GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Correlation of plasma soluble cluster of differentiation 40 ligand, alpha fetoprotein A, and pregnancy-associated plasma protein A with carotid plaque in patients with ischemic stroke

Y.J. Wang, Z.Q. Gong, X.M. Bi and Y.L Li

Department of Neurology, Eighth Hospital of Qingdao, Qingdao, China

Corresponding author: Y.J. Wang
E-mail: wangyongjiu_l@163.com

Genet. Mol. Res. 14 (3): 8091-8099 (2015)
Received December 8, 2014
Accepted March 18, 2015
Published July 17, 2015
DOI http://dx.doi.org/10.4238/2015.July.17.18

ABSTRACT. This study investigated the correlation of plama levels of inflammatory biomarkers [soluble cluster of differentiation 40 ligand (sCD40L), alpha fetoprotein A (fetuin-A), and pregnancy-associated protein A (PAPP-A)] with carotid plaque in patients with acute ischemic stroke. After undergoing color Doppler ultrasonography of the bilateral carotid arteries, 200 patients with acute ischemic stroke were grouped into plaque and non-plaque groups. The plaque group was further divided into stable and unstable plaque sub-groups by carotid plaque stability. Inter-group and -subgroup comparisons included demographic characteristics, current condition and medical history, and clinical laboratory and plama inflammatory biomarker data, and logistic regression explored the correlations between plama inflammatory biomarker levels and carotid plaques. Significantly higher sCD40L and fetuin-A levels were found in the plaque group than

in the non-plaque group (all $P < 0.05$), with odds ratios (plaque *vs* non-plaque) of 6.372 and 4.101, respectively. Increased plama inflammatory biomarker levels were accompanied by a high risk of carotid plaque formation. Similarly, significantly higher plama sCD40L and PAPP-A levels were found in the unstable plaque subgroup than in the stable plaque subgroup (all $P < 0.05$), and the odds ratios (unstable *vs* stable) were 5.290 and 4.125, respectively. Increased plama inflammatory biomarker levels were accompanied by a high risk of carotid plaque instability. The study findings showed that plasma sCD40L, fetuin-A, and PAPP-A levels are associated with carotid plaque formation and instability. Fetuin-A and sCD40L might be predictors of carotid plaque formation, while PAPP-A and sCD40L might be predictors of carotid plaque instability.

Key words: Ischemic stroke; Carotid plaque; Fetuin-A; Soluble cell differentiation antigen 40 ligand; Pregnancy-associated plasma protein-A

INTRODUCTION

Cerebrovascular disease is among the three leading causes of death, and ischemic cerebrovascular diseases account for 60-80% of all cerebrovascular diseases. Early diagnosis, treatment, and recurrence prevention have become a research hotspot in patients with ischemic cerebrovascular disease. Atherosclerosis is a common pathological basis of thromboembolic disease, and inter-arterial thromboembolism after carotid plaque rupture is an important mechanism of the occurrence and development of ischemic cerebrovascular disease. The vulnerability of atherosclerotic plaque is an important factor in the pathogenesis of ischemic stroke, even the risk can exceed the simple stenosis of carotid artery (Ross, 1999). The vulnerable plaque and the atherosclerotic plaque can easily result in thromboembolic complications or may rapidly progress to a "crime" plaque. The morphological features of vulnerable plaques include a thin fibrous cap, large lipid core, macrophage infiltration, reduced vascular smooth muscle cell production, and intraplaque hemorrhage (Hansson et al., 2006). Atherosclerosis is a fatty accumulation and chronic inflammatory process.

Immunoregulation plays a crucial role in this process (Yeh et al., 2001; Zwaka et al., 2001). Accumulating evidence shows that a variety of inflammatory mediators including plasma soluble cluster of differentiation 40 ligand (sCD40L), alpha fetoprotein A (fetuin-A), and pregnancy-associated protein A (PAPP-A) are involved in the development of atherosclerotic plaque and cardiocerebrovascular disease (Novo et al., 2005; Li et al., 2008; Tuttolomondo et al., 2010). This prospective study aimed to explore the correlation of plasma sCD40L, fetuin-A, and PAPP-A levels with the existence and stability of carotid plaques in patients with acute cerebral infarction (ACI). We screened for the serological predictor with the highest clinical value, which may provide evidence for the early prevention and treatment of ischemic cerebrovascular disease.

MATERIAL AND METHODS

Subjects

This study included patients with ACI hospitalized within 48 h of onset at the Department of Neurology of Eighth People's Hospital of Qingdao between October 2010 and March 2012. All patients were diagnosed according to the standards of the Fourth National Cerebrovascular Disease Academic Conference in 1995 (Chinese Medical Association, 1996) and the findings were confirmed by head computed tomography or magnetic resonance imaging. The exclusion criteria were the presence of liver or kidney disease, heart failure, or cancer; history of rheumatic heart disease; concomitant severe systemic infection or symptoms of infection; and history of surgical operation and trauma within the 4 weeks prior to ACI onset. All subjects provided informed consent. The study was approved by the medical Ethics Committee of the local medical institutions.

Data collection

The cerebrovascular disease risk factors in all patients were recorded (including age, gender, smoking, drinking history, and presence of hypertension, hyperlipidemia, diabetes, and coronary heart disease), along with neurological, blood pressure, and general laboratory examinations (including routine blood tests, liver and kidney function, blood lipid levels, and fibrinogen levels).

Carotid artery ultrasonography

All patients underwent carotid ultrasonic examination using a Philips color Doppler ultrasonic diagnostic apparatus (PHILIPS iu22 USA) by experienced physicians. The frequency of peripheral vascular ultrasound was 5-15 Hz. Vertical scanning along the lateral edge of the sternocleidomastoid muscle followed by transverse scanning was performed with the subjects in the supine position with their heads turned to the opposite side of the examined region. We measured the carotid artery diameter (near bifurcation, 2.0 cm) and internal and external carotid artery diameters (beyond bifurcation, 1.0 cm) as well as the carotid artery intima-media thickness (IMT). A plaque was considered present when the IMT was $\geq$1.2 mm (Ebrahim et al., 1999). The carotid atheromatous plaque appeared hypoechoic compared with the surrounding tissue. A plaque with a rough surface was defined as an unstable plaque, whereas one with the strong echo and smooth surface was defined as a stable plaque (Rosvall et al., 2005). The patients were divided into plaque and non-plaque groups according to the carotid ultrasound examination results, and the plaque group was further divided into stable and unstable plaque subgroups according to plaque stability.

Detection of plasma sCD40L, fetuin-A, and PAPP-A levels by enzyme-linked immunosorbent assay (ELISA)

From all patients, 5 mL fasting blood was collected within 48 h of hospitalization, centrifuged for 10 min at a speed of 3000 rev/min, and the plasma was separated and stored at -20°C. The plasma sCD40L, fetuin-A, and PAPP-A levels were measured using ELISA (R&D

Systems, Minneapolis, MN, USA) according to the manufacturer protocol. The accuracy was ≥0.9900, the coefficient R of standard linear regression with the expected concentration. The minimum detectable concentrations were 0.1 pg/mL, 0.1 mIU/L, and 0.1 μg/mL for sCD40L, fetuin-A, and PAPP-A, respectively. No cross-reactions with other soluble structural analogs were seen. The intra- and inter-batch variation coefficients were <6%. The basic operation process was as follows. 1) The sample to be tested was thawed (the ELISA kit was stored at 2-8°C) and kept at room temperature for 20 min before use. 2) The standard, sample, and blank wells were prepared with the addition of different concentrations of standard samples (50 μL standard added to the standard holes, 10 μL samples to be detected added to the sample hole plus sample dilution of 40 μL, and no samples added to the blank holes). 3) Horseradish peroxidase-labeled antibody (100 μL) was added to each hole of the standard and sample groups, sealed with a closure plate membrane, and incubated for 60 min at 37°C. No samples were added to the blank holes. 4) The liquid was discarded and each hole was dried using absorbent paper, filled with washing liquid for 1 min, and dried again using the absorbent paper. This process was repeated five times to wash the plate. 5) Substrates A and B (50 μL) were added to each hole and incubated in the dark for 15 min at 37°C. 6). Fifty microliters of stop solution (2 M H_2SO_4) was added to each hole to stop the reaction and the OD value of each hole at 450 nm wavelength was detected within 15 min. 7) The standard linear regression curve was plotted on Excel worksheets, with the standard concentration as the abscissa and the corresponding OD as the ordinate, and each sample concentration was calculated according to the curve.

Statistical analysis

All data were analyzed using the SPSS 11.5 statistical software (Chicago, IL, USA). The measurement data of normal distribution are reported as means ± SD, those of two samples were compared using a *t*-test, and the count data were analyzed using the χ^2 test. Values of $P < 0.05$ were considered to be statistically significant. Regression analysis was conducted using multivariate logistic regression analyses. In the inter-group comparison, the assignment method of the plasma marker was set to "1" if an individual's average value was higher than that of all subjects; "0" if the individual value was higher than the average group value; and "1" in men with a positive history and "0" otherwise. The variable selection method was the step back technique, the inclusion criterion was $P < 0.1$, and the exclusion criterion was $P > 0.1$.

RESULTS

A total of 221 patients with ACI were chosen for this study. Of them, 21 did not meet the inclusion criteria, and a total of 200 patients (122 men, 78 women; mean age, 60.1 ± 10.3 years; range, 33-37 years) were finally included. They were divided into the carotid plaque group (139 cases) and non-plaque group (61 cases) according to the existence of a carotid plaque after carotid artery ultrasonography. The carotid plaque group was subdivided into the stable plaque (43 cases) and unstable plaque (96 cases) subgroups according to plaque characteristics.

Comparison of demographic and clinical data in the carotid plaque and non-plaque groups

The average age and male gender ratio of the carotid plaque group were significantly

higher than those of the non-plaque group ($P < 0.05$); the prevalences of hypertension, diabetes, and hyperlipidemia were significantly higher than those in the non-plaque group ($P < 0.05$). Some laboratory indexes [total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and fasting blood sugar levels] and levels of inflammatory markers (sCD40L, fetuin-A, and PAPP-A) were also significantly higher in the plaque group than in the non-plaque group ($P < 0.05$), whereas the prevalences of smoking, drinking history, coronary heart disease, and other risk factors were not statistically significant different between the groups ($P > 0.05$) (Table 1).

Table 1. Comparison of demographic and clinical data in the carotid plaque and non-plaque groups.

Variable	Plaque group (N = 139)	Non-plaque group (N = 61)	*t* value or χ^2	P value
Age, years	63.2 ± 8.7	50.3 ± 9.5	t = 10.179	<0.001
Male [N (%)]	95 (68.3%)	27 (44.3%)	χ^2 = 10.336	0.001
Hypertension [N (%)]	99 (71.2%)	33 (54.1%)	χ^2 = 5.540	0.019
Hyperlipidemia [N (%)]	109 (78.4%)	23 (37.7%)	χ^2 = 31.31	0.000
Diabetes mellitus [N (%)]	65 (46.8%)	18 (29.5%)	χ^2 = 5.199	0.023
Coronary heart disease [N (%)]	84 (60.4%)	32 (52.5%)	χ^2 = 1.106	0.293
Atrial fibrillation [N (%)]	27 (19.4 %)	9 (14.8%)	χ^2 = 0.627	0.429
Smoking [N (%)]	80 (57.6 %)	34 (55.7%)	χ^2 = 0.057	0.811
Drinking [N (%)]	77 (55.4%)	33 (54.1%)	χ^2 = 0.029	0.865
TC (mM)	5.7 ± 1.1	5.3 ± 1.0	t = 2.433	0.016
TG (mM)	2.3 ± 0.9	2.2 ± 1.0	t = 0.699	0.485
LDL-C (mM)	4.5 ± 1.0	4.1 ± 0.9	t = 2.683	0.008
HDL-C (mM)	1.2 ± 0.3	1.1 ± 0.4	t = 1.749	0.084
Fasting blood glucose (mM)	7.5 ± 2.5	6.4 ± 2.1	t = 3.002	0.003
sCD40L (pg/mL)	151.4 ± 55.8	102.8 ± 65.9	t = 5.360	0.000
Fetuin-A (μg/mL)	390.1 ± 80.6	352.9 ± 98.6	t = 2.591	0.011
PAPP-A (mIU/L)	11.49 ± 4.67	8.46 ± 3.99	t = 4.409	0.000

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PAPP-A, pregnancy-associated protein A.

Independent predictors of carotid plaque in patients with ischemic stroke

We performed a multivariate logistic regression analysis of the variables with values of $P < 0.1$ in Table 1 as independent variables and the existence of carotid plaque as the dependent variable. Hyperlipidemia, sCD40L level, and fetuin-A level remained independent predictors of carotid plaques in patients with ischemic stroke (Table 2).

Table 2. Independent predictors of carotid plaque in patients with ischemic stroke.

Variables	OR value*	95%CI	P value
Hyperlipidemia	6.582	2.321-18.662	<0.001
sCD40L	6.372	2.174-18.670	0.010
Fetuin-A	4.101	1.012-16.619	0.048

OR, odds ratio; CI, confidence interval; sCD40L, soluble cluster of differentiation 40 ligand; Fetuin-A, alpha fetoprotein A; *OR values of the advantage of carotid plaque *vs* no carotid plaque.

Comparison of demographic and clinical data in the stable carotid plaque subgroup and unstable carotid subgroups

Subjects with plaque were divided into two groups according to plaque stability, and the average age of the unstable carotid plaque subgroup was significantly higher than that of the stable plaque subgroup ($P < 0.05$), while the prevalence of hypertension was

significantly higher in the unstable than in the stable plaque subgroup ($P < 0.05$). Some laboratory indexes (TC, LDL-C, and fasting blood sugar levels) and levels of inflammatory markers (sCD40L and PAPP-A) were significantly higher in the unstable plaque subgroup than in the stable plaque subgroup ($P < 0.05$), whereas the mean high-density lipoprotein cholesterol (HDL-C) level was lower in the unstable group than in the stable plaque subgroup ($P < 0.05$). There were no significant intergroup differences in the other indexes ($P > 0.05$) (Table 3).

Table 3. Comparison of demographic and clinical data in the stable and unstable carotid plaque subgroups.

Variable	Stable plaque group (N = 43)	Unstable plaque group (N = 61)	*t* value or χ^2 value	P value
Age (years)	59.6 ± 9.3	64.1 ± 7.2	t = 3.231	0.002
Male [N (%)]	27 (62.8%)	68 (70.8%)	χ^2 = 0.888	0.346
Hypertension [N (%)]	24 (55.8%)	75 (78.1%)	χ^2 = 7.213	0.007
Hyperlipidemia [N (%)]	33 (76.7%)	76 (79.1%)	χ^2 = 0.103	0.748
Diabetes mellitus [N (%)]	19 (44.2%)	46 (47.9%)	χ^2 = 0.166	0.684
Coronary heart disease [N (%)]	25 (58.1%)	59 (61.5%)	χ^2 = 0.137	0.712
Atrial fibrillation [N (%)]	9 (20.9%)	18 (18.6%)	χ^2 = 0.090	0.764
Smoking [N (%)]	21 (48.8%)	59 (61.5%)	χ^2 = 1.937	0.164
Drinking [N (%)]	23 (53.5%)	54 (56.3%)	χ^2 = 0.092	0.762
TC (mM)	5.4 ± 0.9	6.0 ± 1.1	t = 3.136	0.002
TG (mM)	2.2 ± 0.9	2.4 ± 1.0	t = 1.123	0.263
LDL-C (mM)	4.0 ± 1.2	5.7 ± 1.0	t = 8.696	0.000
HDL-C (mM)	1.2 ± 0.2	1.1 ± 0.3	t = 2.314	0.022
Fasting blood glucose (mM)	7.1 ± 2.3	7.9 ± 1.9	t = 2.147	0.034
sCD40L (pg/mL)	135.3 ± 74.3	176.5 ± 64.5	t = 3.319	0.001
fetuin-A (μg/mL)	387.4 ± 82.5	401.6 ± 68.9	t = 1.055	0.293
PAPP-A (mIU/L)	10.96 ± 5.02	13.98 ± 4.63	t = 3.463	0.001

TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; sCD40L, soluble cluster of differentiation 40 ligand; PAPP-A, pregnancy-associated protein-A.

Independent predictors of unstable carotid plaques in patients with ischemic stroke

We performed a multivariate logistic regression analysis of the variables with values of $P < 0.1$ in Table 3 as independent variables and the existence of an unstable carotid plaque as the dependent variable. Hyperlipidemia, sCD40L level, and fetuin-A level remained in the regression equation through the variable selection step back technique in which HDL-C was a protective factor for atherosclerotic plaque stability and sCD40L and PAPP-A levels were independent predictors of unstable carotid plaques in patients with ischemic stroke (Table 4).

Table 4. Independent predictors of unstable carotid plaques in patients with ischemic stroke.

Variable	OR value*	95%CI	P value
HDL-C	0.234	0.060-0.906	0.022
sCD40L	5.290	1.613-17.351	0.029
PAPP-A	4.125	1.281-13.283	0.021

OR, odds ratio; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; sCD40L, soluble cluster of differentiation 40 ligand; PAPP-A, pregnancy-associated protein-A; *OR values were the advantage of unstable carotid plaque/stable carotid plaque.

DISCUSSION

Approximately 70% of cases of ischemic stroke are caused by carotid artery lesions, the main cause of which is atherosclerosis. Acute cardiovascular disease events are primarily caused by plaque rupture related to atherosclerosis and thrombosis, which almost always occurs in unstable plaques. Therefore, early recognition of carotid plaque vulnerability and effective early intervention before an ischemic event have become research hotspots.

The purpose of this study was to investigate the correlation of plasma inflammatory markers with the existence and stability of carotid atherosclerosis in patients with ACI. The results showed that, in addition to the existence of carotid plaque and recognized risk factors for hyperlipidemia, higher sCD40L and fetuin-A concentrations corresponded to an increased risk of carotid plaque. HDL-C level was a protective factor for carotid plaque in terms of its stability, while increased sCD40L and PAPP-A levels indicated an increased risk of plaque instability.

As in a chronic inflammatory disease (Ross, 1999; Hansson et al., 2006), activation of the inflammatory reaction process may be the main causative factor for unstable plaque. The inflammation markers sCD40L, fetuin-A, and PAPP-A play an important role in plaque formation and stability (Novo et al., 2005; Li et al., 2008; Tuttolomondo et al., 2010).

CD40L is a transmembrane glycoprotein that belongs to the tumor necrosis factor family (Rosvall et al., 2005). Leukocyte differentiation antigen 40 (CD40) and its ligand CD40L are involved in the occurrence and development of patches (Lutgens and Daemen, 2002). CD40 and CD40L are the key mediators of the cell information channel in inflammatory reactions (Heeschen et al., 2003). CD40L is usually stored in the platelets but becomes sCD40L in the blood circulation after stimulation, which combines with CD40 to produce a series of inflammatory reactions. CD40 and CD40L are expressed in endothelial cells, smooth muscle cells, and macrophages in human atherosclerotic plaque (Mach et al., 1997), with low or no expression in normal arteries.

The plasma sCD40L levels of patients with ischemic stroke and carotid plaques were significantly higher than those of asymptomatic patients (Ding et al., 2008), suggesting that an elevated plasma sCD40L level plays an important role in platelet activation and arteriosclerotic thrombosis formation in patients with ischemic stroke. Studies have shown that the CD40 system was upregulated in patients with ACI (Garlichs et al., 2003) and the plasma sCD40L level was significantly elevated (Rosvall et al., 2005), which may be a predictive risk factor for cerebrovascular events (Novo et al., 2005). Similar to the results of the above studies, the results of this study indicated that the plasma sCD40L levels of patients with ACI and carotid plaques were higher than those of patients without carotid plaques, while the plasma sCD40L levels of patients with ACI and unstable plaques were higher than those of patients with stable plaques. This finding suggested that the sCD40L levels have some predictive value for carotid plaque existence and stability.

PAPP-A is a kind of zinc ion-binding metalloprotease with a high molecular weight that belongs to the zinc peptide enzyme superfamily, is produced by the activated cells in unstable plaques, and is released into the extracellular matrix (Oxvig et al., 1994; Lawrence et al., 1999). A large amount of PAPP-A was expressed in coronary and carotid artery corrosion and rupture, and was mainly expressed in monocytes and macrophages of the shoulder fibrous cap but seldom in the stable plaque (Bayes-Genis et al., 2001). PAPP-A is a kind of specific insulin-like growth factor-l agonist that can induce cellular proliferation, differentiation, and migration, inflammatory cell activation, LDL-C uptake, and inflammatory factor release to

promote plaque development and destabilization (Hermus et al., 2010).

Some scholars believe that there is a correlation between plasma PAPP-A levels and plaque instability, a predictive marker of carotid plaque destabilization and rupture (Sangiorgi et al., 2006; Heider et al., 2010), while other scholars suggested that the elevated plasma PAPP-A level is induced by heparin and that PAPP-A is not a marker of unstable plaque (Iversen et al., 2011). The results of this study showed that the plasma PAPP-A levels of patients with carotid plaque were not significantly different from those of patients without carotid plaques, while the plasma PAPP-A levels of patients with unstable plaques were higher than those of patients with stable plaques. These findings suggest that plasma PAPP-A level is a predictive factor for plaque stability.

Fetuin-A belongs to the cysteine protease inhibitor superfamily, and is mainly secreted by the liver and exists widely in the liver, muscle, and blood (Schafer et al., 2003). Fetuin-A is an inhibitor of the natural insulin receptor tyrosine kinase, which can regulate insulin receptor tyrosine kinase levels and inhibit insulin receptor autophosphorylation (Srinivas et al., 1993), and is involved in insulin resistance. Plasma fetuin-A level positively correlated with coronary atherosclerotic plaque formation (Fiore et al., 2007), metabolic syndrome, and atherosclerosis occurrence and development (Ix et al., 2007). In patients with ACI, fetuin-A plays a role in promoting the development of inflammation (Rosvall et al., 2005).

The results of this study indicate that the plasma fetuin-A levels of patients with carotid plaques were significantly higher than those of patients without carotid plaques, while there was no significant difference in the plasma fetuin-A levels between patients with stable plaques and those with unstable plaques. This suggests that fetuin-A levels may predict the presence of carotid plaque.

In conclusion, this study found that levels of the inflammatory markers sCD40L, PAPP-A, and fetuin-A correlated with the presence and stability of carotid plaques. Fetuin-A and sCD40L may be predictive factors for the existence of carotid plaque, while sCD40L and PAPP-A may be predictive factors for carotid plaque stability.

REFERENCES

Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, et al. (2001). Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *N. Engl. J. Med.* 345: 1022-1029.

Chinese Medical Association (1996). All types of cerebrovascular disease diagnostic criteria. *Chin. J. Neurology*. 29: 379-383.

Ding S, Zhang M, Zhao Y, Chen W, et al. (2008). The role of carotid plaque vulnerability and inflammation in the pathogenesis of acute ischemic stroke. *Am. J. Med. Sci*. 336: 27-31.

Ebrahim S, Papacosta O, Whincup P, Wannamethee G, et al. (1999). Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke* 30: 841-850.

Fiore CE, Celotta G, Politi GG, Di Pino L, et al. (2007). Association of high alpha2-Heremans-Schmid glycoprotein/fetuin concentration in serum and intima-media thickness in patients with atherosclerotic vascular disease and low bone mass. *Atherosclerosis* 195: 110-115.

Garlichs CD, Kozina S, Fateh-Moghadam S, Handschu R, et al. (2003). Upregulation of CD40-CD40 ligand (CD154) in patients with acute cerebral ischemia. *Stroke* 34: 1412-1418.

Hansson GK, Robertson AK and Soderberg-Naucler C. (2006). Inflammation and atherosclerosis. *Annu. Rev. Pathol.* 1: 297-329.

Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, et al. (2003). Soluble CD40 ligand in acute coronary syndromes. *N. Engl. J. Med*. 348: 1104-1111.

Heider P, Pfäffle N, Pelisek J, Wildgruber M, et al. (2010). Is serum pregnancy-associated plasma protein A really a potential marker of atherosclerotic carotid plaque stability? *Eur. J. Vasc. Endovasc. Surg.* 39: 668-675.

Hermus L, Lefrandt JD, Tio RA, Breek JC, et al. (2010). Carotid plaque formation and serum biomarkers. *Atherosclerosis* 213: 21-29.

Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, et al. (2007). Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. *Circulation* 115: 2533-2539.

Iversen K, Teisner A, Dalager S, Olsen KE, et al. (2011). Pregnancy associated plasmaserum protein-A (PAPP-A) is not a marker of the vulnerable atherosclerotic plaque. *Clin. Biochem*. 44: 312-318.

Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, et al. (1999). The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasmaserum protein-A. *Proc. Natl. Acad. Sci. U. S. A.* 96: 3149-3153.

Li X, Liu Q, Zhou T, Zhao S, et al. (2008). PAPP-A: a possible pathogenic link to the instability of atherosclerotic plaque. *Med. Hypotheses.* 70: 597-599.

Lutgens E and Daemen MJ (2002). CD40-CD40L interactions in atherosclerosis. *Trends Cardiovasc. Med.* 12: 27-32.

Mach F, Schönbeck U, Sukhova GK, Bourcier T, et al. (1997). Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 94: 1931-1936.

Novo S, Basili S, Tantillo R, Falco A, et al. (2005). Soluble CD40L and cardiovascular risk in asymptomatic low-grade carotid stenosis. *Stroke* 36: 673-675.

Oxvig C, Sand O, Kristensen T, Kristensen L, et al. (1994). Isolation and characterization of circulating complex between human pregnancy-associated plasma protein-A and proform of eosinophil major basic protein. *Biochim. Biophys. Acta*. 1201: 415-423.

Ross R (1999). Atherosclerosis - an inflammatory disease. *N. Engl. J. Med*. 340: 115-126.

Rosvall M, Janzon L, Berglund G, Engstrom G, et al. (2005). Incidence of stroke is related to carotid IMT even in the absence of plaque. *Atherosclerosis* 197: 325-331.

Sangiorgi G, Mauriello A, Bonanno E, Oxvig C, et al. (2006). Pregnancy-associated plasma protein-a is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques: a link between inflammation and cerebrovascular events. *Am. Coll. Cardiol*. 47: 220l-2211.

Schafer C, Heiss A, Schwarz A, Westenfeld R, et al. (2003). The serum protein alpha 2-Heremans-Schmid glycoprotein/ fetuin-A is a systemically acting inhibitor of ectopic calcification. *J. Clin. Invest.* 112: 357-366.

Srinivas PR, Wagner AS, Reddy LV, Deutsch DD, et al. (1993) Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. *Mol. Endocrinol.* 7: 1445-1455.

Tuttolomondo A, Di Raimondo D, Di Sciacca R, Casuccio A, et al. (2010). Fetuin-A and CD40L plasma levels in acute ischemic stroke: differences in relation to TOAST subtype and correlation with clinical and laboratory variables. *Atherosclerosis* 208: 290-296.

Yeh ET, Anderson HV, Pasceri V and Willerson JT (2001). C-reactive protein: linking inflammation to cardiovascular complications. *Circulation* 104: 974-975.

Zwaka TP, Hombach V and Torzewski J (2001). C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation* 103: 1194-1197.