ORIGINAL ARTICLE
Korean Circulation J 2006;36:418-423
ISSN 1738-5520

The Role of Endothelial Dysfunction for Thromboembolic Risk of Patients with Atrial Fibrillation

Hyung-Wook Park, MD[1], Nam-Sik Yun, MD[1], Sang-Yup Lim, MD[1], Sang-Rok Lee, MD[1], SeoNa Hong, MD[1], Kye-Hun Kim, MD[1], Il-Suk Sohn, MD[1], Young-Joon Hong, MD[1], Ju-Han Kim, MD[1], Weon Kim, MD[1], Myung-Geun Shin, MD[2], Young-Keun Ahn, MD[1], Myung-Ho Jeong, MD[1], Jeong-Gwan Cho, MD[1], Jong-Chun Park, MD[1] and Jung-Chaee Kang, MD[1]

[1]Department of Cardiovascular Medicine and [2]Laboratory Medicine, Chonnam National University Hospital, Chonnam National University Research Institute of Medical Sciences, Gwangju, Korea

ABSTRACT

Background and Objectives : Thromboembolism (TE) is a common complication of atrial fibrillation (AF). Although several serum markers for TE in AF patients have been reported, the mechanisms for TE have not been completely determined. **Subjects and Methods :** Seventy four patients with persistent or permanent AF (M : F=39 : 35, mean age: 53±18 years) were enrolled. The epidemiologic risk factors for TE, including old age (≥65 years), diabetes, hypertension, heart failure (HF), valvular heart disease, left ventricular (LV) dysfunction, and a history of TE were investigated. Serum markers for the endothelial function [von-Willebrand factor (vWF) and thrombomodulin (TM)], inflammation [quantitative and high sensitive C-reactive protein (CRP) and interleukin (IL)-6], coagulation [fibrinogen, fibrinogen degradation product (FDP), d-dimer] and platelet activity (p-selectin), and the echocardiographic parameters were measured. **Results :** The vWF was increased in patients with old age, hypertension, HF and a history of TE, and the vWF was positively correlated with age and the left atrium dimension (LAD), respectively. TM was also increased in the patients with old age and a history of TE and the LV dysfunction, and it was positively correlated with age and the LAD. The quantitative CRP was increased with old age, hypertension and LV dysfunction, and it was positively correlated with age and the LAD. High sensitive CRP was increased with old age and LV dysfunction, and it was positively correlated with age and the LAD. IL-6 was increased in diabetic patients. Fibrinogen was increased with old age and hypertension, and it was positively correlated with age and the LAD. FDP and d-dimer were increased in the patients with a history of TE and LV dysfunction. P-selectin was neither increased nor correlated with any other parameters. All the analyzed serum markers, except the markers for coagulation and platelet activity, were correlated with age and the LAD. **Conclusions :** It was shown that endothelial dysfunction plays an important role for the TE in AF patients. The serum markers for endothelial function may be used to screen the AF patients who are at a high risk for TE. **(Korean Circulation J 2006;36:418-423)**

KEY WORDS : Atrial fibrillation ; Thromboembolism.

Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia and it is associated with a substantial risk for stroke and thromboembolism.[1)] The risk of thromboembolism varies widely according to the presence or absence of certain clinical and echocardiographical factors.[2)] Although the mechanism behind stroke and thromboembolism in AF is incompletely understood, the increased risk is mainly due to the embolization of thrombi that are initially formed within the left atrial appendage (LAA).[3)] A reduced LAA blood flow velocity, dense spontaneous echocontrast (an index of left atrial stasis) and the complex aortic plaque seen on transesophageal echocardiography have each been shown to independently predict LAA thrombus and stroke in AF pati-

Received : January 20, 2006
Revision Received : April 3, 2006
Accepted : April 10, 2006
Correspondence : Jeong-Gwan Cho, MD, Department of Cardiovascular Medicine, Chonnam National University Hospital, Chonnam National University Research Institute of Medical Sciences, 8 Hak-dong, Dong-gu, Gwangju 501-757, Korea
Tel: 82-62-220-6242, Fax: 82-62-226-0179
E-mail: chojg@unitel.co.kr

ents.[4)5)] There is also a growing body of evidence that an increased risk of stroke and thromboembolism in AF patients is facilitated by increased thrombogenesis, changes in the left atrial wall and a prothrombotic or hypercoagulable state, with abnormalities of hemostasis, thrombosis and the platelet and endothelial function and with the inflammation in AF patients; this all leads to fulfillment of Virchow's triad.[6-11)]

However, there are few reports about the thromboembolic risks in the Asian population. Thus, this study was performed to determine the contribution of the endothelial function, the coagulation state, inflammation and platelet activation to the thromboembolic risk of Korean patients suffering with persistent and permanent AF.

Subject and Methods

We studied 74 consecutive patients (M : F=39 : 35, mean age: 53±18 years) with persistent or permanent AF that was documented by an electrocardiogram on at least two occasions at least six weeks apart at an outpatient clinic. The previously established and widely accepted epidemiologic risk factors for TE, including advanced age, hypertension, diabetes mellitus, heart failure and a previous embolic history, were investigated and we measured the echocardiographic parameters, including the left ventricular ejection fraction (LV EF) and the left atrial dimension (LAD). LV dysfunction was defined as an LV EF less than 40%. The exclusion criteria were patients with recent (<6 months) myocardial infarction or acute coronary syndrome, stroke, infection or inflammatory disease, surgery, malignancy, thyrotoxicosis or renal/liver impairment.

We measured the blood levels of von Willebrand factor (vWF), and thrombomodulin (TM) for examining the endothelial function, C-reactive protein (CRP, quantitative and high sensitive) and interleukin (IL)-6 for examining the inflammatory activity, fibrinogen, fibrinogen degradation product (FDP) and fibrin d-dimer for examining the coagulation system, and soluble P-selectin for examining the platelet activity. Blood samples were taken by venipuncture atraumatically in the morning from patients after fasting for >12 hours. Blood was drawn without stasis into tubes preloaded with trisodium citrate. Soluble P-selectin, vWF and IL-6 were measured by the enzyme-linked immunosorbent assay (ELISA) technique with using commercial reagents (R & D systems, USA), and the TM was measured by the enzyme immunosorbent assay (EIA) technique with using commercial reagents (Daiichi, Japan). CRP was measured by the latex agglutination method (quantitative CRP, Behring nepherometer analyzer; high sensitive CRP, Olympus AU 5400). Measurements of fibrinogen, FDP and d-dimer were performed via chromogenic assay (Sysmex, CA1500, USA).

Transthoracic m-mode, two-dimensional and Dopper echocardiography exams were performed in all patients. The echocardiographic parameters were measured on the parasternal long axis and on the apical four-chamber views.

The results are expressed as means±standard deviation. Comparisons of the serum markers between the patients who were with and without thromboembolic risk factors were analyzed by using the independent t-test. Correlations of the serum markers with the clinical and echocardiographic risk factors were analyzed using the Pearson correlation test. All statistical calculations were performed using a commercially available statistical package (SPSS, version 12.0, USA). A $p<0.05$ was considered as statistically significant.

Results

The clinical characteristics, including the demographic characteristics, the associated medical conditions and the risk factors of the study population are shown in Table 1.

Hypertension was present in 24 patients (32.7%), diabetes in 5 (6.8%), LV dysfunction in 6 (8.1%), mitral stenosis in 3 (4.1%), a history of TE in 5 (6.8%) and congestive heart failure in 22 (29.7%). The mean duration of the atrial fibrillation and the LV EF were 2.7±4 years and 61±12%, respectively. The left atrial dimension was 38±9 mm. Aspirin was used by 15 (20.3%) patients and warfarin by 59 (79.7%) for antithrombotic therapy.

Table 1. Clinical characteristics of patients

Demographic features	Results
Age (years)	55±15
≥65 years, n (%)	26 (35.1)
Sex (M : F)	39 : 35
LV EF (%)	61±12
LAD (mm)	38±9
Mean duration of AF (years)	2.7±4
Risk factors for TE, n (%)	
Hypertension	24 (32.7)
Diabetes mellitus	5 (6.8)
LV dysfunction	6 (8.1)
Mitral stenosis	3 (4.1)
History of TE	5 (6.8)
Congestive heart failure	22 (29.7)
Antithrombotic therapy, n (%)	
Aspirin	15 (20.3)
Warfarin	59 (79.7)

LV EF: left ventricle ejection fraction, TE: thromboembolism, LAD: left atrial dimension, AF: atrial fibrillation

Markers for endothelial dysfunction

The vWF level was significantly increased in the patients with old age, hypertension, a history of TE and congestive heart failure ($p<0.05$) and the vWF level was positively correlated with age, the NYHA functional class and the LAD ($p<0.05$). The TM level was also increased in patients with old age, a history of TE, congestive heart failure and LV dysfunction ($p<0.05$). The TM level was positively correlated with age, the NYHA functional class and the LAD, and it was negatively correlated with the LV EF ($p<0.05$) (Fig. 1) (Table 2).

Markers for inflammatory activity

The quantitative CRP was significantly increased in patients with old age, hypertension and LV dysfunction ($p<0.05$) (Fig. 2) (Table 3). The quantitative CRP was

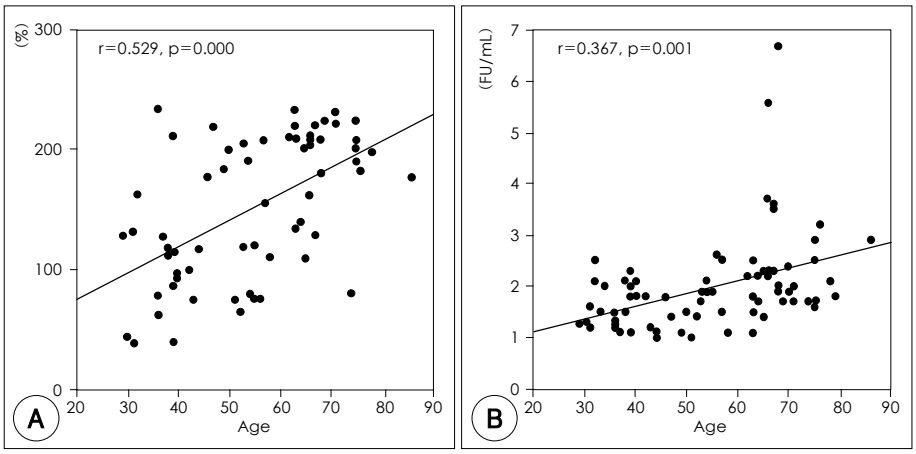

Fig. 1. Correlation of markers for endothelial function with age. von Willebrand factor (A) and thrombomodulin (B) level were positively correlated with age.

Table 2. Comparison of the vWF and TM levels according to the presence or absence of TE risk factors

	vWF (%)	TM (FU/mL)
Age		
≥65 years	190±39†	2.6±1.2†
<65 years	133±59	1.7±0.4
Hypertension		
Yes	178±53*	2.3±1.3
No	131±63	1.8±0.6
History of TE		
Yes	186±36*	3.0±2.2*
No	149±39	1.9±0.7
NYHA class		
I	125±66	1.7±0.4
II	128±66	1.8±0.6
III	179±47†	2.3±1.3
IV	198±54†	2.5±0.7†
MS		
Yes	156±67	2.6±1.6
No	146±65	1.9±0.9
DM		
Yes	138±68	1.5±0.6
No	147±64	2.0±0.9
LV dysfunction		
Yes	163±30	3.7±2.7†
No	147±68	1.8±0.6

*: $p<0.05$, †: $p<0.01$. vWF: von-Willebrand factor, TM: thrombomodulin, TE: thromboembolism, CHF: congestive heart failure, MS: mitral stenosis, DM: diabetes mellitus, LV: left ventricle

Table 3. Comparison of the C-reactive protein and IL-6 levels according to the presence or absence of TE risk factors

	Q-CRP (mg/dL)	Hs-CRP (mg/dL)	IL-6 (pg/mL)
Age			
≥65 years	1.2±1.2†	0.8±1.1†	11±7
<65 years	0.4±0.4	0.2±0.4	12±7
Hypertension			
Yes	0.9±1.1*	0.7±1.0	12±7
No	0.4±0.7	0.3±0.7	11±6
History of TE			
Yes	1.4±0.7	1.2±1.6	13±5
No	1.2±1.8	1.6±1.8	12±7
NYHA class			
I	0.3±0.4	0.3±0.6	12±7
II	0.6±0.5	0.3±0.4	12±7
III	0.8±1.3	0.6±1.2	11±6
IV	0.8±0.9	0.6±0.8	13±5
MS			
Yes	0.4±0.1	0.4±0.1	14±8
No	0.6±0.9	0.4±0.9	11±6
DM			
Yes	0.5±0.5	0.3±0.3	18±4†
No	0.6±0.9	0.4±0.9	11±3
LV dysfunction			
Yes	1.6±2.3*	1.5±2.1*	13±6
No	0.5±0.7	0.3±0.6	11±7

*: $p<0.05$, †: $p<0.01$. TE: thromboembolism, CHF: congestive heart failure, MS: mitral stenosis, DM: diabetes mellitus, LV: left ventricle, Q and Hs: quantitative and high sensitive, CRP: C-reactive protein

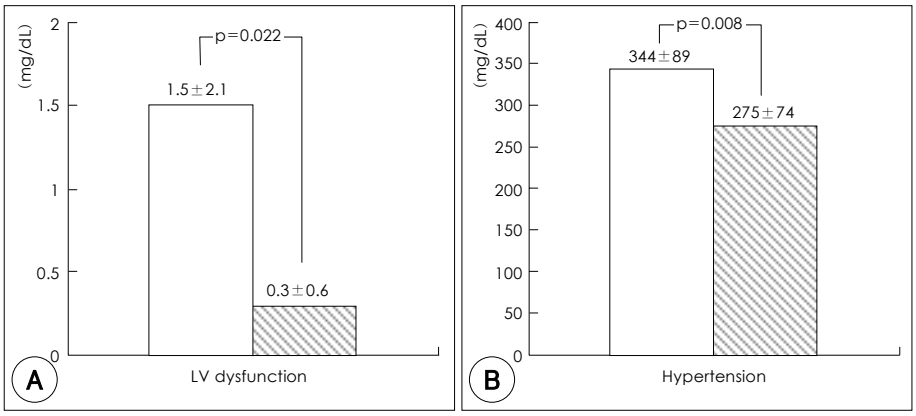

Fig. 2. High sensitive C-reactive protein (A) and fibrinogen level (B) according to presence of left ventricle (LV) dysfunction and hypertension. High sensitive C-reactive protein and fibrinogen level were significantly increased in patients with LV dysfunction and hypertension. Closed and dotted bar denote presence or absence of risk factor, respectively.

Table 4. Correlation coefficient of each marker with clinical characteristics and echocardiograhic parameters

	Age	NYHA class	EF	LAD
Endothelial function				
vWF	0.529†	0.394†	0.092	0.482†
TM	0.367†	0.305*	-0.343*	0.383*
Inflammation				
Q-CRP	0.436†	0.233	-0.345*	0.486†
Hs-CRP	0.378*	0.173	-0.366*	0.495†
IL-6	0.113	0.049	0.146	0.060
Coagulation system				
Fibrinogen	0.360†	0.169	-0.419*	0.517†
FDP	0.112	0.181	-0.345*	0.256
D-dimer	0.094	0.250	0.364	0.420
Platelet activation				
P-selectin	0.087	0.107	0.055	0.046

*: p<0.05, †: p<0.01. vWF: von-Willebrand factor, TM: thrombomodulin, Q-CRP: quantitative C reactive protein, Hs-CRP: high sensitive C reactive protein, IL-6: interleukin-6, FDP: fibrinogen degradation product, LVEDD and LVESD: left ventricular end-diastolic and end-systolic dimension, EF: ejection fraction, LAD: left atrium dimension

positively correl ated with increasing age and LAD (p< 0.05), and it was negatively correlated with the LV EF (p<0.05) (Table 4). High sensitive CRP was also significantly increased in the patients with old age and LV dysfunction (p<0.05). High sensitive CRP was also positively correlated with increasing age and LAD (p<0.05), and it was negatively correlated with the LV EF (p<0.05) (Table 4). IL-6 was significantly increased in the patients with diabetes mellitus (p<0.05) (Table 3).

Markers for coagulation system

Fibrinogen was significantly increased in the patients with old age and hypertension (p<0.05); this was positively correlated with age and the LAD (p<0.05), and it was negatively correlated with the LV EF (p<0.05). The FDP was significantly increased in the patients with a history of TE and LV dysfunction (p<0.05), and it was negatively correlated with the LV EF (p<0.05). Fibrin D-dimer was significantly increased in the patients with a history of TE and LV dysfunction (p<0.05) (Table 4, 5).

Table 5. Markers for coagulation system and platelet activation levels according to TE risks

	Fibrinogen (mg/dL)	FDP (μg/mL)	Fibrin D-dimer (mg/L)	P-selectin (ng/mL)
Age, y				
≥65 years	356±103†	2.9±6.5	0.3±0.3	139±97
<65 years	279±86	2.0±4.3	0.2±0.2	122±62
Hypertension				
Yes	344±89†	3.6±7.7	0.3±0.4	136±72
No	275±74	1.6±2.4	0.2±0.1	129±80
History of TE				
Yes	332±119	5.2±3.6*	0.5±0.5*	109±41
No	304±98	1.9±3.7	0.2±0.2	129±78
NYHA class				
I	286±87	1.6±2.3	0.1±0.1	141±83
II	306±98	1.3±2.9	0.2±0.2	135±92
III	302±81	2.7±2.7	0.3±0.3	121±56
IV	314±99	3.0±2.8	0.2±0.4	145±95
MS				
Yes	323±75	1.4±0.7	0.3±0.2	131±49
No	300±81	2.4±3.1	0.2±0.3	132±78
DM				
Yes	308±124	2.2±2.6	0.1±0.1	157±98
No	298±84	2.3±3.1	0.2±0.2	130±73
LV dysfunction				
Yes	372±26	6.3±1.7†	0.6±0.5†	117±45
No	291±80	1.6±2.3	0.2±0.2	133±81

*: p<0.05, †: p<0.01. TE: thromboembolism, CHF: congestive heart failure, MS: mitral stenosis, DM: diabetes mellitus, LV: left ventricle, FDP: fibrinogen degradation product

Markers for platelet activity

P-selectin was neither significantly changed nor cor-

related with any other risk factors (Table 5).

Discussion

Endothelial dysfunction and inflammation, and the abnormalities of the coagulation system and platelet activation have all been reported to contribute to the development of thromboembolism in AF patients. This study shows the most powerful and consistent correlation of the serum markers for endothelial function with the known epidemiologic thromboembolic risk factors in Koreans suffering with persistent and permanent atrial fibrillation.

Endothelial dysfunction may lead to increased endothelial adhesiveness to the leukocytes, and to the production of procoagulant and vasoactive molecules, cytokines and growth factors.[12]

vWF is a multifunctional plasma protein that plays a very important role in hemostasis following vascular injury. As a consequence of vascular injury, the subendothelial matrix and collagen fibers are exposed to the blood flow. Circulating platelets adhere to the injured site and initiate the process of thromobosis. Subendothelial vWF plays an important role in mediating platelet adhesion at the injured site. vWF is secreted not only from the vascular endothelium, but also from the atrial endocardium in response to vascular injury and disease. Raised plasma levels of vWF were reported to be associated with widespread endothelial damage/dysfunction, atherothrombosis, LA endothelial damage and LA appendage thromobosis. Recently, vWF is generally accepted and used as a marker for endothelial damage/dysfunction.[13][14] Also, thrombomodulin (TM), which is transmembrane spanning protein that can also be cleaved from the membrane to circulate in a soluble form, is one of the major anticoagulant components of the endothelial surface.[15][16] It binds to thrombin to form a 1 : 1 complex. Thrombin bound to TM consequently loses its procoagulant and proinflammatory functions. It cannot cleave fibrinogen or activate platelets and factor XIII.[17] In our study, we found that the markers for endothelial dysfunction are mostly well correlated with the known epidemiologic and echocardiographic risk factors for TE in Korean AF patients, and endothelial dysfunction may play the most powerful role in the development of TE in AF patients.

There is an apparent link between thrombogenesis and inflammation. An established index of inflammation is IL-6, which is a circulating cytokine produced by monocytes, macrophages, T-lymphocytes and endothelial cells. IL-6 can induce a prothrombotic state by increasing the expression of fibrinogen, tissue factor, factor VIII and von Willebrand factor, as well as by activating endothelial cells and increasing the platelet production.[6-8] Elevated CRP levels have been reported in AF patients, which reflects an inflammatory state, and this could promote the persistence of AF.[18] Although the C-reactive protein level was increased and correlated with some of the known risk factors for TE in this study, the IL-6 level was increased only in the patients with diabetes mellitus.

The level of hemostatic activation may also reflect the underlying mechanism of thromboembolism, and this is especially pronounced during cardioembolic stroke.[19] The fibrin D-dimer assay is based on the production of cross-linked fibrin by thrombin, making it a sensitive marker of fibrin turnover and this allows us to recognize activated coagulation.[20] The markers for the coagulation system were substantially increased and correlated with some of the risk factors for TE; however those were increased and correlated to a lesser degree as compared with the markers for endothelial function and inflammation in this study.

Although contribution of platelet activation was not so dominant in this study, platelet activation had been reported in the western population. P-selectin is an α-granule membrane protein that is translocated and expressed on the plasma membrane during platelet activation and degranulation.[21] Although P-selectin has been identified on endothelial cells, it is becoming clear that the majority of the soluble P-selection (sP-selectin), if not all of it, in the plasma arises from the platelets.[22] Platelet surface P-selectin and sP-selectin in the plasma have been studied as potential markers of platelet activation in various disease conditions,[23][24] and especially in the patients with an embolic and preembolic status.[25] Platelet activation was not significant in this study and the dominant role of the endothelium and the lesser role for the platelets might explain the relatively disappointing efficacy of antiplatelet therapy for the prevention of stroke in AF patients.[13]

While the benefits of antithrombotic therapy for preventing stroke in AF patients are being increasingly recognized, further developments in thromboprophylaxis are needed, especially as warfarin confers the inconvenience of regular monitoring of the prothrombin time (PT) and the benefits of aspirin are somewhat inconsistent. Current clinical practice for preventing thromboembolic stroke in AF patients is limited not only by the disappointing efficacy of antiplatelet therapy, but also by the hemorrhagic complications and the need for PT monitoring that is inherent with warfarin therapy.[26]

The finding that, in addition to a relationship between vWF and the stroke risk factors,[13] an increased plasma level of vWF may prospectively predict stroke and also vascular events might have powerful implications for the assessment and management of cardiovascular risk in AF patients. Furthermore, vWF or the vascular endothelial function/integrity may present ta-

rgets for the development of novel thromboprophylactic agents to treat AF patients. Thus, further studies are needed to establish the true mechanism of stroke in patients with increased plasma vWF, to evaluate the outcome of novel antithrombotic therapies targeting the endothelium/endocardium, to investigate the other markers of endothelial dysfunction, and to examine the potential role of plasma vWF as an aid when stratifying the risk of patients with AF.

This study determined the importance of endothelial dysfunction in the development of TE in Asian AF patients, and further research and efforts to improve endothelial dysfunction should be considered.

This study is limited by its cross-sectional design, which only allows us to explore associations. No causality is implied this study as only a prospective cohort study with a large number of AF subjects can confirm the natural history of the AF patient with changing levels of markers that would be measured in the short, medium and long-term.

This study was financially supported by research funds of Chonnam National University in 2003.

REFERENCES

1) Wolf PA, Abbot RD, Kannel WB. *Atrial fibrillation as an independent risk for stroke. Stroke 1991;22:983-8.*
2) Lee SM, Choue CW, Hong SJ, et al. *The joint multicenter study on the atrial fibrillation in Korea. Korean Circ J 2000;30:646-52.*
3) Hart RG, Halperin JL. *Atrial fibrillation and stroke: concepts and controversies. Stroke 2001;32:803-8.*
4) Jeon SH, Park SH. *Multiplane transesophageal echocardiograhic assessment of left atrial appendage. Korean Circ J 2002;32:137-45.*
5) Ha JW, Kang SM, Jang KJ, et al. *Enhanced detection of left atrial spontaneous echo contrast by transthoracic harmonic imaging in mitral stenosis. Korean Circ J 2000;30:1230-7.*
6) Lip GY, Lowe GD, Rumley A, Dunn FG. *Increased markers of thrombogenesis of chronic atrial fibrillation: effects of warfarin therapy. Br Heart J 1995;73:527-33.*
7) Lip GY. *Does atrial fibrillation confer a hypercoagulable state? Lancet 1995;346:1313-4.*
8) Kumagai K, Fukunami M, Ohmori M, Kitabatake A, Kamada T, Hoki N. *Increased cardiovascular clotting in patients with chronic atrial fibrillation. J Am Coll Cardiol 1990;16:377-80.*
9) McEver RP. *Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. Thromb Haemost 2001;86:746-56.*
10) Kerr R, Stirling D, Ludlam CA. *Interleukin 6 and haemostasis. Br J Hematol 2001;115:3-12.*
11) Joseph L, Fink LM, Hauer-Hensen M. *Cytokines in coagulation and thrombosis: a preclinical and clinical review. Blood Coagul Fibrinolysis 2002;13:105-16.*
12) Blann AD. *Endothelial cell activation, injury, damage and dysfunction: separate entities or mutual terms. Blood Coagul Fibrinolysis 2000;11:623-30.*
13) Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. *Prognostic value of plasma von Willebrand factor and soluble P-selectin as indices of endothelial damage and platelet activation in 994 patients with nonvalvular atrial fibrillation. Circulation 2003;107:3141-5.*
14) Roldan V,Marin F, Garcia-Herola A, Lip GY. *Correlation of plasma von Willebrand fator levels, an index of endothelial damage/dysfunction, with two point-based stroke risk stratification scores in atrial fibrillation. Thromb Res 2005;116:321-5.*
15) Califano F, Giovanniello T, Pantone P, et al. *Clinical importance of thrombomodulin serum levels. Eur Rev Med Pharmacol Sci 2000;4:59-66.*
16) Maruyama I. *Thrombomodulin, an endothelial anticoagulant: its structure, function and expression. Jpn Circ J 1992;56:187-91.*
17) Fuentes-Prior P, Iwanaga Y, Huber R, et al. *Structural basis for the anticoagulant activity of the thrombin-thrombomodulin complex. Nature 2000;404:518-25.*
18) Chung MK, Martin DO, Sprecher D, et al. *C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. Circulation 2001;104:2886-91.*
19) Feinberg WM, Erickson LP, Bruck D, Kittelson J. *Hemostatic markers in acute ischemic stroke: association with stroke type, severity, and outcome. Stroke 1996;27:1296-300.*
20) Lip GY, Lowe GD. *Fibrin D-dimer: a useful clinical marker of thrombogenesis? Clin Sci 1995;89:205-14.*
21) Sternberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF. *A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol 1985;101:880-6.*
22) Fijnheer R, Frijns CJ, Korteweg J, et al. *The origin of P-selectin as a circulating plasma protein. Thromb Haemost 1997;77:1081-5.*
23) Amrani DL, Stojanovic L, Mosesson MN, Shalev Y, Mosesson MW. *Development of a whole platelet ELISA to detect circulating activated platelets. J Lab Clin Med 1995;126:603-11.*
24) Goldsmith IR, Blann AD, Patel RL, Lip GY. *von Willebrand factor, fibrinogen and soluble P-selectin levels after mitral valve replacement versus mitral valve repair. Am J Cardiol 2000;85:1218-22.*
25) Li-Saw-Hee FL, Blann AD, Gurney D, Lip GY. *Plasma von Willebrand factor, fibrinogen and soluble P-selectin levels in paroxysmal, persistent and permanent atrial fibrillation: effects of cardioversion and return of left atrial function. Eur Heart J 2001;22;1741-7.*
26) Levine MN, Raskob G, Landefeld S, Kearon C. *Hemorrhagic complications of anticoagulant treatment. Chest 2001;119:108S-21S.*