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Fibrinogen concentrations in ischaemic stroke patients with metabolic disorders

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ABSTRACT

Background. Hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots. It is involved in the process of platelet aggregation, primary haemostasis, and leukocyte-endothelial cell interactions. The aim of our study was to assess the correlations between fibrinogen concentration and particular risk factors for vascular diseases and atherosclerotic changes in stroke patients.

Methods. The study group consisted with 94 patients with acute ischaemic stroke with normo- or hyperglycaemia and normo- or hyperlipidemia. 21 healthy subjects served as a control group. Fibrinogen level, HbA_{1c} and lipid profile were measured in all patients. Using a flow cytometer, we assessed CD61-positive microparticles which were defined as platelet-derived microparticles (PDMPs). The level of sP-selectin in serum was measured using the ELISA method.

Results. A significant positive correlation was observed between fibrinogen concentration and sP-selectin ($p = 0.001$), HbA1c ($p < 0.05$) level, and percentage of PDMPs ($p < 0.05$) in the study patients. Furthermore, we noticed a significant negative correlation between fibrinogen concentration and the level of HDL ($p < 0.05$). No correlation was observed between fibrinogen and TC, LDL and TG levels.

Conclusions and clinical implications. Our findings suggest that an elevated fibrinogen level may represent a marker of prothrombotic condition exacerbated in the state of hyperglycaemia and activation of platelets and endothelial cells. This suggests an important role played by fibrinogen in the process of thrombogenesis.

Key words: fibrinogen, sP-selectin, PDMPs, hyperglycaemia, hyperlipidemia

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Introduction

A significant role in the pathogenesis of ischaemic stroke is played by changes in the vascular endothelium and platelets activation. This process leads to atherothrombotic complications, and as a result contributes to brain infarct.

Metabolic abnormalities such as hyperglycaemia and hyperlipidemia are very common concomitant diseases observed in patients with ischaemic stroke. They are significant risk factors for vascular diseases and they play a key role in platelets activation and the development of atherosclerosis. It is also known that hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots.

Studies have shown that a high plasma concentration of fibrinogen — an acute phase protein involved in the process of platelet aggregation, primary haemostasis, and leukocyte-endothelial cell interactions — is associated with an increased risk of total stroke [1, 2]. Hyperfibrinogenemia has been identified as an independent risk factor for both venous and arterial thrombosis [3] and might contribute to the formation and progression of atherosclerotic plaques [4]. However, is also associated with early signs of atherosclerosis, even in asymptomatic individuals [5].

It must be emphasised that an increased level of fibrinogen after acute ischaemic stroke is also associated with a worse neurological outcome [6] and reduced efficacy for thrombolysis after stroke [7–9], caused by increased thrombus resistance to thrombolysis [10]. The adhesion molecule, P-selectin, also plays an important role in atherogenesis and plaque formation. Increased concentration of the soluble form of P-selectin

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(sP-selectin) has been observed in the plasma of patients with vascular diseases and this also reflects the activation of endothelial cells and platelets [11]. Furthermore, sP-selectin is also associated with the formation of platelet-derived microparticles (PDMPs) which participate in the haemostatic response to vascular injury [12]. This can exert procoagulant activity and might play an important role in thrombotic disorders.

Clinical rationale for study

The aim of our study was to assess the correlation between fibrinogen concentration and other risk factors for vascular diseases and atherosclerotic changes such as hyperlipidemia, hyperglycaemia, sP-selectin and PDMPs concentration. Better understanding of the mechanisms of interaction between the factors involved in the coagulation processes may play an important role in the prevention of ischaemic diseases of the central nervous system. An elevated fibrinogen level may indicate a prothrombotic condition of stroke patients, supporting the important role of this factor in the process of thrombogenesis. Indeed, it could be a simple marker of an increased risk of ischaemic events.

Material and methods

The study group consisted of 94 patients who were admitted to the Department of Neurology and Stroke at the Medical University of Lodz, Poland with a diagnosis of acute non-lacunar ischaemic stroke. The diagnosis of stroke was established by a combination of medical history, clinical examination, and cerebral CT or MRI scans.

The stroke patients presented different concentrations of lipids and glycaemia ranges (presented as HbA_{1c} percentages) from low to high. The control group consisted of 21 normolipidemic and normoglycaemic patients with no history of cerebrovascular diseases, who were hospitalised in the Department of Neurology and Stroke due to discopathy or tension-type headache. The exclusion criteria were a history of infection shortly before stroke, severe liver disease, renal failure, evidence of malignant, chronic inflammatory diseases, and haemorrhagic diathesis. The risk factors for ischaemic stroke (arterial hypertension, ischaemic heart disease) were similar in the study and the control groups.

Plasma samples were taken no more than seven days after the onset of symptoms. Fibrinogen, $HbA_{1c,}$ total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoproteins cholesterol (LDL-C) levels were measured in a fasting state in all patients. Serum LDL-C levels were calculated using Friedewald's formula. Biochemical determinations were performed with an Olympus AU640 Analyser (Olympus Optical Co Ltd, Shizuoka, Japan). Blood plasma was obtained from EDTA-anticoagulated samples after 10 min of centrifugation and stored at –80°C until measurement. Blood concentration of sP-selectin was measured, according to the manufacturers' instructions, with commercially available ELISA kits (R&D Systems, Abingdon, UK).

Flow cytometry (FACScan, Becton Dickinson, San Jose, CA, USA) was used to measure the PDMPs. To avoid platelet activation, blood was withdrawn without stasis. The sample contained 0.1 mL of blood and 1 mL of a 0.5% solution of paraformaldehyde in PBS. All platelet measurements were performed within 90 min of blood withdrawal. The antibody anti-CD61-FITC (Dako) — a fluorescein-isothiocyanate-conjugated antibody to glycoprotein IIIa — was used as an activation-independent marker of platelets. To assess the extent of the nonspecific association of protein with platelets, a control tube containing antiCD61-FITC and nonfractionated PE conjugated IgG (Becton Dickinson) was used for each blood sample. The reaction mixture was incubated in a dark room, at room temperature, for 30 min. Then, the antibody-bound platelets were fixed with 200 µl of FACS flow liquid and analysed. Platelets were subtracted from other blood cells and identified by flow cytometry based on the size and platelet-specific CD61 surface expression. CD61-positive microparticles were defined as platelet-derived microparticles (PDMPs). They were distinguished from other platelets on forward scatter histograms based on their size < 0.2 µm. WinMDI 2.8 was used to analyse the data collected by flow cytometry.

Since all study variables did not pass the D'Agostino normality test, differences between groups were analysed using a Kruskal-Wallis test followed by a post-hoc Duna test for multiple comparisons adjustment. A Spearman's correlation was run to assess the relationship between continuous variables. All statistical analyses were performed using Statistica for Windows v. 8.0. The null hypothesis was rejected if $p < 0.05$.

The study was approved by the Ethics Committee of the Medical University of Lodz, Poland (No. RNN/465/11/KB).

Results

The stroke patients presented different concentrations of lipids: total cholesterol, LDL and HDL cholesterol and glycaemia ranged from normal to high values (patients with normo- or hyperlipidemia and normo- or hyperglycaemia). Mean concentrations of fibrinogen in patients were within the normal range (Tab. 1).

The results of our study showed a significantly higher sP-selectin concentration and a significantly higher percentage of PDMPs in stroke patients compared to control subjects ($p < 0.0001$; Tab. 2). Moreover, we observed a significant positive correlation between fibrinogen concentration and sP-selectin level in the group of stroke patients ($p = 0.001$; Fig. 1). A positive correlation was also noted between the concentration of fibrinogen and PDMPs ($p < 0.05$; Fig. 2). The results of our study also indicated a positive correlation between fibrinogen concentration and the level of HbA_{1c} ($p < 0.05$; Fig. 3).

Table 1. Clinical characteristics of study groups

	Stroke patients (n = 94) mean ± SD	CS (n = 21) mean ± SD
Sex, male/female	44/50	10/11
Age, years	71.43 ± 10.3	60.2 ± 14.6
TC [mmol/L]	4.68 ± 1.2	4.06 ± 0.5
LDL [mmol/L]	3.44 ± 1.35	2.45 ± 0.13
HDL [mmol/L]	1.15 ± 0.3	0.95 ± 0.17
TG [mmol/L]	1.56 ± 0.72	1.31 ± 0.48
HbA_{1c} [%]	6.5 ± 1.25	5.22 ± 0.37
FBG [mg/dL]	349 ± 87	322 ± 84
CRP	10.3 ± 14	5.8 ± 6.8

CS — control subjects; SD — standard deviation; TC — total cholesterol; LDL — low-density lipoprotein cholesterol; HDL — high-density lipoprotein cholesterol; TG — triglyceride; HbA_{1c} — glycosylated haemoglobin A_{1c}; FBG — fibrinogen; CRP — C Reactive Protein

Table 2. Percentages of platelet derived microparticles (PDMPs) and soluble P-selectin concentration in study group

Study parameters	Median	
	Stroke patients	CS
PDMPs [%]	12.78*	8.3
sP-selectin [ng/mL]	115.45*	83.32

*$p < 0.0001$ *vs* control

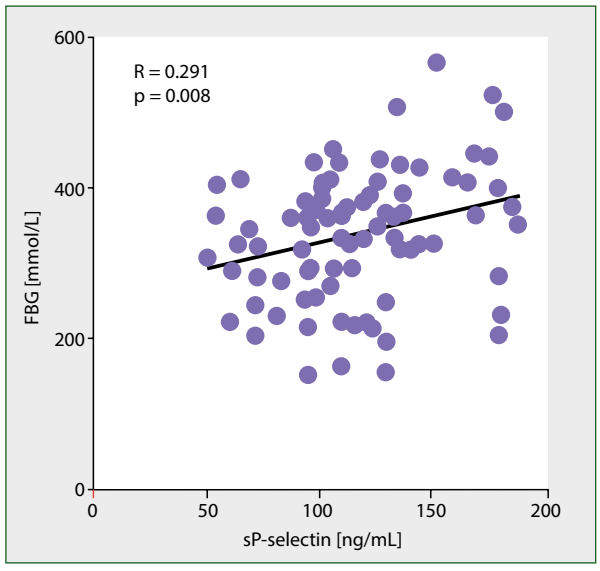

Figure 1. Correlation between fibrinogen concentration and sP-selectin level

Based on our previous studies, we expected that we would observe a positive correlation of atherogenic lipid fractions with fibrinogen. In fact, we did not observe any relation between fibrinogen concentration and TC, LDL, and TG levels. However, we found a significant negative correlation between fibrinogen concentration and the level of HDL in our study patients ($p < 0.05$; Fig. 3).

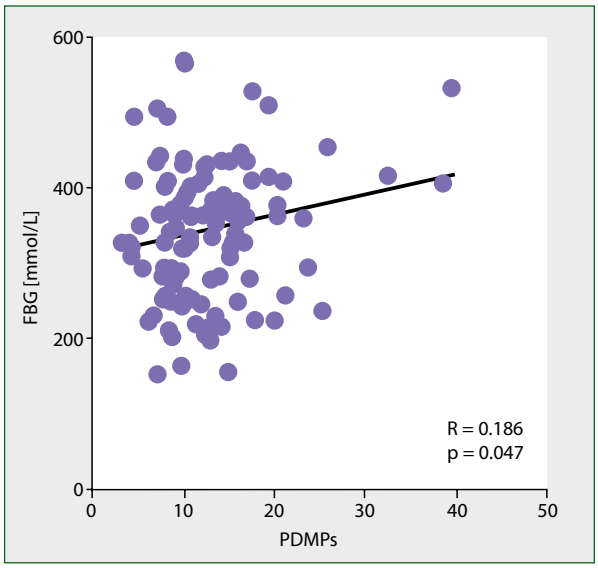

Figure 2. Correlation between fibrinogen concentration and % of PDMPs

Discussion

The principal finding of our study is a positive association between plasma fibrinogen concentration and the risk factors for atherosclerotic changes in patients with acute ischaemic stroke.

It is known that metabolic disturbances like hyperlipidemia and hyperglycaemia are factors with strong atherogenic properties, and that hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots. Atherogenesis and atherothrombotic complications are also initiated in part by fibrin deposition [13, 14]. Fibrinogen also accelerates platelet aggregation, and increases its reactivity [15].

Our study found a positive correlation between fibrinogen level and serum concentration of sP-selectin. This suggests the important role they play in inflammation and haemostasis disorders. It has been proposed that soluble cell adhesion molecules, such as sP-selectin, could be a marker of the endothelial damage preceding atherosclerosis [16]. However, the increased plasma concentration of soluble P-selectin reflects also platelet activation related to the release of this adhesion molecule from activated platelets [17] and exerts procoagulant activity resulting from their high levels in the blood [18]. A previous study has demonstrated that fibrinogen increases platelet intracellular P-selectin level and affects P-selectin expression on the surface of platelets [19], leading to their

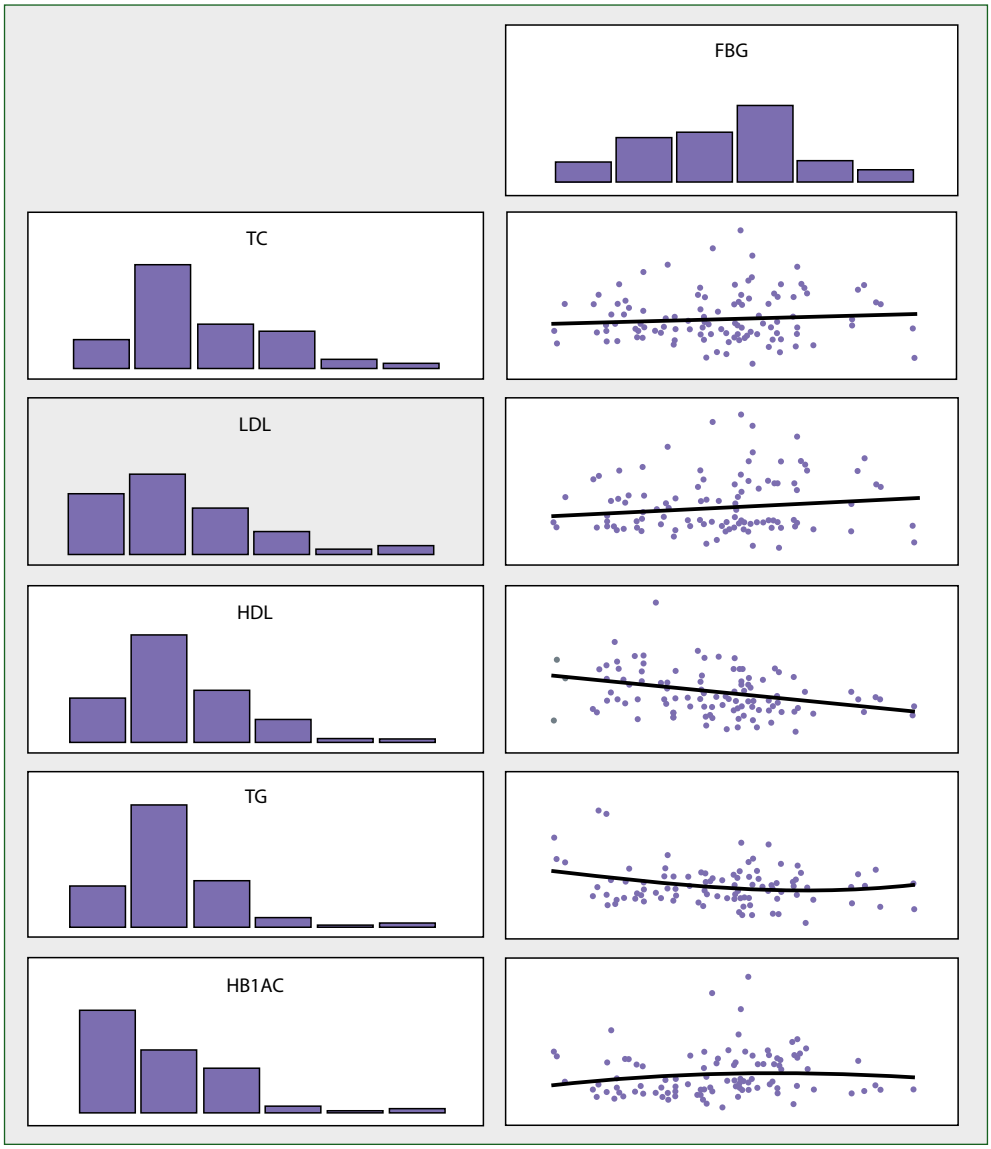

Figure 3. Correlations between fibrinogen concentration and TC, LDL, HDL, TG, HBA1c levels

activation. Because P-selectin expression on the platelet surface is short lasting, circulating degranulated platelets rapidly lose the surface P-selectin, and its level rises in the plasma pool [20]. It is important also to note that activated platelets cause Weibel-Palade body release, leading to P-selectin–mediated leukocyte rolling [21].

These findings taken together suggest that platelet P-selectin plays a crucial role in the process of inflammation and atherogenesis. In our study, we could not be sure of the origin of s-P selectin level. It could reflect platelets activation as well as endothelial cells damage. However, in both cases, fibrinogen plays an important role as the activator of the thrombogenic process. The role of blood platelets in the development of atherosclerotic lesions and in the enhancement of the prothrombotic state is also significant and mostly results from their interactions with damaged endothelial cells [22].

PDMPs play an important role in coagulation. So, an increased PDMPs level can lead to the state of hypercoagulability [23]. It has been reported that PDMP blood concentrations are significantly higher in hyperlipidemic patients with diabetes mellitus (DM), suggesting that PDMPs may participate in atherosclerosis development [24]. An elevated level of PDMPs observed in patients with ischaemic stroke may suggest their thrombogenic potential [24, 26].

The results of our study confirmed these observations, because we noticed in ischaemic stroke patients a positive correlation between fibrinogen and PDMPs level, the two potential athero- and thrombogenic factors. Another study also showed that in the acute phase of cerebral infarction, an increased fibrinogen level was associated with elevated levels of platelet-derived microparticles [27]. Thus, this correlation may reflect the influence of fibrinogen on platelet activation

and the role of these factors in the process of clot formation. It is also possible that the local generation of PDMPs in atherosclerotic arteries may promote arterial occlusion.

The next most important finding of our study was the significant positive correlation between fibrinogen concentration and the level of HbA_{1c} in patients with acute ischaemic stroke. A similar observation indicating a correlation between HbA_{1c} and fibrinogen levels was found in a study of diabetic patients with cardiovascular diseases [28]. Diabetes mellitus and hyperglycaemia lead to a hypercoagulable state, and several factors contribute to the prothrombotic condition which characterises patients with DM. The most important of these are increased coagulation, impaired fibrinolysis, endothelial dysfunction, and platelet hyperreactivity [29].

Our results indicate that a concomitance of hyperglycaemia and hyperfibrinogenemia may accelerate vascular complications. This conclusion is confirmed by the study of Lee et al. [30] which suggested that hyperfibrinogenemia in patients with acute stroke and diabetes mellitus was associated with early neurological deterioration. In DM patients, prolonged glycation related to insulin resistance increases the risk of thrombosis [31]. It has been shown that in patients under diabetic conditions fibrinogen is glycated. That leads to changes in the fibrin clot structure that reduce permeability and decrease fibrinolysis [32, 33]. These findings may explain the worse neurological outcome in patients with acute ischaemic stroke and DM.

The findings of our study do not indicate an influence of hyperlipidemia on fibrinogen concentration, although we found a negative correlation between fibrinogen and HDL levels in our study patients. A similar observation was found in the study by Pacilli et al. [29]. They noted a negative correlation between HDL level and fibrinogen concentration in diabetic patients with coronary artery disease. These results suggest an influence of poor glycaemic control and low HDL level on atherosclerotic processes.

Clinical implications

To sum up, hyperfibrinogenemia plays an important role in thrombotic disorders. In patients with acute ischaemic stroke the fibrinogen concentration is strongly correlated with atherogenic factors like hyperglycaemia, increased level of sP-selectin, and PDMPs, which reflect both atherosclerosis progression and platelet activation. Our findings suggest that an elevated fibrinogen level may represent a marker of prothrombotic condition exacerbated in the state of hyperglycaemia. Our findings indicate an important role played by fibrinogen in the process of thrombogenesis.

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