RELATIONSHIP BETWEEN FIBRINOGEN AND ATHEROMA ARIA AT ATHEROSCLEROSIS SENESCENT PATIENTS

Simona **Opris,** Cici **Gainaru,** Anton **Valuch**

"Ana Aslan" National Institute of Gerontology and Geriatrics Bucharest, Romania

Abstract. Increased concentrations of fibrinogen in cardiovascular diseases have led to the idea that fibrinogen is an independent risk factor for developing cardiovascular morbidity and mortality. We have studied senescent patients with carotidian atherosclerosis ATS1 (n = 15) vs. control C1 (n = 9). In addition, a group-ATS2 patients (n = 12) with advanced stenosis (70-90%) highlighted by carotid Doppler. Data have shown for fibrinogen a statistically significant increase in ATS 1 vs. C1 (523.3 vs. 360.89, p <0.001). The equations of the linear regression showed a strong correlation between the levels of fibrinogen and atheroma aria at ATS2 (p <0.05). In terms of total atheroma area we observed a highly significant increase in ATS1 vs. ATS2 (p <0.005). Study results suggest that possible alterations in the levels of fibrinogen are associated with atherosclerosis. **Key words:** fibrinogen, atherosclerosis, atheroma aria, aging

Rezumat. Concentraţiile crescute de fibrinogen în bolile cardiovasculare au condus la ideea că fibrinogenul este un factor de risc independent pentru creşterea morbidităţii cardiovasculare şi mortalităţii acestor boli. S-au luat în studiu pacienti senescenti cu ateroscleroză carotidiană ATS1 (n=15) vs. control C1 (n=9). În plus, s-a mai luat în studiu un lot- ATS2 de pacienţi (n=12) cu grad de stenoză avansată (70-90%) evidenţiate prin Doppler carotidian. Pentru fibrinogen s-a obţinut o creştere semnificativă statistic la ATS 1 vs. C1 (523.3 vs. 360.89 ; p<0.001). Ecuaţiile de regresie liniară au arătat o corelaţie puternică între nivelele de fibrinogen şi aria ateroamelor la lotul ATS2 (p<0.05). Din punct de vedere al ariei totale de ateroame se observă o creştere înalt semnificativă la ATS1 faţă de ATS2 (p<0.005). Rezultatele studiului sugerează că posibilele alterări ale nivelelor de fibrinogen sunt asociate cu ateroscleroza.

INTRODUCTION

The first correlation of fibrinogen with cardiovascular disease was made in 1950 when high levels were found in patients with cardiac ischemia. Fibrinogen is a liver protein whose changes in circulating levels have implications for various diseases. It also acts as an acute phase protein and increase after myocardial injury reaching a maximum 5 days after.

The role of elevated fibrinogen levels as an independent risk factor for coronary, cerebral, and peripheral vascular disease is well established on the basis of clinical and epidemiological studies. In cardiovascular disease, fibrinogen has been mainly considered as being involved in thrombotic occlusion and hence in the final stage of atherothrombosis. However, a number of investigators have suggested that fibrinogen may play a more active role in the development and progression of atherosclerotic plaque. The simultaneous presence of fibrinogen, its degradation products, and LDL cholesterol has been observed to influence atherogenesis in the arterial wall. Fibrinogen is involved in platelet aggregation and the formation of fibrin substrate. Fibrin appears to be a multi-potential component of atherogenesis, intervening at virtually all stages of lesion development. Fibrin and microthrombus deposition on normal intima is associated with endothelial disruption and intimal oedema, and oedema is a primary characteristic of early proliferative lesions. Fibrin strands on or in the intima encourage smooth muscle cell (SMC) migration and proliferation, and contribute to the growth of plaques. Fibrin also provides a continuing source of fibrin degradation products, and these have mitogenic activity which will sustain SMC proliferation in growing plaques, and act as chemoattractants for blood leucocytes.

Atherogenic factors include: elevated LDL cholesterol, low HDL cholesterol, elevated triglycerides, oxidized LDL, hypertension,

elevated C-reactive protein, elevated Lp-PLA_2, elevated omega-6:omega-3 ratio, elevated glucose, excess insulin, elevated homocysteine, elevated fibrinogen, insufficient vitamin D, insufficient vitamin K, low testosterone and excess estrogen (in men), insufficient CoQ_{10}, nitric oxide deficit.

Elevated fibrinogen in obesity, diabetes and cardiovascular disease have led to the idea that fibrinogen is an independent risk factor (1,2) to increase cardiovascular morbidity and mortality of these diseases, which should be added to the profile cardiovascular risk factors.

Also, an age-related increase in fibrinogen has been reported in many epidemiological studies (3). However, little is known about the biochemical mechanism of this increase in fibrinogen with age. Measurement of circulating fibrinogen could give important information about a potential association between elevated plasma levels of this protein and aging.

Under these conditions we intend to study, in senescent patients with carotid atherosclerosis, pathological changes in the levels of fibrinogen with aging and the correlations between them and total atheroma area.

MATERIALS AND METHODS

Subjects

Patients distributed in 2 groups:

- control C1 - 60-75 years (n=9);
- carotidian atherosclerosis ATS1 - 60-75 years (n=15) were studied.

Additionally, a group –ATS2 (n=12) with advanced stenosis (70-90%).

Patients (diabetes mellitus, acute and chronic inflammatory state, neoplasie were excluded) with significant atherosclerotic injury, evidenced by carotidian Doppler (minimum total atheroma aria considered as 6 mm^2) were compared with elderly subjects with the same age, apparently healthy, without significant atherosclerosis injury. For accurate evaluation of atherosclerosis process, clinical and laboratory exams were performed: vascular Doppler, total cholesterol, HDL, LDL, triglycerides, creatinine, urea, uric acid.

Doppler extracranian ultrasonography was performed with an ultrasound probe Interspec pencil Apogee Cx 5-MHz for spectral Doppler and sectorial pencil with variable frequency for ecotomografie.

Samples processing

For fibrinogen assay, blood samples were drawn by venopuncture into the tubes containing sodium citrate, centrifuged, diluted 1/10 and then determinated with a cuagulometer (excluded lipemic and hemolisated samples).

Fibrinogen assay

Was performed with a 1-channel optical cuagulometru COATRON M1, which measures the basic parameters of haemostasis - stage II using the Clauss method and clotting time obtained is inversely proportional to the amount of fibrinogen. Diluted serum samples are coagulated by trombine and clotting time was obtained by extrapolation of the sample on the standard curve, the results were expressed in mg/dl (normal values: 200-400 mg/dL).

Statistical analysis

Results are presented as means ± S.D. Statistical analyses were done by Student's "t" test and $p<0.05$ was considered to be statistically significant. The relationship between age, fibrinogen and total atheroma aria was assessed using a linear regression model.

RESULTS AND DISCUSSIONS

From biochemical an immunological point of view (Table 1), are noticed minor variations. This emphasize that usual clinical data give only an overview of the occurrence or progression of a disease, but it cannot provide additional fine detail and accurate diagnosis.

Table 1. Clinical features at ATS patients vs. Control

	ATS 1	C1	ATS 2 –advanced stenosis
AGE (years)	72.2±3.144	71.89±5.41	61.58±8.78
GLYCEMIC (mg/dL)	97.6±12.43	93.89±11.61	95.16±15.54
CHOLESTEROL (mg/dL)	245.86±46.78	220.89±48.97	206.58±36.96
TRIGLYCERIDES (mg/dL)	149.73±92.22	136.55±90.79	189.08±47.53
HDL-CHOLESTEROL (mg/dL)	52.3±13.26	56.52±16.71	43.81±8.54
LDL-CHOLESTEROL (mg/dL)	143.2±41.25	145.61±25.8	128.11±26.95
CREATININE (mg/dL)	0.9±0.137	0.8±0.16	1.25±0.39
UREA (mg/dL)	32.01±12.66	28.33±6.5	48.58±20.05
URIC ACID(mg/dL)	4.96±1.26	4.13±1.17	6.63±1.87
IgG(UI/mL)	178.4±55.11	179.55±68.07	126.67±66.52
IgA(UI/mL)	150.66±58.82	168.44±48.01	157.67±82.53
IgM(UI/mL)	226.1±193.8	218.89±79.28	152.67±56.84
C_3(mg%)	88.93±38.23	84±39.79	61.16±4.62

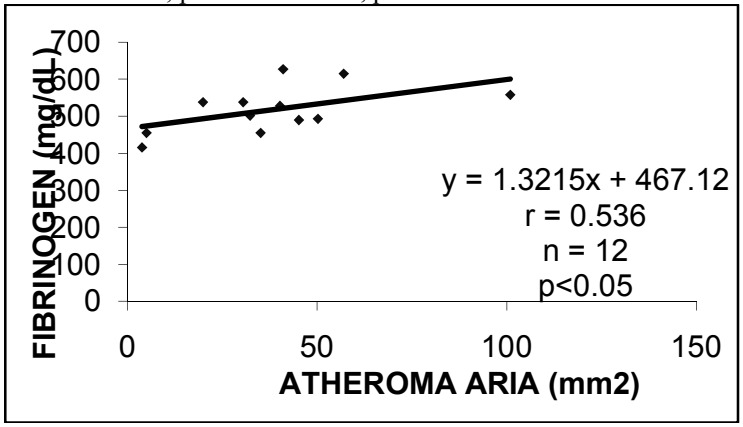
Results are presented as means±D.S.

Our data (Table 2) revealed a statistically significant increase for fibrinogen at ATS1 vs. C1 (523.3 vs. 360.89, $p < 0.001$). The association between increased levels of fibrinogen and atherosclerosis observed in the present study are in agreement with other studies (4,5,6). Other authors (7) too, have shown that plasma fibrinogen levels are increased in patients with coronary atherosclerosis. Some researchers (8) have shown a positive association of high levels of fibrinogen and atheroma area. Regression analysis of our data also showed a strong correlation (Fig. 1) between fibrinogen levels and atheroma area at ATS2 ($p < 0.05$).

Table 2. Studied parameters at ATS patients vs. Control

	ATS 1	C1	ATS 2 advanced stenosis
FIBRINOGEN (mg/dL)	523.33±108.57**	360.89±101.08	517.91±63.09
PLAQUES NUMBER	2.4±1.05	non-disclosure	2.91±1.16
TOTAL ATHEROMS ARIA(mm^2)	14.33±13.44tt	non-disclosure	38.43±25.6

Results are presented as means ±D.S.; $p^{**}<0.001$ vs. C1, $p^{tt}<0.005$ vs. ATS2



Curve fitting was by linear regression. r = correlation coefficient

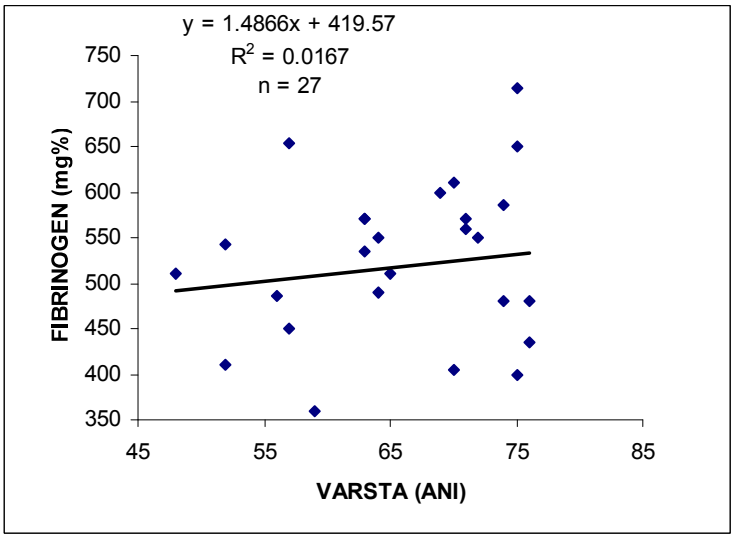
Fig. 1 Correlation between fibrinogen and total atheroma aria at ATS2

The presence of an inflammatory status increases plasma levels of fibrinogen. The tissues reaction to these stimuli lead to degradation products of fibrinogen which in turn acts on macrophages to release of the hepatocyte-stimulating factor and thereby increases hepatic synthesis of fibrinogen (9). This increase reflects hypercoagulability, hyperviscosity and increased platelet aggregation, suggesting that plasma fibrinogen penetrate the vascular wall (10). Therefore, the more plasma levels of fibrinogen increases, more fibrinogen penetrate the vessel wall, leading to the progression of atherosclerosis.

In terms of total atheroma area (Table 2) there is a highly significant increase in ATS1 vs. ATS2 ($p < 0.005$). This underlines the importance of early examination for taking preventive measures before installation and progression of atherosclerosis.

Normal aging is characterized by specific age-related changes in cardiovascular structure and function. These changes can be accelerated by diseases like atherosclerosis, mainly in the elderly population (11). In addition, aging of the other systems (neuroendocrine, respiratory) may change the aging cardiovascular process and contribute to the total clinical phenotype.

The relationship between aging and the studied parameters by linear regression equations show a statistically insignificant increase in fibrinogen in patients with atherosclerosis. Other authors have found too, high concentrations of fibrinogen in the elderly patients (12).



Curve fitting was by linear regression. r = correlation coefficient

Fig 2. Correlation between age and fibrinogen at ATS patients

CONCLUSIONS

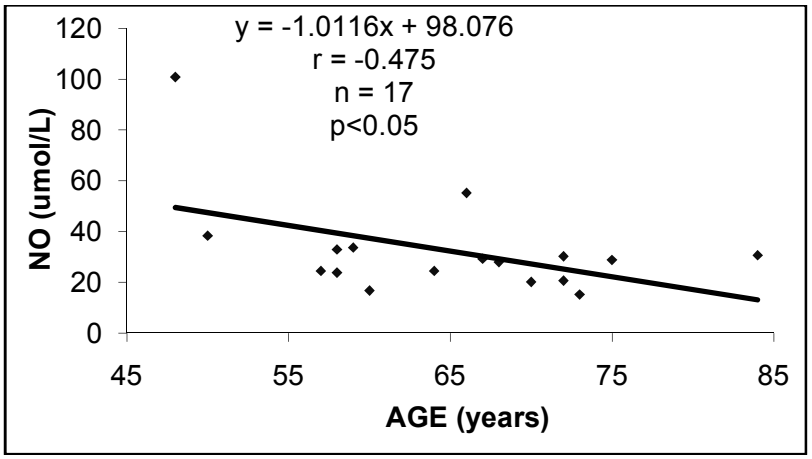
Ageing is an independent risk factor in the development of atherosclerosis and is associated with a progressive decline in endothelial-dependent vasodilation. Study results suggest that the possible alterations in the levels of fibrinogen are associated with atherosclerosis, but detailed studies are needed to evaluate the mechanisms and interactions of these biochemical changes that lead to disease. If fibrinogen is accepted as an independent risk factor and marker for chronic inflammatory processes that reflect atherogenesis would be very important to know what factors influence its levels.

In terms of total area of atheroma (Table 1) is observed highly significant increase in groups ATS2 and ATS1 vs. ATS3 ($p < 0.005$). This underlines the importance of early examination for taking preventive

measures before installation and progression of atherosclerosis.

Normal aging is characterized by specific age-related changes in cardiovascular structure and function. These changes can be accelerated by diseases like atherosclerosis, mainly in the elderly population (17). In addition, aging of the other systems (neuroendocrine, respiratory, etc) may change the aging process and contribute to the clinical phenotype of cardiovascular total.

The relationship between aging and the studied parameters, by linear regression equation, show a significant decrease of plasma levels of NO (Fig. 3) in the control group ($p < 0.05$).



y = linear regression equation ; r = correlation coefficient

Fig. 3 Correlation between NO levels and age at control groups

CONCLUSIONS

Ageing is an independent risk factor in the development of atherosclerosis and is associated with a progressive decline in endothelial-dependent vasodilatation. Endothelial dysfunction can have a pathogenic role in the development of atherosclerosis and its complications in elderly patients. The effect of aging on endothelial vasodilatation in the arteries is characterized by a significant decrease response to acetylcholine in blood flow. The decrease, linked to the aging, of the production of NO, increased degradation of NO or increased vasoconstriction factors in vascular wall may contribute to this effect.

Study results suggest that the possible alterations of plasma levels of NO are associated with atherosclerosis, but detailed studies are needed to evaluate the mechanisms and interactions of these biochemical changes that lead to disease. It also remains to be determined why the normal physiological production of NO can prevent atheroma formation and overproduction after induction of iNOS is harmful.

REFERENCES

[1] Handa K, Kono S, Saku K, Sasaki J, Kawano T, Sasaki Y, et al. Plasma fibrinogen levels as an independent indicator of severity of coronary atherosclerosis. Atherosclerosis, 1989, 77, 209-213.

[2] Ernst E. Plasma fibrinogen—an independent cardiovascular risk factor. J Intern Med, 1990, 227, 365-72.

[3] Ernst E, Resch KL.Fibrinogen as a cardiovascular risk factor a meta-analysis and review of the literature . Ann Int Med, 1993, 118, 956-963.

[4] Berderly M, Graff E, Reicher-Reis H, Behar S, Brunner D, Goldbourt U. Fibrinogen is a predictor of mortality in coronary heart disease patients. Arterioscler Thromb Vasc Biol, 1996, 16, 351-356.

[5] Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovscular disease. The Framingham study. J Am Med Assoc, 1987, 258, 1183-1186.

[6] Levenson J, Giral P, Razavian M, Gariepy J, Simon A. Fibrinogen and silent atherosclerosis in subjects with cardiovascularnrisk factors. Arterioscler Thromb Vasc Biol, 1995, 15, 1263-1268.
[7] Lowe GDO, Drummond MM, Lorimer AR, Hutton I, Forbes CD, Prentice CRM et al. Relation between extent of coronary artery disease and blood viscosity. Br Med J, 1980, 282, 673.
[8] Small M, Lowe GDO, Beattie JM. Severity of coronary artery disease and basal fibrinolysis. Haemostasis, 1987, 17, 305.
[9] Ritchie DG, Fuller GM. Hepatocyte-stimulating factor: a monocyte-derived acute-phase regulatory protein. Ann N Y Acad Sci, 1983, 408, 490.
[10] Haust MD, Wyllie JC, More RH. Atherogenesis and plasma constituents. Atherosclerosis, 1964, 44, 255.
[11] Wolk R, Somers VK. Overview of cardiovascular aging. Advances in cell aging and gerontology, 2002, 11, 1-22.
[12] Fu A, Nair KS. Age effect on fibrinogen and albumin synthesis in humans. Am J Physiol Endocrinol Metab, 1998, 275, E1023-E1030.