.30)
ACA IgM (MPL)	2.00 (0.80–5.10)	1.75 (0.60–4.10)	2.00 (1.20–8.10)	2.40 (0.80–5.20)

\*results are expressed as median (interquartile range)

*Relationship between PAPP-A levels, markers of lipid metabolism and renal function and ACA IgG and IgM (Table 3).*

A positive correlation between PAPP-A and total cholesterol in patients of group 3 ( $r=0.497$ ,  $p<0.05$ ) was observed. A slight, but not significant correlation between PAPP-A and total cholesterol was observed in the group of all patients ( $r=0.297$ ,  $p=0.063$ ). Serum PAPP-A levels in all studied patients correlated positively with serum creatinine ( $r=0.395$ ,  $p<0.05$ ). ACA IgG and IgM did not correlate with PAPP-A neither in individual groups nor in the group of all patients.

In summary, PAPP-A levels were increased in patients with cerebrovascular disease, but less than in patients with acute coronary events, and positively correlated with cholesterol and serum creatinine. ACA levels were similar in patients with ischaemic stroke as well as in those with intracranial haemorrhage.

## Discussion

Pregnancy-associated plasma protein-A (PAPP-A) isolated from human pregnancy serum was first described by Lin et al. [5]. Its concentration during pregnancy rises with the gestational age [6]. Low PAPP-A maternal serum levels in the first trimester of pregnancy are associated with a trisomy 21 in foetus [7, 8]. In non-pregnant subjects, PAPP-A mRNA synthesis has been demonstrated in various reproductive as well as non-reproductive tissues such as colon, kidney, bone marrow cells, breast and breast carcinoma cells [9]. Immunochemically PAPP-A was localised in various genital and extragenital organs from foetuses, infants and adults [10].

The function of PAPP-A remained unknown for a long time. Because of the presence of an elongated zinc-binding motif in amino acid sequence, PAPP-A is classified as a member of the pappalysins which belong to the metzincin superfamily of metalloproteinases [11]. Possible role of the surface-bound PAPP-A may be explained by activating insulin-like growth factor (IGF) through cleaving

**Table 3 – Correlation of serum PAPP-A levels with serum total cholesterol, creatinine, ACA IgG and IgM levels in patients with cerebrovascular diseases**

Correlation PAPP-A with	Total (n=43)		Group 1 (n=16) ischaemic stroke		Group 2 (n=10) intracranial haemorrhage		Group 3 (n=17) both ischaemic stroke and coronary artery diseases	
	r	p	r	p	r	p	r	p
Total cholesterol	0.297	n.s.	-0.085	n.s.	0.166	n.s.	0.497	<b>0.042</b>
Creatinine	0.395	<b>0.011</b>	0.156	n.s.	-0.010	n.s.	0.247	n.s.
ACA IgG	-0.129	n.s.	-0.137	n.s.	-0.450	n.s.	-0.202	n.s.
ACA IgM	-0.036	n.s.	0.208	n.s.	0.236	n.s.	-0.149	n.s.

insulin-like growth factor binding protein 4 (IGFBP-4) in proximity of an IGF receptor. This mechanism increases the concentration of free IGF near receptors and the probability that IGF binds to them [12].

PAPP-A may function in injury/repair responses. PAPP-A is expressed by activated wound fibroblasts and staining for PAPP-A is also prominent in activated wound macrophages suggesting a participation of PAPP-A in tissue remodelling. The cleavage of IGFBP-4 by PAPP-A can amplify local IGF bioactivity in wound healing [13].

By similar mechanisms, PAPP-A may contribute to the proliferative process within the atherosclerotic plaques. PAPP-A expression is enhanced in vascular repair. Bayes-Genis et al. [14] showed that PAPP-A is expressed in vascular smooth muscle cells and that the expression is markedly upregulated in neointimal hyperplasia after coronary angioplasty. By this way, PAPP-A may act in autocrine or paracrine fashion, during vascular repair after balloon injury. These experiments were supported by clinical studies. Elevated serum PAPP-A levels were found in patients with acute coronary syndromes. A threshold level of 10 mIU/l identified patients with acute coronary syndrome with the best sensitivity and specificity [4]. However, the release patterns of PAPP-A in patients with acute coronary syndromes are very variable, with 2-10-fold differences. Increases in PAPP-A levels can be seen as early as 2 h or as late as 30 h after the onset of the chest pain [15]. PAPP-A levels are even elevated in hypercholesterolemic and diabetic subjects without clinical signs of atherosclerosis [16]. Moreover, we found increased PAPP-A levels in dialysed chronic renal failure patients where accelerated atherosclerosis and cardiovascular events are more frequent [17, 18, 19]. Similarly as in our previous study [17] dealing with the patients with mild, moderate and advanced renal failure but not dialysed we found a positive correlation between PAPP-A and serum creatinine in the patients with cerebrovascular diseases, too. This finding can suggest that the association between PAPP-A and renal function is not typical only for the renal diseases but can be a more common phenomenon.

It seems that the action of PAPP-A may not be limited to coronary arteries only. Recently, it was reported that elevated PAPP-A levels represent a possible marker of the atherosclerotic plaques echogenicity degree in carotid arteries of asymptomatic hyperlipidemic patients [20]. Our present study demonstrates for the first time the increased PAPP-A levels in patients with cerebrovascular stroke. However, the PAPP-A elevation is less expressed than in patients with acute coronary syndromes. As PAPP-A levels were more increased in patients with ischaemic stroke at high cardiovascular risk in comparison with ischaemic stroke only; it is probable that the elevation may reflect rather the extent of atherosclerosis than the specific cerebrovascular disease. It is in good agreement with the study of Cosin-Sales et al. [21], who found that PAPP-A levels were significantly higher in patients with multivessel coronary artery disease than in those with single-vessel disease. Moreover, the relationship between the atherosclerotic process and PAPP-A is

supported by the finding of a positive correlation between PAPP-A and total cholesterol in a group of patients with both ischaemic stroke and coronary disease.

Antiphospholipid antibodies (APL) are associated with a variety of neurological syndromes, especially with cerebral ischaemia. The increased serum ACA concentrations predict the risk of future transient ischaemic attacks and ischaemic stroke in women [22]. The presence of APL in an older stroke population ranges from 10 to 18% [23]. Similarly, in our patients the ACA IgG or IgM > 10 GPL or MPL were found in 11.6%.

### Conclusion

PAPP-A levels are increased in patients with intracranial haemorrhage and ischaemic stroke with coronary artery diseases. The atherosclerotic process may contribute to increased serum PAPP-A levels. PAPP-A may be a marker of increased risk of atherothrombotic events in general. Nevertheless further and more detailed studies will be needed to confirm the theory indicated by our preliminary results. A follow-up PAPP-A levels in a dynamic manner in the period immediately after stroke as well as a comparison of them in the group of younger patients and older ones should be performed.

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