in vivo *28*: 99-104 (2014)

Activated Partial Thromboplastin Time Correlates with Methoxyhydroxyphenylglycol in Acute Myocardial Infarction Patients: Therapeutic Implications for Patients at Cardiovascular Risk

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Abstract. *Background/Aim: Acute myocardial infarction (AMI) is associated with increased coagulation which in the presence of unstable atheroma or endothelial damage leads to occlusive coronary vessel thrombosis. AMI is usually characterized by increased levels of catecholamines. It is possible there may be a link between catecholamines and hypercoagulation, but this still remains to be determined. In the current study we sought to verify whether hypercoagulation is associated with hypersympathetic activity in AMI patients, and whether there is a correlation between increased Methoxyhydroxyphenylglycol (MOPEG) levels (a metabolite of catecholamines) and shorter APTT (a marker of hypercoagulation). Results: Shorter APTT values were detected in the plasma of AMI patients, which had also increased MOPEG levels. A linear correlation between APTT and MOPEG values was observed. High levels of the coagulation marker prothrombin (fragments 1+2) were also found. Conclusion: Shortened APTT demonstrates hypercoagulation and high MOPEG levels indicate increased catecholamine metabolism. A direct correlation between APTT and MOPEG was found herein, demonstrating a link between catecholamines and the process of coagulation. Catecholamines may interact with the α2-adrenergic receptors located on platelets and convert factor XII to XIIa or through the kallikrein-kinin system, they may activate factor XII. The activation of factor XII initiates the intrinsic coagulation pathway, which is monitored by APTT. It is suggested to control patients with a shortened APTT and increased sympathetic activity with the aim of preventing secondary coagulation and cardiovascular accidents by administering anti-thrombotic and anti-adrenergic agents.*

Acute myocardial infarction is associated with hypercoagulation which in the presence of unstable atheroma or endothelial damage leads to occlusive coronary vessel thrombosis (1). The origin of the hypercoagulation is multi-factorial, but an important role is played by a fissured and unstable atherosclerotic plaque induced by conditions of stress (2, 3).

In previous articles of our group in AMI patients, both an increase of prothrombin fragments F1+2, demonstrating a hypercoagulation state and an increase of matrix metalloproteinase-9 (MMP-9), a metalloproteinase secreted by inflammatory cells infiltrating the atherosclerotic plaque, capable of priming coagulation through tissue factor and factor VII, was shown (4). Several other factors may also trigger hypercoagulation.

Fissures in the caps of atherosclerotic plaque allow for blood to penetrate the arterial wall, where a thrombus forms in the intima that may be followed by thrombosis in the lumen (2). It has been shown that factor XII binds to the surface of endothelial cells, which express a matrix protein capable of binding it (5). At the site of a fissured atheroma, materials such as the polyphosphates released by activated platelets, collagen exposure, and oligonucleotides contribute to activating factor XII (6, 7), which in turn can activate factor XI and the intrinsic coagulation cascade (7). The activity of the factors of the intrinsic coagulation pathway can be monitored by means of activated partial thromboplastin time (APTT), a global coagulation test (8).

Abbreviations: AMI, acute myocardial infarction; H, healthy subjects, MOPEG, methoxyhydroxyphenylglycol; F1+2, prothrombin fragments F1+2; APTT, activated partial thromboplastin time.

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Key Words: Acute myocardial infarction (AMI), methoxyhydroxy-phenylglycol (MOPEG), activated partial thromboplastin time (APTT), prothrombin fragments F1+2 (F1+2), hypersympathetic activity, hypercoagulation, intrinsic coagulation pathway.

Table I. *Marker values [median, 1st (Q1) and 3rd (Q3) quartiles, minimum (min), maximum (max), mean, standard deviation (S.D.), coefficient of variation % (CV%)] for patients with acute myocardial infarction (AMI) and healthy subjects (H).*

	Subjects	F1+2 pmol/L	MOPEG ng/ml	APTT Ratio
Median	H	187.5	4.1	1.06
	AMI	1013.0***	6.1*	0.89**
min	H	140.0	2.8	0.96
	AMI	530.0	3.8	0.79
Q1	H	160.0	3.5	1.03
	AMI	833.0	5.0	0.87
Q3	H	219.8	5.1	1.10
	AMI	1620.0	8.1	0.94
max	H	294.0	5.9	1.17
	AMI	2342.0	10.0	1.10
Mean	H	196.5	4.3	1.07
	AMI	1221.0	6.5	0.91
S.D.	H	49.9	1.1	0.06
	AMI	604.0	1.9	0.09
CV%	H	25%	25%	5.6%
	AMI	49%	29%	1.0%

F1+2, Prothrombin fragments 1+2; MOPEG, methoxyhydroxyphenylglycol; APTT, activated partial thromboplastin time. *$p<0.05$; **$p<0.02$, ***$p<0.001$.

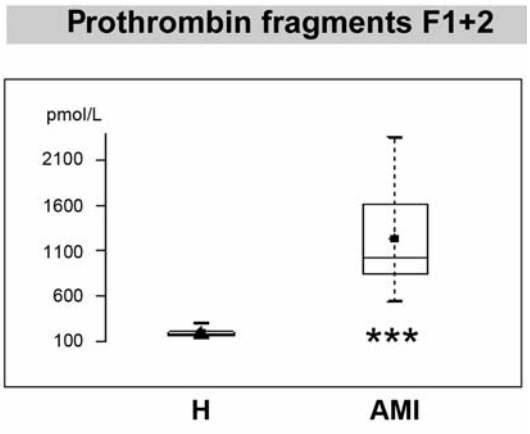

Figure 1. *Box and Whisker plots for the coagulation marker prothrombin fragment F1+2. The top and bottom edges of the box are the 3rd and 1st quartiles, the horizontal line inside the box is the median, the dot is the mean, the "whiskers" represent the highest and lowest values. H, Healthy subjects; AMI, patients with acute myocardial infarction. ***p<0.001.*

It is important to observe the hyperactivity of catecholamines which may affect coagulation. We have previously shown, in rabbits, that injection of the catecholamine isoproterenol induces a shortening of partial thromboplastin time (PTT) and this hypercoagulation induces acute myocardial infarction in rabbits (9).

Atherosclerotic plaque disruption may be induced by stress and may be associated with an elevation of catecholamines, and thrombus formation (3). An increase in the levels of adrenomedullary catecholamines is found after injection of factor XII, responsible for promoting the intrinsic pathway of coagulation (10, 11). Thus the hypersympathetic activity may be associated with hypercoagulation. A relationship may exist between sympathetic activity and coagulation but the link between catecholamines and hypercoagulation has not been yet clearly demonstrated.

The aims of the present study were to verify whether hypercoagulation is associated with hypersympathetic activity in AMI patients, measuring APTT and MOPEG and whether there is a correlation between increased MOPEG levels and a shorter APTT.

Patients and Methods

Ten selected male patients with AMI aged 50-65 years, with a history of hypertension and 10 healthy males of the same age were chosen in order to verify whether coagulation and sympathetic markers are interrelated in the processes leading to AMI.

Patients with metabolic syndromes or kidney diseases were excluded. This preliminary study was approved by the Ethics Committee of Niguarda Ca' Granda Hospital, Milan, Italy. Patients did not receive any medication before withdrawing the blood.

The presence of AMI was demonstrated by means of a clinical evaluation and by measuring plasma c-troponin T (c-TnT) levels with a commercial kit provided by Roche (Roche, Milan, Italy) (12). Plasma prothrombin fragment 1+2 (F1+2) was measured using an immuno-enzymatic procedure (Enzygnost F1+2 monoclonal, Siemens, Milan, Italy) (13).

APTT was evaluated with the APTT reagent Pathromtin SL obtained from Siemens Healthcare Diagnostics. The test was performed according to the manufacturer's recommendations. Results for APTT were expressed as a ratio of test: reference coagulation times, using as reference normal plasma tested in parallel with test plasmas (14).

MOPEG is a catecholamine metabolite which expresses sympathetic activity (15). MOPEG levels were determined by means of high-performance liquid chromatography (HPLC) with amperometric detection (16).

Statistical analysis. Descriptive statistics (median values, quartiles, minimum and maximum values, mean values, standard deviations, and coefficients of variation) were used to describe the markers in the healthy subjects and AMI patients. The differences in median values were tested using Wilcoxon's signed-rank test. In accordance with Šidák's correction for multiple comparisons (17), each comparison was considered significant if the *p*-value was less than $1-(1-\alpha)^{1/n}$, where α is the overall type I error probability and n the number of comparisons: an overall α of 0.05 (n=7) means that the significance of each comparison is 0.01 (one-tailed test). Spearman's rank correlation coefficient (r) was calculated in order to evaluate the relationships between the markers. The correlations were considered weak if r was ≤0.35, moderate if r was 0.6-0.66, and strong if r was >0.66 (18).

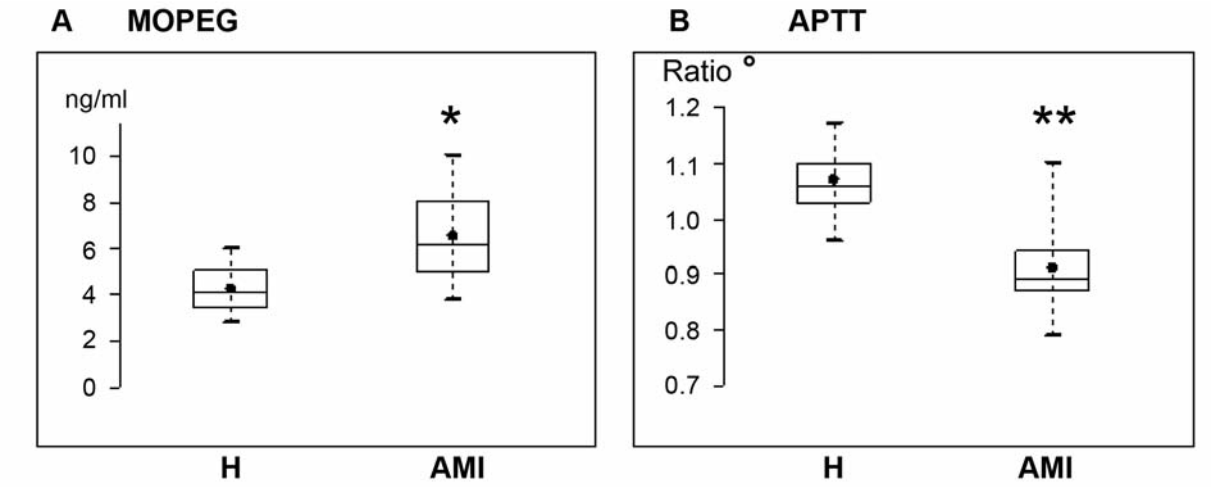

Figure 2. *Box and Whisker plots for sympathetic metabolite methoxyhydroxyphenylglicol (MOPEG) (A) and activated partial thromboplastin time (APTT) (B) in healthy subjects and AMI patients. The top and bottom edges of the box are the 3rd and 1st quartiles, the horizontal line inside the box is the median, the dot is the mean, the "whiskers" represent the highest and lowest values. H, Healthy subjects; AMI, patients with acute myocardial infarction. °Ratio values between analyzed samples and the plasma reference pool. *p<0.05; **p<0.01.*

Results

Mean plasma c-TnT levels were greatly higher in AMI patients compared to healthy subjects (mean±SE: 3.07±0.98 *vs*. <0.01±0.001 ng/ml) ($p<0.001$), thus demonstrating the presence of AMI.

Table I shows the descriptive statistics related to F1+2, APTT and MOPEG. Figure 1 shows box and whisker plots of F1+2 levels: the values were significantly higher in the AMI patients ($p<0.001$). Figure 2A and 2B show box and whisker plots of MOPEG levels and APTT, both of which were significantly different for the two groups: In AMI *vs*. healthy subjects MOPEG was found to increase, while APTT became shorter for AMI patients.

Figure 3 shows the scatter plot and linear correlation coefficient (r) between APTT and MOPEG levels in AMI patients: r≥0.67 indicating a strong correlation.

Discussion

The presence of myocardial infarction was demonstrated by high c-TnT levels, electrocardiographic alterations and a clinical evaluation (12). Furthermore, the increase in F1+2 levels demonstrated up-regulated coagulation due to increased thrombin generation (4, 19). Increased F1+2 values were previously shown by our group to be associated with high levels of matrix metalloproteinase-9 (MMP-9), released from inflammatory cells infiltrating the atherosclerotic plaque (4).

AMI patients also had a shorter APTT, with involvement of factors of the intrinsic coagulation pathway (14). Localized coagulation requires for specific binding factors on surfaces, such as phospholipids, collagen and other various factors docking at the lesion site (6). In particular factor XII is activated and initiates the intrinsic coagulation cascade (7) evaluable with APTT analysis.

The present study demonstrated a correlation between prothrombin fragment F1+2 and APTT (r=0.736; $p=0.020$).

However, bearing in mind that APTT is a global and non-selective marker, its shortening proves the existence of hypercoagulation representing high risk of thromboembolism (14). When APTT is shortened, it could contribute to thrombotic risk (20-22).

Our patients also had increased MOPEG levels, thus demonstrating a high degree of sympathetic activity in the presence of hypercoagulation, and there was a direct reciprocal relationship between MOPEG levels and APTT (r=0.67; $p=0.031$). This shows the existence of a link between hypersympathetic activity and hypercoagulation in AMI patients.

It is important to explain how hypersympathetic activity relates to hypercoagulation. Our findings are in line with data from experimental observations. It has been described that epinephrine binds to alpha-2 adrenoreceptors of platelets and activates them (23). Upon activation platelets release large amount of polyphosphates, by which factor XII is activated priming intrinsic coagulation (24).

The activation by contact with negatively-charged surfaces of the complex HMWK-prekallikrein leads to release of kallikrein, which cleaves the factor XII into active factor XIIa, inducing the intrinsic coagulation cascade (5, 25).

It is important to observe that among the factors activating the complex prekallikrein–HMWK there are catecholamines.

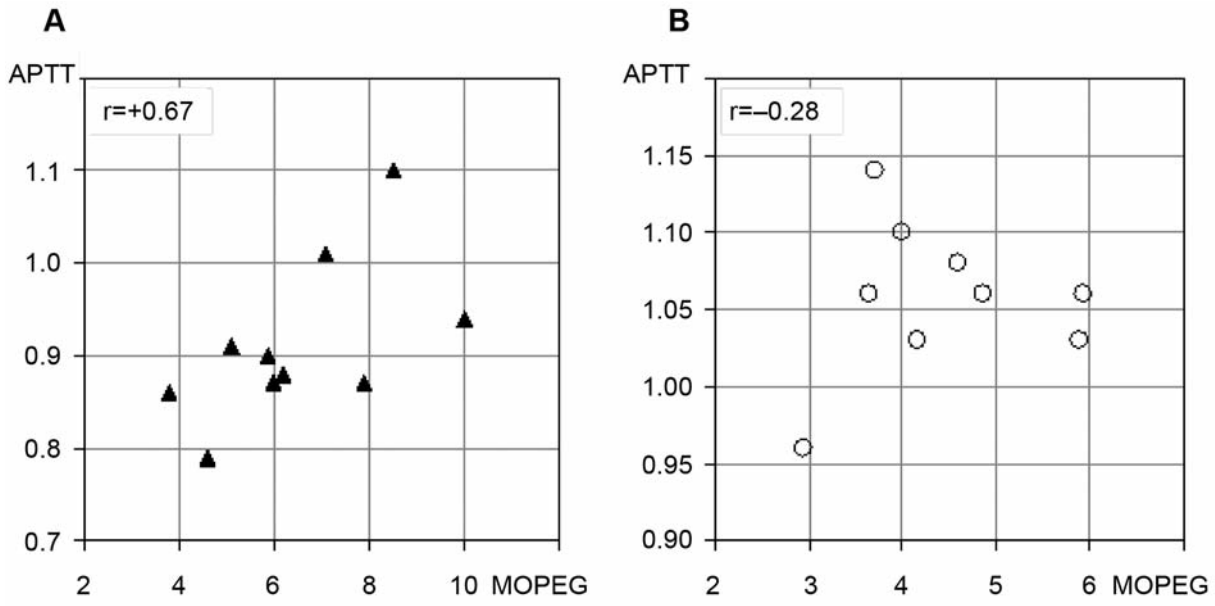

Figure 3. *Scatter plot and linear correlation coefficient (r) between activated partial thromboplastin time (A) and metabolite methoxyhydroxyphenylglicol (MOPEG) (B). Spearman's correlations. (A) r=+0.67; p<0.031. (B) r=–0.28; not significant.*

A lowering of kininogen in rat blood by catecholamines has been shown (26). Also consumption of kininogen, formation of kinins in rat plasma in presence of L-adrenaline have been reported (27).

In the current study we demonstrated a correlation between hypersympathetic activity (increased MOPEG levels) and hypercoagulation (shortened APTT values).

It is probable that in our patients sympathetic activity, activates kallikrein and consequently factor XII and the intrinsic coagulation pathway. These observations are in line with other reports describing that epinephrine activates the kallikrein-kinin system (28) and the intrinsic coagulation cascade. Thus, factor XII (Hageman factor) appears to be the missing link between stress (epinephrine) and hypercoagulability (23). The inter-relationship between hypersympathetic activity and hypercoagulation is further supported by observations that injection of pro-coagulant factor XII causes increased elevation of epinephrine (10, 11).

It has been reported that the hypercoagulation expressed by short APTT correlates with poor survival in head trauma patients and may be related to a high catecholamine values (29-31).

Since the activation of factor XII initiates the intrinsic coagulation cascade, the pharmacological regulation of this factor may be a new target to control for pathological coagulation (5).

It is important to monitor patients with shortened APTT values and increased sympathetic activity with the aim of preventing cardiovascular accidents, especially those with hypertension associated with increased sympathetic activity. Hypertensive subjects have a strong "adrenergic drive" (32) and increased catecholamine levels (33), and it is known that epinephrine is involved in the genesis of hypertension (34). Such patients should be given both low anticoagulant doses capable of prolonging APTT (35,36) and anti-adrenergic drugs capable of counteracting the adrenergic drive (32).

It would also be important to monitor coronary ischemic syndrome patients with shortened APTT values and increased MOPEG levels as there is arteriographic evidence of coronary arterial spasm in patients with AMI (37). Alpha-2 adrenergic activation reduces coronary blood flow by microvascular constriction. Both alpha-1 and alpha-2 adrenergic epicardial and microvascular constrictions are augmented by atherosclerosis and can induce myocardial ischemia. Epinephrine-increased levels expose patients affected by coronary artery disease (CAD) to AMI (38).

Administration of low anti-coagulant doses to prolong APTT may prevent from secondary coagulation in subjects with acute coronary syndrome (35, 36), and anti-adrenergic treatment is recommended to prevent from adrenergic effects in such patients (38).

Conclusion

Given the limitation of the study (low number of patients), our findings clearly demonstrate a relationship between MOPEG levels (hypersympathetic activity) and APTT (hypercoagulation) in AMI patients. Subjects at cardiovascular risk with shortened APTT values and

increased MOPEG levels should be closely monitored, and it is advisable to administer anti-coagulants capable of prolonging APTT and anti-adrenergic agents to block the effects of catecholamines on coagulation in order to prevent cardiovascular accidents.

Conflicts of Interest

None of the Authors has any conflicts of interest related to this manuscript.

Acknowledgements

The Authors are grateful to Mr Fausto Tarozzi – President of Azienda Agricola S. Paolo, via Calzavecchio 23 -Casalecchio di Reno (BO), for supporting this research.

References

1 Robbins and Cotran: Pathological Basis of Diseases, *In*: Kumar V, Abbas AK and Fausto N (eds.). Philadelphia, USA, Elsevier Saunders, 7th edition, pp. 516-525, 571-586, 2005.
2 Richardson PD, Davies MJ and Born GV: Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. Lancet *21*: 941-944, 1989.
3 Ni M, Wang Y, Zhang M, Zhang PF, Ding SF, Liu CX, Liu XL, Zhao YX and Zhang Y: Atherosclerotic plaque disruption induced by stress and lipopolysaccharide in apolipoprotein E knockout mice. Am J Physiol Heart Circ Physiol *296*: H1598-H1606, 2009.
4 Pinelli A, Trivulzio S, Brenna S, Rosato V, Rivoltini L and Rossoni G: Identification of factors causing sudden coagulation in patients with acute myocardial infarction. J Clin Exp Cardiol *3*: 7, 2012.
5 Schousboe I: Endothelial cells express a matrix protein which binds activated factor XII in a zinc-independent manner. Thromb Haemost *95*: 312-319, 2006.
6 Maas C, Oschatz C and Renné T: The plasma contact system 2.0. Semin Thromb Hemost *37*: 375-381, 2011.
7 Müller F and Renné T: Novel roles for factor XII-driven plasma contact activation system. Curr Opin Hematol *15*: 516-521, 2008.
8 Tripodi A, Chantarangkul V, Martinelli I, Bucciarelli P and Mannucci PM: A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. Blood *104*: 3631-3634, 2004.
9 Pinelli A, Trivulzio S, Tomasoni L, Brenna S, Bonacina E and Accinni R: Isoproterenol-induced myocardial infarction in rabbits. Protection by propranolol or labetalol: a proposed non-invasive procedure. Eur J Pharm Sci *23*: 277-285, 2004.
10 Amfilochiadis AA, Backx PH and Floras JS: Adrenomedullary catecholamine, pressor and chronotropic responses to human coagulation beta-FXIIa mediated by endogenous kinins. J Hypertens *26*: 61-69, 2008.
11 Papageorgiou PC, Yeo EL, Backx PH and Floras JS: Activated plasma coagulation β-Factor XII-induced vasoconstriction in rats. Clin Sci *122*: 581-590, 2012.
12 Rottbauer W, Greten T, Muller-Bardorff M, Remppis A, Zehelein J, Grunig E and Katus HA: Troponin T: a diagnostic marker for myocardial infarction and minor cardiac cell damage. Eur Heart J *17(Suppl)*: F3-F8, 1996.
13 Pelzer H, Schwarz A and Stuber W: Determination of human prothrombin activation fragment 1 + 2 in plasma with an antibody against a synthetic peptide. Thromb Haemost *65(2)*: 153-159, 1991.
14 Korte W, Clarke S and Lefkowitz JB: Short activated partial thromboplastin times are related to increased thrombin generation and an increased risk for thromboembolism. Am J Clin Pathol *113*: 123-127, 2000.
15 Goodman and Gilman's: The Pharmacological Basis of Therapeutics. *In*: Brunton L. (ed), B. Chabner and B. Knollman Associated Editor, New York, USA, Mc Graw Hill, 12th edition, pp. 202, 2011.
16 Krstulovic AM, Bertani-Dziedzic L, Dziedzic SW and Gitlow SE: Quantitative determination of 3-methoxy-4-hydroxyphenylethyleneglycol and its sulphate conjugate in human lumbar cerebrospinal fluid using liquid chromatography with amperometric detection. J Chromatogr *223*: 305-314, 1981.
17 Abdi, H: "Bonferroni and Šidák corrections for multiple comparisons". *In*: Salkind NJ (ed.). Encyclopedia of Measurement and Statistics. Thousand Oaks, CA 2007: Sage. http://www.utdallas.edu/~herve/Abdi-Bonferroni2007-pretty.pdf.
18 Taylor R: Interpretation of the Correlation Coefficient. A Basic Review. J Diagn Med Son *6*: 35-39, 1990.
19 Paramo JA: Prothrombin fragments in cardiovascular disease. Adv Clin Chem *51*: 1-23, 2010.
20 Mina A, Favaloro EJ and Koutts J: Relationship between short activated partial thromboplastin times, thrombin generation, procoagulant factors and procoagulant phospholipid activity. Blood Coagul Fibrinolysis *23*: 203-207, 2012.
21 Abdullah WZ, Moufak SK, Yusof Z, Mohamad MS and Kamarul IM: Shortened activated partial thromboplastin time, a hemostatic marker for hypercoagulable state during acute coronary event. Transl Res *155*: 315-319, 2010.
22 Mina A, Favaloro EJ, Mohammed S and Koutts J: A laboratory evaluation into the short activated partial thromboplastin time. Blood Coagul Fibrinolysis *21*: 152-157, 2010.
23 Eggers AE: Factor XII (Hageman factor) is a missing link between stress and hypercoagulability and plays an important role in the pathophysiology of ischemic stroke. Med Hypotheses *67*: 1065-1071, 2006.
24 Müller F and Renné T: Platelet polyphosphates: the nexus of primary and secondary hemostasis. Scand J Clin Lab Invest *71*: 82-86, 2011.
25 Douglas WV: Polypeptides, angiotensin, plasma kinins and other: The Pharmacological Basis of Therapeutics. *In*: Goodman and Gilman's (eds.). Goodman LS, Gilman A, Editor, New York, USA, Mc Millan Publishing Company, 6th edition, pp. 647-667, 1980.
26 Rothschild AM, Cordeiro RS and Castania A: Lowering of kininogen in rat blood by catecholamines. Involvement of non-eosinophil granulocytes and selective inhibition by Trasylol. Naunyn Schmiedebergs Arch Pharmacol *282*: 323-326, 1974.
27 Rothschild AM, Castania A and Cordeiro RS: Consumption of kininogen, formation of kinin and activation of arginine ester hydrolase in rat peritoneal fluid cells in the presence of l-adrenaline. Specific sensitivity to acetylsalicylic acid. Naunyn Schmiedebergs Arch Pharmacol *285*: 243-256, 1974.
28 Rothschild AM, Gomes JC and Castania A: adrenergic and cholinergic control of the activation of kallikrein-kinin system in the rat blood: Kinins, pharmacodynamics and biological roles, *In*: Sicuteri F, Back N and Haberland GL (editors), New York and London, Plenum Press, pp. 195-200, 1976.

29 Kearney TJ, Bentt L, Grode M, Lee S, Hiatt JR and Shabot MM: Coagulopathy and catecholamines in severe head injury. J Trauma *32*: 608-611, 1992. discussion 611-612.
30 Johansson PI. Stensballe J. Rasmussen LS and Ostrowski SR: High circulating adrenaline levels at admission predict increased mortality after trauma. J Trauma *72*: 428-436, 2012.
31 Johansson PI, Stensballe J, Rasmussen LS and Ostrowski SR A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients. Ann Surg *254*: 194-200, 2011.
32 Opie LC and Gersh BJ: Drugs for the heart. *In*: Saunders, Philadelphia, USA, 7th edition, pp. 200, 2009.
33 Goldstein DS: Plasma catecholamines and essential hypertension. An analytical review. Hypertension *5*: 86-99, 1983.
34 Floras JS: Epinephrine and the genesis of hypertension. Hypertension *19*: 1-18, 1992.
35 Verheugt FW: Low-dose anticoagulation for secondary prevention in acute coronary syndrome. Am J Cardiol *111*: 618-626, 2013.
36 Hankey GJ and Eikelboom JW: Antithrombotic drugs for patients with ischaemic stroke and transient ischaemic attack to prevent recurrent major vascular events. Lancet Neurol *9*: 273-284, 2010.
37 Oliva PB and Breckinridge JC: Arteriographic evidence of coronary arterial spasm in acute myocardial infarction. Circulation *56*: 366-374, 1977.
38 Baumgart D, Haude M, Gorge G, Liu F, Ge J, Grosse-Eggebrecht C, Erbel R and Heusch G: Augmented alpha-adrenergic constriction of atherosclerotic human coronary arteries. Circulation *99*: 2090-2097, 1999.

Received September 17, 2013
Revised November 11, 2013
Accepted November 12, 2013