ORIGINAL RESEARCH

Mehmet N. Akkus¹ MD

Adil Orman² MD

Sabri Seyis³ MD

Çagdas Baran⁴ MD

Aysegül Görür⁵

Mehmet N. Bilen² MD

Plasma EMMPRIN levels in acute myocardial infarction and stable coronary artery disease

The work was conducted in Department of Cardiology and Clinical Biochemistry, Faculty of Medicine, Mersin University

¹ Department of Cardiology, Faculty of Medicine, Mersin University,

² Department of Cardiology, Medical Park Elazig Hospital,

³ Department of Cardiology, Dogus Hospital Mersin,

⁴ Department of Cardiovascular Surgery, Faculty of Medicine, Ankara University.

⁵ Department of Clinical Biochemistry, Faculty of Medicine, Mersin University,

Abstract

Purpose: The purpose of this study was to determine whether the plasma levels of soluble extracellular matrix metalloproteinase inducer (EMMPRIIN) differed among the patients with ST-elevation myocardial infarction (STEMI), non-STEMI (NSTEMI) and stable coronary artery disease (CAD) and the healthy controls, and to identify the factors associated with the differences in plasma levels of this protein among patients in these groups.

Methods: Plasma EMMPRIN levels were compared among four age- and sex-matched groups of patients with STEMI, NSTEMI and stable CAD and healthy controls (n=44 per group), then logistic regression and correlation analyses were conducted for the whole acute myocardial infarction (AMI) patients group.

Results: EMMPRIN levels were significantly higher in the STEMI ($39.4 \pm 9.2 \text{ ng/mL}$) and NSTEMI ($37.1 \pm 10.5 \text{ ng/mL}$) groups than in either the stable CAD ($27.5 \pm 4.7 \text{ ng/mL}$) or control ($24.5 \pm 5.8 \text{ ng/mL}$) groups ($p < 0.001$ for each comparison), whereas the differences between the STEMI and NSTEMI groups, and between the stable CAD group and the controls, was not significant. Moreover, in the whole AMI patients group, the elevation of EMMPRIN was independent of the other variables (Odds ratio=1.25, 95% confidence intervals=1.16-1.34, $p < 0.001$), and EMMPRIN levels were correlated significantly and positively with the plasma levels of peak creatine kinase-MB ($r=0.22$, $p < 0.05$), peak cardiac troponin T ($r=0.27$, $p < 0.05$) and admission C-reactive protein ($r=0.26$, $p < 0.05$), and correlated significantly and inversely with left ventricular ejection fraction ($r=-0.22$, $p < 0.05$).

Conclusion: EMMPRIN seems to play a role in the pathogenesis of AMI and its measurement in the plasma may play a role in the diagnosis of this disease.

Manuscript submitted 10th June, 2015

Manuscript accepted 31st March, 2016

Clin Invest Med 2015; 39 (3): E79-E87.

Correspondence to:

Mehmet Necdet Akkus, MD

Department of Cardiology, Faculty of Medicine, Mersin University,

Çiftlikköy Kampüsü, Yenisehir,

Postal Code: 33343, Mersin, Turkey

e-mail: mnecdetakkus@hotmail.com

The clinical manifestations of coronary artery disease (CAD), namely, stable CAD and acute coronary syndrome (ACS), are mainly related to the composition and vulnerability of the underlying atheroma: when the plaque mass encroaches on the vessel lumen and obstructs the coronary blood flow, clinical manifestations of stable CAD occurs, whereas when the plaque ruptures or ulcerates, it becomes a focus for the aggregation of platelets, leading to thrombus formation and ACSs, including unstable angina (UA), ST-elevation myocardial infarction (STEMI), non-ST-elevation myocardial infarction (NSTEMI) and sudden death [1]. Extracellular matrix metalloproteinase inducer (EMMPRIN) is a member of the immunoglobulin superfamily of adhesion molecules and has a role in the activation of inflammatory factors [2,3] and several matrix metalloproteinases (MMPs) [4,5], which contribute to the atheroma vulnerability [6,7]. EMMPRIN has been implicated either in the atheroma development [5,8,9] and consequently in stable CAD [10,11] or in the destabilization and rupture of the atheroma [12-15] and consequently in ACS [10,12-16]. Clinical studies comparing the circulating levels of soluble EMMPRIN in CAD populations to those in a control group are limited. There are only two studies on the matter: one compared soluble EMMPRIN levels between stable CAD patients and healthy controls [11], whereas the other study compared EMMPRIN levels between AMI patients and healthy controls [16].

In this study, we aimed to compare soluble EMMPRIN levels in plasma among patients with STEMI, NSTEMI and stable CAD and healthy controls.

Methods

The study has been approved by, and carried out according to the instructions of, our Institutional Ethics Committee. Written informed consents were obtained from all participants.

Forty-four consecutive patients with STEMI who underwent coronary angiography at our institution between February 2013 and June 2014 comprised the STEMI group. Three additional groups, also from the patients who underwent angiography at our Institution, consisting of 1) patients with NSTEMI, 2) patients with stable CAD and 3) normal healthy subjects, matched according to age and gender with the STEMI group and to each other, were formed ($n=44$ for each). Whereas the STEMI and NSTEMI groups were recruited from patients who presented at our emergency department, the stable CAD and control groups were recruited from subjects who underwent angiography for

diagnostic purposes (i.e., for the further evaluation of chest pain, or a positive non-invasive test result). Patients with significant valvular heart disease (at least moderate aortic or mitral stenosis) and nonischemic cardiomyopathy on Doppler echocardiography and/or coronary angiography, those with inflammatory, immunologic, renal (serum creatinine $>2.0\text{mg/dL}$), or hepatic disease (aspartate transaminase or alanine transaminase >1.5 times upper limit of normal), and those with a history or the presence of neoplastic disease, were excluded.

First, the plasma levels of EMMPRIN were compared among the four groups. Then the STEMI and NSTEMI groups were combined to form a new group (the whole acute myocardial infarction [AMI] patients group), and a logistic regression was conducted on this group to evaluate whether plasma EMMPRIN levels were elevated, independently of some potential confounding factors; including, age, gender, body mass index (BMI), the presence of diabetes, hypertension, dyslipidemia, cigarette smoking, a positive family history for CAD, and the use of an angiotensin converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB), nitrate, statin, aspirin, β blocker, diuretic and calcium channel blocker (CCB). Finally, a correlation analysis was conducted in the whole AMI patients group between the plasma levels of EMMPRIN and peak creatine kinase-MB (CK-MB), peak cardiac troponin T (cTnT) and admission C-reactive protein (CRP) levels, and left ventricular ejection fraction (LVEF).

AMI was defined according to the third universal definition of myocardial infarction [17]. Briefly, AMI was diagnosed in patients with a cTnT level of 0.1 ng/mL and with at least one of the following: (1) symptoms of ischaemia: chest pain lasting ≥ 30 minutes; (2) new, or presumed new, significant ST-segment-T wave changes or a new left bundle branch block (LBBB) in the electrocardiogram (ECG); (3) development of pathological Q waves in the ECG; (4) imaging evidence of new loss of viable myocardium or new regional wall-motion abnormality; and, (5) identification of an intracoronary thrombus by angiography. STEMI was defined as a new, or presumably new, ST-segment elevation $\geq 0.2\text{ mV}$ for men and $\geq 0.15\text{ mV}$ for women in V_2-V_3 , and $\geq 0.1\text{ mV}$ in other leads or a new LBBB. NSTEMI was defined as absence of these changes. Among the non-AMI subjects, those who had $\geq 50\%$ lumen diameter narrowing in a major coronary artery or one of their major branch on angiogram were assigned into the stable CAD group and those who had $<20\%$ narrowing were assigned to the control group.

TABLE 1. Subject characteristics

	Asthma (n=14)	AAHR (n=14)	P
Age (yr) (range)	27 (20-41)	28 (20-46)	0.87
Baseline FEV ₁ *	101.7 ± 10.3	95.9 ± 13.2	0.20
Baseline FVC*	110.6 ± 8.8	99.0 ± 13.6	0.01
Baseline FEF _{25-75%} *	98.4 ± 24.3	99.5 ± 20.2	0.90
Baseline IC*	104.1 ± 18.9	101.0 ± 18.4	0.67
PC ₂₀ on screening methacholine(mg/ml) (Mean and range)	2.19 (0.30-16)	2.19 (0.30-16)	0.13

AAHR = asymptomatic airway hyperresponsiveness; FEV₁ = forced expiratory volume in one second; FEF_{25-75%} = forced expiratory flows 25-75%; FVC = forced vital capacity; IC = inspiratory capacity; PC₂₀ on screening methacholine = provocative concentration of methacholine inducing a 20% decrease in FEV₁.

*Data are presented as mean % of predicted values ± SD

All blood samples for the measurement of EMMPRIN were drawn just before angiography. Blood (15 mL) was collected using venipuncture, by gentle aspiration without a tourniquet, using a 20 G intravenous cannula. The first 5 mL was collected in plain tubes and analyzed for cTnT, CK-MB and CRP. The final 10 mL of blood was transferred to tubes containing K₂EDTA. The EDTA-containing tubes were centrifuged at 1000 g for 15 minutes at 4°C; the plasma was then removed and stored at -80°C for the assessment of EMMPRIN. EMMPRIN was measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Quantikine ELISA-Human EMMPRIN (R&D Systems, Inc. Minneapolis, USA) following the manufacturer's instructions on a Dynex DSX four-plate automated ELISA processor (Dynex technologies Inc. Virginia, USA). cTnT was measured using a third-generation cTnT assay, CK-MB was measured using the immunoinhibition method, and high-sensitivity CRP was measured using particle-enhanced immunonephelometry.

Statistics

All the continuous variables were normally distributed as determined using the Kolmogorov-Smirnov test and were expressed as mean±SD. For more than two groups, a one-way analysis of variance (ANOVA) was used, followed by Tukey's *post hoc* test, when appropriate. The continuous variables between the two groups were compared using the Student's t-test. Categorical variables were expressed as n (%), and analysed using a χ^2 or Fisher's exact test. In the multiple logistic regression analysis, the dependent variable was the presence or absence of STEMI or NSTEMI. Among the

independent variables, gender, the presence of diabetes, hypertension, dyslipidemia, cigarette smoking and family history of CAD, and use of ACEI or ARB, nitrate, statin, aspirin, β blocker, diuretic and CCB, were entered as categorical variables; while EMMPRIN, age, and BMI were entered as continuous variables. For the categorical variables, an indicator contrast with the first category as the reference was chosen. Correlation analyses were performed using the Pearson's test. A p value <0.05 was considered to be significant. The statistical software used was SPSS 17.0.

Results

Baseline clinical characteristics

The groups were well matched regarding age and gender. As expected, the percentages of the subjects with individual CAD risk factors were higher both in the STEMI and NSTEMI groups than in both the stable CAD and control groups; however, none of these differences were of statistical significance, except for BMI, which was significantly higher in both the STEMI and NSTEMI groups than in the controls (p<0.05 for both). On the other hand, and also as expected, there were significant differences among the groups with regard to LVEF and the use of some cardiovascular drugs: the use of a nitrate, aspirin, and β blocker differed significantly amongst the groups; however, the differences in the use of ACEI/ARB, statin, diuretic, and CCB were not statistically significant (Table 1).

TABLE 1. Baseline clinical characteristics of study population.

	STEMI group (n=44)	NSTEMI group (n=44)	Stable CAD group (n=44)	Controls (n=44)	p for overall test
Age, years	54.9±10.6	54.7±10.7	55.4±10.1	54.6±9.4	NS
Gender, men	34(77.3)	35(79.6)	34(77.3)	33(75.0)	NS
Risk factors for CAD					
Diabetes	7(15.9)	8(18.2)	4(9.1)	3(6.8)	NS
Hypertension	5(11.4)	3(6.8)	3(6.8)	1(2.3)	NS
Cigarette smoking	7(15.9)	6(13.6)	3(6.8)	3(6.8)	NS
Family history of CAD	2(4.6)	3(6.8)	4(9.1)	2(4.6)	NS
Dyslipidemia	15(34.1)	16(36.4)	11(25.0)	10(22.7)	NS
BMI	27.6±3.3	27.9±3.5	27.2±3.6	25.7±3.6	<0.05*
LVEF, %	50.1±13.4	53.5±12.6	59.3±5.4	61.2±5.0	<0.001†
Medication on admission					
ACEI/ARB	6(13.6)	3(6.8)	3(6.8)	1(2.3)	NS
Nitrate	3(6.8)	3(6.8)	10(22.7)	1(2.3)	<0.01‡
Statin	7(15.9)	7(15.9)	6(13.6)	2(4.6)	NS
Aspirin/clopidogrel	6(13.6)	7(15.9)	18(40.9)	2(4.6)	<0.001§
β blocker	8(18.2)	7(15.9)	22(50.0)	3(6.8)	<0.001¶
Diuretic	4(9.1)	3(6.8)	4(9.1)	0(0.0)	NS
CCB	2(4.6)	4(9.1)	4(9.1)	0(0.0)	NS

*p<0.05 for STEMI group vs. controls and for NSTEMI group vs. controls; †p<0.001 for STEMI group vs. controls and for STEMI group vs. stable CAD group, p<0.01 for NSTEMI group vs. controls, and p<0.05 for NSTEMI group vs. stable CAD group; ‡p<0.01 for stable CAD group vs. controls and p<0.05 for stable CAD group vs. STEMI group and for stable CAD group NSTEMI group; §p<0.001 for stable CAD group vs. controls and p<0.01 for stable CAD group vs. the STEMI group and for stable CAD group vs. NSTEMI group; and ¶p<0.001 for stable CAD group vs. controls and p<0.01 for stable CAD group vs. STEMI group and for stable CAD group vs. NSTEMI group.

Angiographic and laboratory characteristics of the patients groups

There was a significantly higher number of patients with three-vessel disease in the STEMI and NSTEMI groups compared with the stable CAD group (p<0.01 for both); however, the percentage of patients with two-vessel disease was statistically similar between these groups (Table 2). Similarly, the distribution of patients with single- or multi-vessel disease and the distribution of infarct-related arteries (IRAs) were not significantly different between the STEMI and NSTEMI groups, except for the percentage of patients with left circumflex artery (LCx), as the IRA was significantly higher in

the NSTEMI group than in the STEMI group (p<0.05). On the other hand, peak cTnT and peak CK-MB levels were significantly higher in the STEMI group compared with the NSTEMI group (p<0.001 for both). Finally, the mean admission serum CRP level was significantly higher in the STEMI group compared with either the stable CAD or NSTEMI groups, and significantly higher in the NSTEMI group compared with the stable CAD group (p<0.001 for each).

TABLE 2. Angiographic and laboratory characteristics of the STEMI, NSTEMI and stable CAD groups.

	STEMI Group	NSTEMI Group	Stable CAD Group	p for overall test
One-vessel disease	9(20.5)	8(18.2)	18(40.9)	<0.05*
Two-vessel disease	11(45.0)	14(31.8)	17(38.6)	NS
Three-vessel disease	23(52.3)	22(50.0)	8(18.2)	<0.001†
LMCA	1(2.3)	0(0.0)	1(2.3)	NS
Infarct Related Artery				
LAD	21(47.7)	15(34.1)	-	NS
LCx	8(18.2)	17(38.6)	-	<0.05
RCA	14(31.8)	12(27.3)	-	NS
LMCA	1(2.3)	0(0.0)	-	NS
Time from onset of pain to blood sampling, h	4.5±3.4	5.2±3.3	-	NS
Peak cTnT (µg/L)	8.3±4.3	3.7±2.0	-	<0.001
Peak CK-MB (IU/L)	196.9±111.6	99.0±51.3	-	<0.001
CRP on admission (mg/L)	12.0±3.8	9.1±2.5	1.2±0.4	<0.001‡

*p<0.05 for stable CAD group vs. STEMI group and for stable CAD group vs. STEMI group; †p<0.01 for STEMI group vs. stable CAD group and for NSTEMI group vs. stable CAD group; ‡p<0.001 for STEMI group vs. stable CAD group, for STEMI group vs. NSTEMI group, and for NSTEMI vs. stable CAD group.

FIGURE 1. Comparison of plasma EMMPRIN levels among the groups.

*p<0.001 compared with either the controls or stable CAD group and p=NS compared with the NSTEMI group; †p<0.001 compared with either the controls or stable CAD group; ‡p=0.076 compared with controls.

Comparison of plasma EMMPRIN levels among the groups

ANOVA revealed that there was a significant difference among the groups ($p<0.001$): the *post hoc* test showed that plasma EMMPRIN levels were significantly higher in the STEMI group (39.4 ± 9.2 ng/mL) than in either the control (24.5 ± 5.8 ng/mL) or stable CAD groups (27.5 ± 4.7 ng/mL) ($p<0.001$ for both). Similarly, the plasma EMMPRIN levels were significantly higher in the NSTEMI group (37.1 ± 10.5 ng/mL) than either in the control or stable CAD groups ($p<0.001$ for both); however, the differences between the STEMI and NSTEMI groups and between the stable CAD and control groups were not statistically significant (Figure 1).

Multiple logistic regression

In the whole AMI patients group, multiple logistic regression revealed that the elevation of plasma EMMPRIN levels were independent of age, gender, diabetes, hypertension, cigarette smoking, family history for CAD, dyslipidemia, BMI and the use of ACEI/ARB, nitrate, statin, aspirin, β blocker, diuretic

Table 3. Multiple logistic regression analysis for adjustment of possible confounders on the association between EMMPRIN and AMI.

	Odds Ratio	95% Confidence Intervals	p
EMMPRIN	1.25	1.16-1.34	<0.001
Age	1.02	0.97-1.07	NS
Gender	1.03	0.30-3.59	NS
Risk factors for CAD			
Diabetes	2.37	0.58-9.65	NS
Hypertension	1.25	0.23-6.98	NS
Cigarette smoking	3.26	0.72-14.78	NS
Family history of CAD	0.51	0.85-3.08	NS
Dyslipidemia	2.24	0.57-8.75	NS
BMI	1.16	1.02-1.32	<0.05
Medication on admission			
ACEI/ARB	7.27	1.28-41.33	<0.05
Nitrate	0.29	0.06-1.43	NS
Statin	1.05	0.16-6.65	NS
Aspirin	0.57	0.16-2.02	NS
β blocker	0.38	0.14-1.07	NS
Diuretics	0.77	0.10-5.79	NS
CCB	1.47	0.19-11.42	NS

and CCB (Odds ratio=1.25, 95% confidence intervals=1.16-1.34, $p<0.001$, Table 3).

Correlation analysis

In the whole AMI patients group, plasma EMMPRIN levels were significantly and positively correlated with the plasma levels of peak CK-MB ($r=0.22$, $p<0.05$), peak cTnT ($r=0.27$, $p<0.05$) and admission CRP ($r=0.26$, $p<0.05$), and significantly inversely correlated with LVEF ($r=-0.22$, $p<0.05$).

Discussion

EMMPRIN is composed of a 187 residue extracellular domain, a 24 residue trans-membrane domain and a 40 amino-acid cytoplasmic region [18], and has been shown to be expressed on many cell types. In ocular fluids, synovial fluids, HEp-2 human laryngeal epidermoid carcinoma cells and human platelets or plasma, naturally soluble forms of EMMPRIN

have also been discovered [19-22]. It has been shown that this protein can function both in the cell membrane and after being released from cells; however, knowledge on the functions of soluble EMMPRIN is relatively limited. It is known that the extracellular forms of EMMPRIN stimulate their own expression, and high levels of both MMPs and pro-inflammatory cytokines are continually secreted due to these extracellular EMMPRIN forms [23-25]. In the eye, both cellular and extracellular EMMPRIN are implicated in normal retinal development, as well as retinoblastoma [19,26]. According to the literature, EMMPRIN is released from tumor cells in at least two different ways. A significant amount of intact EMMPRIN is released from tumor cells, possibly contained within the membrane vesicles [22,27]. The other pathway is proteolytic shedding. EMMPRIN is likely to be cleaved and shed by MMPs, or other metalloproteinases, because the shedding of EMMPRIN is inhibited by zinc chelator [28,29].

A number of animal or *in vitro* human studies suggested that EMMPRIN performs an important function both in the development of atheroma and in its rupture; for instance, it has been demonstrated that pro-atherogenic stimuli, such as low-density lipoproteins, CRP, advanced glycation end-products, and high glucose levels, can stimulate EMMPRIN expression in inflammatory cells [30-32], whose activity is among the major determinants of the vulnerability of an atheroma to rupture [14]. It has also been shown that EMMPRIN can initiate the activation of nuclear factor κ B, which is involved in the inflammatory and proliferative responses of cells linking to atherogenesis [2,3]. EMMPRIN has also been shown to activate platelets [2], which play a key role in the progression of ACS [1].

Likewise, the results of *in vivo* human studies, the vast majority of which have been performed with the cellular expression of EMMPRIN, is also in agreement with the notion that this protein plays an important role in of atheroma rupture and ACS onset. Significantly elevated expression of EMMPRIN on circulating monocytes from patients with ACS relative to either those from stable CAD patients or from healthy controls has been demonstrated in several studies [12,14,15]. Furthermore, Yang *et al.* [13] more directly showed that higher EMMPRIN expression on circulating monocytes of a mixed CAD population (consisting of stable CAD and ACS patients) was significantly associated with more vulnerable plaque types.

Conversely, to our knowledge, there is only one study comparing circulating levels of soluble EMMPRIN between ACS patients and a control group [16]. Consistent with our result, the plasma EMMPRIN levels of ACS patients were significantly higher than in the healthy controls in that study.

On the other hand, there are only two *in vivo* human studies that addressed the potential association between cellular EMMPRIN expression and stable CAD. Pennings *et al.* [10], in 2010, demonstrated an elevated EMMPRIN expression in the circulating leukocytes from a mixed CAD population consisting of stable CAD and ACS patients than those in healthy controls and Yong *et al.* [33], in 2013, demonstrated in a group of stable CAD patients that the platelet expression of EMMPRIN was significantly higher in the coronary sinus than in the peripheral vein. A study by Pennings *et al.* [11], in 2014, is unique in that it compares the plasma levels of soluble EMMPRIN between a population consisting of patients with stable CAD and healthy controls. Consistent with our study, the difference reported by Pennings was not statistically significant.

To summarize, data from the above-mentioned animal and human studies suggest that EMMPRIN performs an important function in atheroma formation, destabilization and rupture, and, consequently, in the stable CAD and ACS. The present study provides additional data supporting this notion. Moreover, the results of the above-mentioned study by Mao *et al.* [16], in combination with our results, suggest that the measurement of soluble EMMPRIN in plasma can be a useful tool in the diagnosis of AMI.

Knowledge on the temporal changes in EMMPRIN in AMI is limited, with only two published studies [14,34] on this matter. One study [14] reported that the expression level of EMMPRIN on monocytes was significantly elevated in AMI patients whose onset of chest pain was >24 hours previously, than in those whose onset of chest pain was <24 hours. In the other study [34], the baseline levels of circulating soluble EMMPRIN were determined median one day following chest pain onset, and these levels were significantly reduced after two to three weeks. In our study, blood was sampled 4.5±3.4 and 5.2±3.3 hours after chest pain onset in the STEMI and NSTEMI patients, respectively. More studies are needed to determine the time course of EMMPRIN changes in ACS.

On the other hand, with regard to whether elevated soluble EMMPRIN is a causative factor, or only an associated biomarker of AMI, a strong increase in EMMPRIN expression around the zone of necrosis in AMI patients has been shown previously [35]. It has also been reported that in AMI patients, soluble EMMPRIN is positively correlated with infarct size, measured by single-photon emission tomography and peak CK, and inversely correlated with LVEF [34]. It can be concluded that these results, and our results demonstrating that EMMPRIN is significantly positively correlated with peak CK-MB and peak cTnT, inversely correlated with LVEF, are in favour of the notion that EMMPRIN is a causative factor in AMI.

Meanwhile, our result of positive correlation between EMMPRIN and CRP observed in the AMI patients may indicate that there is an acute phase reactant property in this protein. It has been demonstrated that CRP induces EMMPRIN expression in macrophages [32]. In one of the aforementioned studies [10], in a mixed CAD population consisting of stable CAD and ACS patients, EMMPRIN expression levels in leucocytes were not correlated with CRP, whereas its platelet expression levels were correlated positively. Concordant with our relevant result, no significant correlation was observed between the soluble EMMPRIN and CRP levels in stable CAD patients in an above-mentioned study [11].

Contrary to AMI, based on our results for stable CAD patients and the result of the above-mentioned study by Pennings *et al.* [11] we concluded that soluble EMMPRIN is not a good biomarker candidate for stable CAD; however, this conclusion does not mean that EMMPRIN plays no role in the development of atheroma. It is clear from the above-mentioned animal and human studies that there is a discrepancy in the relationship of soluble [11,33] and cell-associated [2,3,10,30-33] EMMPRIN with stable CAD. This phenomenon is probably due to the protein not naturally being found in adequate amounts in peripheral circulation, or possibly at least partially due to use of the drugs that can affect plasma levels of EMMPRIN in this population. Some data in favor of the former notion was provided in the above-mentioned study by Yong *et al.* [33], in which EMMPRIN expression in circulating platelets obtained from the coronary sinus of stable CAD patients was significantly higher than in those obtained from their peripheral vein, the difference was not significant for soluble EMMPRIN levels in the plasma. There are also data in favor of the latter notion: it has been shown that aspirin reduced the platelet expression of P-selectin and PAC-1 [36] and clopidogrel reduced the platelet expression of PAC-1 and circulating levels of soluble P-selectin [37], and that both of these proteins were positively and highly correlated with platelet EMMPRIN expression [10]. Similarly, fluvastatin, has been shown to reduce EMMPRIN expression in macrophages [32]. Actually, 40.9% of our stable CAD patients were using aspirin/clopidogrel and 13.6% were using statins, whereas this percentage was 4.6 for both medication classes among the controls. Although the data the utilization rate of aspirin, clopidogrel and anti-lipid drugs were presented only for the entire population in the study by Pennings *et al.* [11], it can easily be estimated that the percentages of use of these drugs were higher in the stable CAD population than those in the controls, as in our study. Finally, it is possible that our result that soluble EMMPRIN levels did not differ significantly between stable CAD patients and controls is due to the relatively small sample size of these groups.

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