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Matrix Metalloproteinases and psychosocial factors in acute coronary syndrome patients

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Highlights

- Hostility positively correlates with MMP-2 activity in low social support.
- Hostility explained up to a 28% of the variance of MMP-2 activity.
- Social support was related with ejection fraction at acute myocardial infarction.
- The number of diseased vessels was related with social support.

Abstract:

Psychosocial factors have been linked to cardiovascular diseases independently of traditional risk factors. The impact of psychosocial factors on plaque destabilizing factors, such as matrix metalloproteinases (MMPs) has been proposed although scarcely studied.

Objective: to evaluate the relationships between hostility, perceived stress and social support with MMPs activity in patients after an Acute Myocardial Infarction (AMI).

Methods: Blood samples were obtained from 76 patients on admission, post-angioplasty, 24 hours, 7 days and 3 months after AMI. Hostility, perceived stress and social support were evaluated by validated questionnaires.

Results: Social support was positively correlated with patients´ ejection fraction ($r=0.453$, $p=0.009$). Patients with higher infarct size presented increased MMP-2 activity at admission ($p=0.04$). Patients with one diseased vessel had more social support than those with three diseased vessels ($p=0.05$). The highest values of MMP-2 and MMP-9 activity were observed at the acute event, decreasing, with the lowest activity at 3 months post-AMI ($p<0.001$). Only in patients with low social support, hostility correlated with MMP-2 activity, from AMI onset ($r=0.645$, $p=0.013$), to 7 days post AMI ($r=0.557$, $p=0.038$). Hostility explained up to 28% of the variance in MMP-2 activity ($R^2=0.28$, $p=0.005$). Finally, in patients with high hostility, MMP-9 was positivelycorrelated with IL-1β ($r=0.468$, $p=0.02$).

Conclusions: this study adds weight to the idea that two psychosocial factors, namely hostility and social support, acting jointly, may affect MMP-2 activity. Moreover, in hostile patients,

there is a link between IL-1β and MMP-9. These findings support the role of psychosocial factors in plaque destabilization and in the inflammatory process in AMI.

Key words: Metalloproteinase; Acute coronary syndrome; social support; hostility

1. Introduction

Cardiovascular diseases (CVD) and especially coronary heart disease (CHD) represents the leading cause of death in the world in both men and women (Alwan 2011). Traditional risk factors for CVD such as smoking, hypertension, diabetes and dyslipidemia have been widely studied, yet these factors do not predict all new CVD cases and often multiple risk factors combine in the onset of CVD (Wilson and Culleton 1998; Faletra et al 2009).

Though the impact of psychosocial factors on CVD is more controversial, several meta-analyses of longitudinal studies have shown that a number of psychological characteristics including hostility, depression and job-stress have been linked to the onset of coronary artery disease (CAD) independent of traditional biomedical risk factors (Chida and Steptoe 2009; Kivimäki et al 2013). Several studies have found that hostility, reflecting angry feelings, cynical thoughts about others and antagonistic behavior (Barefoot et al, 1992), assessed by various methods, is predictive of future cardiovascular morbidity and mortality (Everson et al 1997). In a meta-analysis of prospective studies associating anger and hostility with future CHD, Chida and Steptoe (2009) found that high levels of anger and hostility were associated with not only increased CHD events in initially healthy populations, but also with poor prognosis in cardiac patients. Previous studies show that depression and hostility may interact to influence sympathetic (β-adrenergic) cardiac control and hemodynamic indices (Hawkins et al 2011). Moreover, different psychosocial factors could moderate the relationship between hostility and sympathetic hyperactivation or disease progression. For example, CAD patients with both high anger-out and low social support were found to have a higher risk of atherosclerosis progression, than other patients, reflecting a synergistic interaction between hostility and social support in relation to CAD (Angerer et al 2000). In another study, trait anger and social inhibition were associated with an increased prevalence and severity of coronary artery plaques (Compare et al 2014). Together, these results support the buffering hypothesis of social support, stress and health (Cohen and Wills, 1985) and extend them to social support's moderating effect on hostility in relation to CAD.

How can hostility and little social support act at the biological level to contribute to CAD? Based on converging empirical evidence, the impact of psychosocial factors on the onset of the acute coronary

syndrome (ACS) is thought to be due to their impact on inflammatory and plaque destabilizing factors, hemodynamic plaque rupturing forces (e.g., coronary spasms, elevated blood pressure) and thrombosis (Gidron et al 2002; Rozanski et al1999). One study found that life events, hostility and little social support were positively correlated with monocyte recruitment levels in ACS patients soon after admission (Gidron et al 2003). Pro-inflammatory cytokines such as IL-1 induce leukocyte chemoattraction to the endothelium, and activate plaque monocytes (macrophages). The macrophages then produce matrix matalloproteinases (MMPs) causing coronary plaque instability (Ross 1999).

Atherosclerotic plaque rupture is a major cause of ACS (de Nooijer et al 2006). Several mechanisms such as matrix degradation have been implicated in this process and MMPs play an important role in plaque instability (Pasterkamp et al 2000; Newby 2008). Different MMPs, have been identified in atherosclerotic plaques and in regions of foam cell accumulation and have been directly associated with plaque remodeling as well as plaque vulnerability (Pasterkamp et al 2000; Newby 2008) and with myocardial remodeling (Dhillon et al 2009). Moreover, they have been suspected to be partly responsible for the pathogenesis of CAD (Ross 1999). Furthermore, levels of MMP-2 were found to be independent predictors of survival in patients after a myocardial infarction (Dhillon et al 2009).

However, the role of psychosocial factor implicated in CAD, in elevation of MMPs, has received little scientific attention. It has been reported that psychosocial factors like hostility and depression are positively associated with MMP-9 levels (Lutgendorf et al 2008), and that sense of coherence (a resilience factor reflecting comprehensibility, manageability and meaningfulness), is associated with decreases in MMP-9, in a middle aged normal population (Garvin et al 2009). In contrast, Jönsson et al (2014) reported that there were no relationships between depressive mood and MMP-9 expression in patients after AMI.

To our knowledge, the impact of psychosocial factors like hostility and social support on different MMPs activity has not been studied in ACS patients. Furthermore, it has been reported that social support may moderate the effects of stress on cardiac responses to stress (Gerin et al 1992). André-Petersson et al (2007) found that social support moderated the effects of high demand and low control

(reflecting job strain) on future myocardial infarctions in women. However, it remains unknown whether social support may moderate the effects of adverse psychological factors such as hostility or stress on biological factors, which directly contribute to the onset of CAD and ACS.

This study, through a prospective design, examined the relationships between hostility, perceived stress and social support with MMP-2 and MMP-9 activity, pro-inflammatory cytokines and hemodynamic factors in patients with ACS. We hypothesized that hostility and stress will be positively correlated with MMPs and inflammation, while social support would be inversely related to such outcomes. Finally, social support was expected to buffer (moderate) the effects of hostility and stress on such plaque-destabilizingfactors.

2. Materials and Methods:

The study population was comprised of 76 consecutive patients (64 men, 59±11 years and 12 women, 61±15 years) who were hospitalized at the Argerich Hospital, Buenos Aires, with an ST-segment elevation acute myocardial infarction (STEMI), referred for primary angioplasty, within 24 hours of onset of symptoms. The STEMI was defined by prolonged chest pain and ST-segment elevation $\geq$ 1mm in at least two consecutive leads, or a new or presumably new left branch bundle block.

Full clinical details were recorded for all patients by pro-forma, including demographic data, smoking, coronary risk factors, previous clinical history and treatment as well as, educational level and marital status. Patients with cancer, stroke, and other severe inflammatory diseases and other pulmonary or hepatic diseases, endocrine disorders, thyroid disease, patients with Cushing's disease or syndrome, as well as chronic renal failure patients on hormonal treatment, were excluded from the subsequent analyses. We also excluded patients with cardiogenic shock at admission and other cardiovascular diseases. Previous myocardial infarction (MI) was established by evidence of previous hospital admission and a discharge diagnosis of MI (Thygesen et al 2012). All studies were performed in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written informed consent obtained from all participants before coronary

angiography. The protocol was approved by the Ethics Committee of the Argerich Hospital and the Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Coronary angiography and primary angioplasty were performed according to standard protocols and techniques. All the patients were referred to the catheterization laboratory, Hemodynamic Unit, within 30 minutes after arriving at the Emergency Department, where they received 100 mg of aspirin, 300 mg of clopidogrel and intravenous nitroglycerine.

For each patient, the Killip and Kimbal index was evaluated at admission. The infarct size was established according to the territory threatened in accordance with the electrocardiographic compromise, so a large acute myocardial infarction (AMI) was considered when more than four electrocardiographic leads with grade 3 ischemia were involved (Birnbaumet al 2014). We used this technique as a surrogate marker of the cardiac magnetic resonance imaging (MRI). The ejection fraction was calculated as: [(final diastolic volume – final systolic volume)/ final diastolic volume].

2.1 Biological tests:

Samples: Blood samples were obtained on admission (time 1); during coronary angiography after angioplasty (time 2); the morning after the routine cardiac protocol, between 0800h-0900h(time 3); 7 days after MI (time 4); and 3 months later (time 5). In each time period, serum and plasma samples were separated by centrifugation at 1500 × *g* for 5 min and stored at -70°C. Serum was used for cortisol, IL-1β, high sensitive CRP (hs-CRP), creatin kinase (CK), CK-MB determinations and MMP-9 concentration, and plasma samples for MMP-2 and MMP-9 activity determination.

Cortisol, IL-1β, hs-CRP, CK and MMP-9 determinations: Cortisol and IL-1β were determined by a chemoluminiscent method (Immuliteautoanalyzer 1, Siemens, LA, USA). The intra-assay (CVi) and inter-assay (CVe) variation coefficients for cortisol were <5% and <9.7% respectively and for IL-1β were <2.8% and <7.7%. Hs-CRP was measured by a high sensitivity immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany) (CVi and CVe<3%), CK by UV-test (CVi<2.3%, CVe<3.3%) and CK-MB by UV-immunologic test (CVi<1.7% and CVe<8.1%) standard methods, in a Cobas C-501 autoanalyzer. MMP-9 concentration was determined by a solid phase ELISA assay

(Quantikine-ELISA, R&D Systems). The CVi was <2.0% and the CVe was <7.9%. Regarding the possibility that the acute event could mask possible associations between IL-1β and the other variables, IL-1β and MMP-9 concentrations were measured at time 4.

MMP-2 and MMP-9 activity: MMP-2 and MMP-9 activity was measured in plasma by gelatinolyticzymography as previously described (Muzzio et al 2009). MMP-9 (84 kd [active form]) and MMP-2 (67 kd [active form]) were identified by molecular weight. Conditioned media from the promyelocyte U-937 cell line was used as activity standard. Coefficients of variation were 4.8% (CVi) and 8.6% (CVe). Enzyme activity was detected as colorless bands quantified using Scion-Image J software (Scion Corporation, Frederick, MD), and relative activity was expressed as a ratio to the internal standard.

2.2 Psychological measures

After angioplasty, when patients were in a lucid state, we assessed their hostility (Gidronet al 2001), perceived stress (Cohenet al 1988) and social support (Timmerman et al 2000) by validated questionnaires. The hostility and social support scales presented adequateinternal reliabilities with a Cronbach´s alpha of 0.740 and 0.827 respectively.However, the perceived stress questionnaire presented an unacceptably low Cronbach´s alpha of 0.166. Patients total scores for each scale were computed.

2.3 Statistical analysis:

We first tested the distribution of variables using normality tests (kurtosis and skewness). Spearman correlations were computed between independent and dependent variables and between potential confounders and dependent variables. To investigate the differences between groups we used the Mann-Whitney test. To verify differences within subjects among variables at different times, we used the Wilcoxon test.To examine the possible impact of psychosocial variables on MMPs we used Spearman correlations. We then divided the sample at the median, into high and low social support, as well as, high and low hostility to examine the possible moderating role of social support. A Spearman's correlation controlling for different covariates (age, BMI, smoking, previous treatment

with aspirin and β-blockers and infarct size), and a linear regression analysis, were performed, to test the relationship between hostility and MMPs, separately in patients with low and high psychosocial factors, according to the median value. We tested the unstandardized residuals for normality in order to perform the regression analysis.

3. **Results:**

The characteristics of patients are presented in Table 1. As expected CK and CK-MB were higher in those patients with larger infarct size (p=0.019 and p=0.022, respectively).

Median values of hostility, social support and perceived stress are shown in table 2. A positive correlation was observed between hostility scores and perceived stress (r=0.33 p=0.012). None of the three psychological variables were related to age, body mass index (BMI), waist circumference, or smoking status, nor were they related to common cardiovascular risk factors or medication status. Social support was positively correlated with patients´ ejection fraction (r=0.453, p=0.009). Finally, patients who had one diseased vessel reported higher levels of social support than those with three diseased vessels (17 (2-20) vs 14 (4-18), p=0.05).

3.1 Cortisol and biomarkers of inflammation and vulnerable plaques:

As seen in Table 3 cortisol, one of the stress hormones, was increased at admission, and decreased at 24 hours. No correlation was found between cortisol, hostility, social support and perceived stress at any time (p=ns).

Hs-CRP showed a significant increase at 24 hours and significantly decreased at 7 days after MI, reaching normal values at 3 months after angioplasty (Table 3).

MMP-9 and MMP-2 activity presented the highest values at the acute event, showing a slow decrease with the lower activity at 3 months (Figure 1a and 1b). No correlation between MMP-9 concentration and activity was observed.

When patients were divided according to infarct size, those with larger infarct size presented increased MMP-2 activity at time 1 (RU: 1.04 (0.81-1.35) vs 0.96 (0.76-1.39), p= 0.04)) and increased MMP-9 activity at time 4 (RU: 1.07 (0.88-1.25) vs 0.97 (0.77-1.18), p=0.05). Importantly, MMP-2 activity was inversely associated with the ejection fraction, at time 1 (r=-0.461, p=0.031), time 2 (r=-0.423, p=0.044), time 3 (r=-0.472, p=0.026) and time 4 (r=-0.429, p=0.046). Moreover, patients with higher degree of Killip and Kimbal index presented higher MMP-2 activity at time 1 (RU: 0.98 (0.76-1.39) vs 1.04 (1.00-1.21), p=0.03).

3.2 Association between psychosocial factors and MMPs over time

There was no correlation between neither MMP-9 and MMP-2 activity nor MMP-9 concentration and the psychosocial factors at any time. However, when the studied population was split by the median value of social support, only in patients with low social support, hostility strongly and positively correlated with MMP-2 activity at time 1 (r=0.645, p=0.013); time 2 (r= 0.568, p=0.034); time 3 (r=0.692, p=0.006) and time 4 (r=0.557, p=0.038), after controlling for age, BMI, smoking, previous treatment with aspirin and β-blockers and infarct size. Thereafter, a regression model was performed to evaluate the impact of hostility on MMP-2 activity in patients with low social support. Hostility explained 16% of the variance in MMP-2 activity after the AMI (F=4.75 p=0.038), 28% of the variance at 24 hours post-AMI (F=9.43 p=0.005) and 22% of the variance 7 days post-AMI (F=6.84 p=0.015) (Figure2)

In the whole population, IL-1β(0.30 (0.13-0.67) pg/ml) tended to be significantly related to MMP-9 concentration(611 (88-1787) ng/ml) at time 4 (r=0.266, p=0.078). We then split the studied population according to the median value of hostility, revealing a positive and significant association between IL-1β and MMP-9 concentration (r=0.468, p=0.020) only in those patients with high hostility. These results remained significant after controlling for age, BMI, infarct size and previous treatment with β-blockers (r=0.598 p=0.009).

4. **Discussion**:

The current study examined the interplay between psychosocial variables and MMPs in ACS patients. Our results show that hostility positively correlates with circulating levels of MMP-2 activity in patients with low social support, but not in patients with high social support. It is notable that this association remained significant after statistically controlling for different confounders. Moreover, hostility explained up to 28% of the variance in MMP-2 activity in patients with low social support. Furthermore, in patients with high hostility, IL-1β was associated with MMP-9 concentration. Social support was positively correlated with ejection fraction soon after the acute event, and patients with more diseased vessels presented lower social support.

MMPs play an important role in extracellular matrix (ECM) remodeling during all phases of atherosclerosis. Focal accumulation of cells that overexpress activated forms of MMPs may promote local destruction of ECM in atheroma, leading to plaque destabilization and rupture (Dollery et al 1995). Increased peripheral blood levels of MMP-2 and MMP-9 were observed in patients with ACS (Kai et al 1998; Hlatky et al 2007). Moreover, Dhillon et al (2009) reported that MMP-2 levels were an independent predictor of mortality post-ACS in men and women. In the present study, we observed initially plasma MMP-2 and MMP-9 activity in ACS patients declining from 24 hours post-AMI. MMP-2 activity at the acute event inversely correlated with the ejection fraction and was associated with the surrogate estimation of infarct size and the Killip index. These results are in accordance to previous findings reported by Nilsson et al (2012) who observed positive associations between MMP-2 concentrations and infarct size evaluated by cardiac MRI. Moreover, in our study, MMP-9 activity was also positively correlated with infarct size. No correlation between MMP-9 concentration and activity was observed. This finding can be result of the fact that the activity of an enzyme is not necessarily related to its plasma concentration.

Hostility and low social support are associated with worse lifestyle behaviors, including smoking, poor diet, obesity, and alcoholism (Bernstein et al 2014; Stickley et al 2014). It has been reported that hostile individuals may also manifest diminished vagal modulation of heart function and increased platelet reactivity, while low social support is associated with activation of the autonomic nervous system (Rozanskiet al 1999). In this study, we found that only in patients with low social support, hostility was positively associated with MMP-2 activity, independent of other risk factors. Previous

findings reported that negative psychosocial factors such as depression were positively correlated with enhance MMPs expression in ovarian carcinoma cells (Lutgendorf et al 2008). Moreover, previous results reported that hostility and depression were positively correlated with MMP-9 levels in middle age healthy people (Garvin et al 2009). It has also been proposed that a dysfunctional hypothalamic pituitary adrenal axis, a marker of chronic stress, promotes an increase in MMP-9 in patients with CAD (Szymanowski et al 2011). One possible mechanism involved in these relationships could be the link between psychological factors and the inflammatory response. Supporting this claim, we found a positive correlation between IL-1β and MMP-9, only in high-hostile patients, in the present study. Gidron et al (2003) reported in ACS patients that percentage of monocytes was positively correlated with adverse psychological characteristics, including hostility and life-events, while protective psychological factors, including perceived-control and emotional-support, were inversely correlated with percentage of monocytes. Pro-inflammatory cytokines, primarily secreted from monocytes, can induce vulnerable plaques through different pathways (Gidron et al 2003). In vitro and animal studies have identified the ability of cytokines to regulate the transcription and synthesis of various MMPs, specifically IL-1 which promotes ECM remodeling by enhancing cardiac fibroblast MMP expression in vitro (Berg et al 2011). Furthermore, hostility has been associated with higher production of pro-inflammatory cytokines in women (Suarez et al 2004) and in men (Suarez et al 1998). Since hostility is also related to lower vagal modulation (Sloan et al 1994) and because the vagus has profound anti-inflammatory effects (Tracey 2009), one mechanism linking hostility to high MMP levels (in patients with little social support) could include excessive inflammation via reduced vagal activity. Our results extend these to AMI patients, proposing that the synergism between hostility and low social-support is related to plaque-destabilizing factors.

Different studies have reported an association observed between cortisol, as stress hormone, and psychological measures, at diverse situations (Evolahti et al 2006; Fabre et al 2014), however in the present study, no relationship among these parameters was observed. One possible explanation could be the acute response of the HPA axis soon after an ACS, which could mask the association.

There are some limitations in this study. One limitation is that only one single measure of morning serum cortisol concentration was obtained 24 hours after AMI, which is not the optimal way to

evaluate links between HPA activity and cardiovascular disease. Nevertheless, it is a feasible approach for screening studies and has been previously applied (Weigensberg et al 2008; Gidron et al 2011; Fabre et al 2013). Furthermore, psychosocial variables were only assessed once, however, hostility is a stable psychological train not expected to change over short time periods. In addition, this study included a short follow up period of the patients as well as a relatively small sample size. Thus, these results should be replicated in a larger cohort of patients. Nevertheless, this study adds weight to the proposition that two psychosocial factors, namely hostility and little social support acting jointly, may affect MMP-2 activity, which has a major role in the pathogenesis of vulnerable plaques and in the acute coronary syndrome.

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Figure 1: MMPs activity in the study population (n=76), from admission to 3 months post infarct. A) MMP-2p<0.001 vs Admission; B) MMP-9 p<0.001 vs Post Angioplasty. RU: Relative Units. Wilcoxon test.

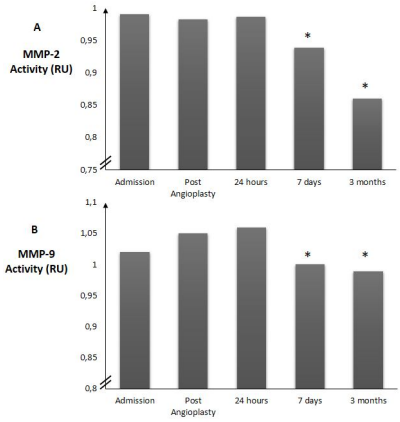

Figure 2: In the study population (n=76), hostility explained 16% of the variance in MMP-2 activity after the Acute Myocardial Infarction (AMI) (R^2=0.16, p=0.038), 28% of the variance at 24 hours post-AMI (R^2=0.28, p=0.005) and 22% of the variance 7 days post-AMI (R^2=0.22, p=0.015).

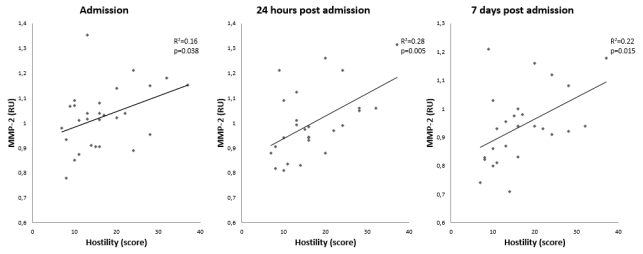

Table 1: Baseline,infarct and hemodynamic characteristics of the study population(n=76).

Variable	**Patients**
Age (years)	59 (11.7)
Male sex	64 (84.2%)
Cardiovascular riskfactors	
Hypertension	41 (53.9%)
Dyslipidemia	28 (36.8%)
Diabetes mellitus	12 (15.8%)
Smoking	41 (53.9%)
Formersmokers	17 (22.4%)
Obesity	29 (38.2%)
Sedentarism	45 (59.2%)

Table 1: Baseline,infarct and hemodynamic characteristics of the study population(n=76).

Systolic blood pressure (mm Hg)	130.2 (22.8)
Diastolic blood pressure (mm Hg)	77.7 (12.6)
Heartrate (bpm)	79.5 (17.1)
Weight (kg)	81.8 (14.8)
Height (cm)	171.3 (8.3)
BMI (kg/m^2)	27.8 (4.4)
Waistcircumference (cm)	97.7 (12.7)
CK basal (U/L)	1379 (379 – 3012)
CK peak (U/L)	2710 (1245 – 4082)
CK-MB basal (U/L)	172.5 (67.5 – 307.5)
CK-MB peak (U/L)	277 (167 – 490)
Previoustreatment	
Aspirin	17 (22.4%)
β-blockers	12 (15.8%)
ACE inhibitor	16 (21.1%)
Angiotensin II receptor antagonists	9 (11.8%)
Calciumchannelblocker	4 (5.3%)
Nitrates	3 (3.9%)
Statins	6 (7.9%)
Fibrates	1 (1.3%)
Location of MI	
Anterior	33 (43.4%)
Inferior	35 (46.0%)
Lateral	4 (5.3%)
Combined	4 (5.3%)
Size of MI (established by location)	
Large (anterior orcombined)	37 (48.7%)
Small (Inferior or lateral)	39 (51.3%)
Killip and Kimbalclassification	
Killipclass I	61 (80.3%)
Killipclass II	9 (11.8%)
Killipclass III	6 (7.9%)
Killipclass IV	0 (0%)
Killip class II + III + IV	15 (19.7%)

Ejectionfraction (%)	43 (5-69)
Number of diseasedvessels	
Onediseasedvessel	43 (56.1%)
Twodiseasedvessels	25 (33.3%)
Threediseasedvessels	8 (10.6%)

Variable data are presented as mean (SD), median (range) or n (%). BMI: bodymassindex; ACE: angiotensin-convertingenzyme

Table 2: Psychological factors evaluated in the study population (n=76).

	Perceived stress	**Hostility**	**Social support**
Score, median (range)	11 (5-15)	16 (7-37)	15 (2-20)
Patients below the median n (%)	43 (56.6%)	43 (56.6%)	38 (50%)
Patients above the median n (%)	33 (43.4%)	33 (43.4%)	38 (50%)

Table 3: Cortisol, hs-CRP, MMP-2 and MMP-9 at different times from admission (n=76). Median (range).

	Cortisol (nmol/L)	HS-CRP (mg/l)	MMP-2 (RU)	MMP-9 (RU)
Admission	963 (193-1749)	4.58 (0.47-90.8)	0.99 (0.76-1.39)	1.02 (0.86-1.43)
Post Angioplasty	1037 (195-1936)	4.30 (0.68-78.3)	0.98 (0.78-1.41)	1.05 (0.86-1.41)
24 hours	386 (99.3-1749)*	17.4 (1.55-143)*	0.98 (0.75-1.47)	1.06 (0.91-1.39)
7 days	411 (210-1010)*	15.3 (1.86-196)*	0.93 (0.71-1.26)*	1.00 (0.77-1.25)**
3 months	345 (135-770)*	3.26 (0.38-30.0)	0.85 (0.58-1.14)*	0.99 (0.71-1.19)**

RU: Relative Units. * p <0.001 vs Admission; ** p<0.001 vs Post Angioplasty, Wilcoxon test.