Evidence of prolonged disturbances in the haemostatic, hemorheologic and inflammatory profiles in transmural myocardial infarction survivors

A 12-month follow-up study

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Summary
Haemostatic, hemorheologic and inflammatory disturbances have been associated with acute coronary syndromes. Most knowledge is reported in cross sectional studies and are without time dependent evolution of these profiles.
The aim of this study was to evaluate, during the first year, the evolution of the haemostatic, hemorheologic and inflammatory profiles determined at hospital discharge in survivors with transmural myocardial infarction (MI).
Eighty eight (79 male; 9 female) mean age of 58 ± 11 years, survivors of a transmural MI were prospectively studied at discharge, 6 months and one year after the event. Haemostatic (protein C, antithrombin III and plasminogen activator inhibitor I), hemorheologic (blood fluidity and components) and inflammatory profiles (polymorphonuclear elastase and leukocyte count) were determined using standard methodology.

The results of the study can be summarized as follows: (1) Protein C decreased (p < 0.05) over time while PAI-I only varied significantly until 6th month. (2) Plasma viscosity and fibrinogen (p < 0.001) decrease over time, while erythrocyte aggregation (p < 0.001) and haematocrit increased. Whole blood viscosity did not vary. (3) Leukocyte decreased (p < 0.001) and elastase did not (4). Those patients with cardiovascular events (n = 7) had higher PAI-I concentration (p<0.05) and leukocyte count (p < 0.01), at discharge (5) Left ventricle ejection fraction correlated significantly with plasma viscosity (r = 0.35 p < 0.05).
The results of this longitudinal study show dynamic modifications of the haemostatic, hemorheologic and inflammatory profiles during the first year of a transmural myocardial infarction. In addition, there are interrelations between them and the clinical profile that could help to explain the clinical evolution of this group of patients.

Keywords
Myocardial infarction, protein C, elastase, plasminogen inhibitor I and erythrocyte aggregation, longitudinal study

Introduction
Coronary heart disease (CHD) is a major cause of death in developed countries, relating to several factors, some biological, other of social behavior. Classic risk factors explain only a third of all myocardial infarctions (1-3).

Regardless of the initial causes, atherosclerosis is a systemic and chronic inflammation of the arterial wall (4, 5). The progression of the atherosclerotic plaque is associated with a systemic (4, 6) thrombogenic and inflammatory state. On the other hand, atherosclerosis and its consequences may contribute to hemorheologic and haemostatic disturbances. Hence, it will
be relevant to evaluate the haemostatic, inflammatory and hemorheological profile of the patients, establishing their time evolution tendency and its predictive value. During the past decades, several studies have shown acute coronary syndromes, stable angina, arterial hypertension, and diabetes and several other vascular disease disturbances in the hemorheological (5, 7-9), inflammatory (11-14) and haemostatic (14-18) profile. Some studies are epidemiological or population based studies or in patients with several vascular diseases (7, 10, 19) and other clinical trials (8).

Various epidemiological studies have investigated the possible link between blood rheology (i.e. blood ability to deform, that could influence blood flow) and coronary heart disease incidence (19-21). Some involved whole blood viscosity, but most involved its major determinants: haematocrit, plasma viscosity, and erythrocyte aggregation. All together, there is a possible relationship between the determinants of blood rheology and CHD, however with unknown importance.

In acute coronary syndromes (ACS) several stimulus, local (plaque disruption) and systemic (thrombotic factors) may contribute to the occurrence, extent, persistence of coronary thrombosis and its clinical sequels. Epidemiological studies indicate several markers of haemostatic and thrombotic function as potent predictors for ACS (14-18), stroke (24), stable angina (25, 26) and sudden death (14). Such markers include those related to fibrinolysis (endogenous plasminogen activators and clot lyser time), and inflammatory (plasma fibrinogen, C reactive protein, amiloid A). Tissue plasminogen activator (tPA), plasminogen activator inhibitor type 1 (PAI-1) and dimers D plasma levels are associated with myocardial infarction (MI) survivors submitted to thrombolysis, to MI extent and to silent ischemia detected in the 24 hours holter (27). This disturbance in the fibrinolytic system characterized by an increase of PAI-1 and tPA could relate to delays in reperfusion and/or reinfarction after thrombolysis (28). Patients with unsuccessful thrombolysis have higher levels of PAI-1 (28). As a compensatory mechanism in hypercoagulable states, there is an increase of protein C levels and of the activated protein C inhibitor complex (29). PAI 1 and tPA levels decrease from the second to the nineteenth day after the AMI and return to baseline levels after 4 weeks (17).

Nevertheless, the haemostatic response is an integrated process requiring the participation of plasma and cellular elements. Normal erythrocytes participate by exposition of the pro-coagulant phospholipids and induction of thrombin generation (30). After an AMI, there is erythrocyte membrane rigidification (12) that increases over a 12-month follow-up. One or the most important hemodynamic effects introduced in vascular disease is stenosis (fixed or dynamic), which decreases the distal intraluminal pressure and the effective perfusion pressure (22, 23), increasing local haematocrit and possibly local erythrocyte aggregation. Rheological disturbances amplify these local changes.

One epidemiological study showed the association between PAI-1 and leukocyte elastase concentrations and to death and/or AMI in patients with angina pectoris (31). In other words, disturbed fibrinolysis and leukocyte activation in patients with angina pectoris could associate to the development of thrombotic events in a 5-year clinical follow-up. The hypothesis that a chronic inflammatory state may play a role in destabilizing the atherosclerotic plaque is well supported by the presence of inflammatory cells in the site of plaque erosion or rupture (8, 32-34), and the presence in circulation of activated leukocytes (9) increased reactive C protein and amiloid A (11, 12). The inflammatory response (34, 35) increases the expression of adhesion molecules (36).

The hemorheological (9), haemostatic (50), inflammatory (13), membrane fluidity (12) and platelet activation (37) detected during the acute phase in survivors of an AMI evolves after hospital discharge and for months later. To our knowledge, there is no study analyzing the evolution of these during the first year after a transmural AMI, and the relationship between them.

The objective of the present study was to determine in survivors of a transmural AMI, the evolution at 6 and 12 months of the haemostatic, hemorheological and inflammatory profiles determined at hospital discharge; and the relationship between them and the patient’s clinical profile.

Patients and methods

Study population

Eighty-eight (79 male; 9 female) survivors of a transmural myocardial infarction (44 anterior and 44 inferior), mean age of 58 ± 11 years, proceeding from a coronary unit were consecutively enrolled from 1994 to 1996. This study was part of a hemorheologic-clinical follow-up project of the Portuguese Society of Hemorheology and Microcirculation in association with the Lisbon Medical School (Biochemistry Institute and the Cardiology Department, UTIC-AC). Table 3 also includes the “normal” range values, characterized by the 95% confidence interval. These values were determined during the same period in our Laboratory in 40 apparently healthy subjects (36 male) mean age of 50 ± 10.0 years, without main cardiovascular risk factors. The “normal” range is only included as a reference, thus in the results there is no reference to comparisons with a normal population.

Clinical investigation

At the coronary unit and immediately before hospital discharge the patient’s medical history was assessed. A 20 ml fasting blood sample was drawn from the antecubital vein by venipuncture into the appropriate tubes. Blood samples were drawn from 8.30 a.m. to 9.30 a.m. in order to minimize diurnal variations in the levels of the hemorheological, haemostatic and inflammatory factors.
Laboratory evaluation
Plasma viscosity (PV) was determined by the Harkness method in a capillary Plasmatic viscometer from Coulter Counter Electronic Ltd; whole blood viscosity (WBV) was determined in native and with standard hematocrit 45% correction at low (22.5 s\(^{-1}\)) and high (225 s\(^{-1}\)) shear stress in a Brookfield Viscometer, hematocrit and leukocyte count in a Cell Dyn 1600 from Abbott; erythrocyte aggregation index (EAI) was determined in a MA 1 Myrenne aggregometer (Roetgen, Germany) at 10s after rotation at 600 s\(^{-1}\). Protein C, antithrombin III and plasminogen activator inhibitor type 1 (PAI-1) determined by colorimetric technology in a Micro Reader Hyperion, PMN elastase by immunoassay with Merck & Co., Diagnostic Kit.

12-month follow-up
Full clinical assessment and laboratory determination was made in three periods: at hospital discharge, after 6 months (n = 43) and after one year (n = 30). In order to determine: (i) the hemorheological, haemostatic and inflammatory profile variation during the first year after a myocardial infarction, (ii) the incidence of cardiovascular events: composite of cardiovascular death, non-fatal myocardial infarction and unstable angina, (iii) the incidence of coronary revascularization.

Definition of cardiovascular events
Baseline transmural myocardial infarction was defined as two of three indicators: electrocardiograph alterations suggestive of transmural (MI), Creatininokinase (CK) 2 fold and the isozyme MB elevation (> 20%) and chest pain. Patients were defined as having a cardiovascular event if they had experienced a fatal or non-fatal myocardial infarction, or unstable angina. The definitions used for myocardial infarction and unstable angina were adapted from the American Heart Association. The events were recorded only once.

Statistical analysis
Laboratory factors were expressed as mean ± standard deviation. A two-way analysis of variance (ANOVA) for repeated measures, one-way ANOVA and student-paired t-test were performed to analyze the differences over time (at discharge, 6 and 12 months) and the clinical profile effect on the laboratory profile. The relationship between the analytical parameters achieved with the Pearson correlation coefficient. A 5% significant level was used. Data analyzed with SPSS 10.01 statistical package.

Results
Clinical profile (Tables 1 and 2)
Nearly half of the patients had arterial hypertension (45%) or smoking habits (46%). A third had known ischemic heart disease (33 stable angina, 11 MI) and fourteen (16%) had diabetes. The cardiovascular risk factors profile was similar for both infarction locations.

<table>
<thead>
<tr>
<th>Cardiovascular events</th>
<th>7 (8%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non fatal MI</td>
<td>2</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Revascularization</th>
<th>26 (29.5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CABG</td>
<td>23</td>
</tr>
<tr>
<td>PCI</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: MI myocardial infarction, CABG coronary artery bypass graft; PCI percutaneous intervention.

Table 1: Cardiovascular events and coronary revascularization in survivors of a transmural acute myocardial infarction.

<table>
<thead>
<tr>
<th>No Events (n)</th>
<th>With Events (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>80</td>
</tr>
<tr>
<td>Age</td>
<td>56.7 ±12.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>60</td>
</tr>
<tr>
<td>F</td>
<td>7</td>
</tr>
<tr>
<td>Angina</td>
<td>25</td>
</tr>
<tr>
<td>AMI</td>
<td>11</td>
</tr>
<tr>
<td>DM</td>
<td>8</td>
</tr>
<tr>
<td>AHT</td>
<td>34</td>
</tr>
<tr>
<td>Dislipidemia</td>
<td>16</td>
</tr>
<tr>
<td>Smoking</td>
<td>27</td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>25</td>
</tr>
<tr>
<td>Inferior</td>
<td>32</td>
</tr>
<tr>
<td>Killip Kimball</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>56</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
</tr>
<tr>
<td>Thrombolyis</td>
<td>27</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>24</td>
</tr>
</tbody>
</table>

Abbreviation: AHT arterial hypertension, M male, F female, DM diabetes mellitus, AMI acute myocardial infarction.

Table 2: Clinical profile of transmural myocardial infarction survivors according to the clinical events. There was one drop-out.
Thrombolysis (mainly streptokinase) was performed in 39 (38%), 29 with clinical criteria for reperfusion, and 6 to rescue angioplasty. No patient submitted to primary angioplasty or to glycoprotein IIb IIIa inhibitors.

The majority (76%) of the patients was in Killip-Kimball class I. Fifteen patients have left ventricle ejection fraction, by echocardiography, less than 40%.

In the 12-month follow-up, seven (8%) of patients had a cardiovascular event. Two had a non-fatal MI and five died. Twenty-six (29.5%) needed coronary revascularization (angioplasty-6, CABG 23). Of the 88 patients included in the study at hospital discharge, laboratory determinations were made in 43 patients at 6 months and in 30 at 12 months. Clinical follow-up at 12 months was achieved in all but one patient.

**Hemorheological profile variation during the 12-month period (Table 2)**
There was a significant variation in the plasma viscosity (Fig. 1; p < 0.001), fibrinogen concentration (Fig. 2; p < 0.001), haematocrit (p < 0.001) and the erythrocyte aggregation index (Fig. 3; p < 0.001). There was no significant variation in whole blood viscosity.

Haematocrit increased (p < 0.001) over time, with significant differences between 6-month (p < 0.001) and 12-month (p < 0.001) determinations in relation to the discharge value.

Plasma viscosity and fibrinogen fell (p < 0.001) over the 12-month period. These parameters decreased significantly between the discharge evaluation, the 6-month (p < 0.001), and the 12-month (p < 0.001) determinations (Table 2, Figs. 1 and 2). A fibrinogen cut-off value of 370 determined and used, was considered as the upper limit of the normal range because of the methodology in our laboratory. Patients with fibrinogen above the cut off value had lower (p < 0.05): haematocrit (42.6 ± 2.9% vs. 40.8 ± 3.6 %), erythrocyte aggregation (15.6 ± 6.1 vs. 13.2 ± 4.9), WBV at 225 s-1 native (7.58 ± 0.48 mPa.s vs. 7.31 ± 0.53 mPa.s) and with haematocrit 45% correction (7.41 ± 0.82 mPa.s vs. 7.31 ± 0.77 mPa.s). Thus, high fibrinogen and high plasma viscosity are not associated as expected to high levels of WBV.

**Figure 1:** Plasma viscosity (PV) and fibrinogen (Fib) concentration variation during the time of the study (discharge, 6 and 12 months). There was a significant decrease of the PV (p < 0.001) and of the fibrinogen concentration (p < 0.001). Fibrinogen is a major determinant of the plasma viscosity, and of the whole blood viscosity.

**Figure 2:** Erythrocyte aggregation index (EAI) variation during the time of the study (discharge, 6 and 12 months). There was a significant increase (p < 0.001) of its value.

**Table 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Discharge</th>
<th>6 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fib</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Erythrocyte aggregation index increased (p < 0.001) over time, significantly between 6-month (p < 0.01), and 12-month (p < 0.001) determinations in relation to the values determined at discharge (Fig. 2)

### Haemostatic profile variation during the 12-month period (Table 3)

Values of protein C (Fig. 3) activity varied significantly (p < 0.05) between the three time points (discharge, 6 and 12 months), significant (p < 0.05) between the 6, 12 months values and the discharge determination. PAI-1 (Fig. 3) did not vary with time, however, there was a significant decrease from discharge to 6 months (p < 0.05). There was no significant variation in the antithrombin III during the 12 months.

### Inflammatory profile variation during the 12-month period (Table 3)

The leukocyte count (Fig. 4; p < 0.001) decreased significantly over time: between the discharge determination, and the 6-month (p < 0.01), and 12-month (p = 0.05) determinations. PMN elastase (Fig. 4) did not vary significantly, however there was a significant decrease of the elastase levels between discharge and 6 months (p < 0.05), 12 months (p < 0.01).

### Relationship between the haemostatic, hemo-rheologic and inflammatory profiles

The interrelationship between the parameters of the hemorheological, haemostatic and inflammatory profiles are influenced by the cardiovascular events during the three determinations.

1. Patients who had an event (n = 7) during the follow-up showed at discharge: (1) fibrinogen correlates directly with plasma viscosity (r = 0.89 p < 0.01), (2) elastase correlates inversely with WBV at high shear stress native (r = -0.84 p < 0.05) and with haematocrit 45% correction (r = -0.82 p < 0.05), (3) erythrocyte aggregation index correlates directly with PAI 1 (r = 0.96 p < 0.01) and PMN elastase (r = 0.85 p<0.05) while elastase correlates directly with PAI 1 (r = 0.98 p < 0.01). (Figs. 5 and 6).

2. In the patients with no events (n = 81): (1) At discharge there was a positive correlation (r = 0.60 p < 0.001) between plasma viscosity and fibrinogen, erythrocyte aggregation correlates indirectly with plasma viscosity (r = -0.36 p < 0.01) and fibrinogen (r = -0.36 p < 0.01) and directly with PAI 1 (r = 0.31 p < 0.05). Haematocrit correlates inversely with fibrinogen (r = -0.37 p < 0.01), (2) At 6 (Fig. 7; r = 0.48 p < 0.01) and 12 months (r = 0.48 p < 0.05), there was a direct correlation between the leukocyte count and the plasma viscosity (3) At 12

### Table 3: Mean ± standard deviation of all analytical determinations at discharge, 6 and 12 months, in survivors of a transmural myocardial infarction; and the 95% confidence interval for the “normal” range of these parameters.

<table>
<thead>
<tr>
<th>“Normal” Range</th>
<th>Discharge</th>
<th>6 Months</th>
<th>12 Months</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht (%)</td>
<td>40.38 – 43.93</td>
<td>41.38 ±3.45</td>
<td>43.60 ±3.21</td>
<td>44.21 ±3.40</td>
</tr>
<tr>
<td>WBV 225±1 nat.</td>
<td>6.796 – 7.300</td>
<td>7.399 ±0.530</td>
<td>7.286 ±0.495</td>
<td>7.488 ±0.630</td>
</tr>
<tr>
<td>WBV 225±1 nat.</td>
<td>4.199 – 4.593</td>
<td>4.704 ±0.503</td>
<td>4.781 ±0.555</td>
<td>4.724 ±0.274</td>
</tr>
<tr>
<td>WBV 225±1 cor.</td>
<td>6.670 – 7.136</td>
<td>7.160 ±0.801</td>
<td>7.093 ±1.035</td>
<td>7.523 ±0.808</td>
</tr>
<tr>
<td>WBV 225±1 cor.</td>
<td>3.901 – 4.449</td>
<td>4.549 ±0.388</td>
<td>4.507 ±0.650</td>
<td>4.657 ±0.629</td>
</tr>
<tr>
<td>PV (mPa.s)</td>
<td>1.170 – 1.240</td>
<td>1.388 ±0.120</td>
<td>1.273 ±0.073</td>
<td>1.265 ±0.081</td>
</tr>
<tr>
<td>Fib (mg/dL)</td>
<td>273.0 – 254.0</td>
<td>426.4 ±105.6</td>
<td>306.4 ±92.2</td>
<td>294.3 ±57.2</td>
</tr>
<tr>
<td>EAI (nd)</td>
<td>10.3 – 13.7</td>
<td>14.0 ±2.4</td>
<td>14.5 ±3.9</td>
<td>20.1 ±4.1</td>
</tr>
<tr>
<td>PAI-1 (U/mL)</td>
<td>0.3 – 3.5</td>
<td>4.3 ±2.0</td>
<td>3.4 ±1.9</td>
<td>4.5 ±2.0</td>
</tr>
<tr>
<td>Protein C (%)</td>
<td>87.32 – 106.68</td>
<td>93.3 ±23.2</td>
<td>87.5 ±21.8</td>
<td>87.7 ±17.7</td>
</tr>
<tr>
<td>AT III (%)</td>
<td>90.54 – 105.88</td>
<td>90.3 ±12.7</td>
<td>95.1 ±15.6</td>
<td>87.9 ±14.1</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>5000.0 – 9000.0</td>
<td>8523.1 ±2362.1</td>
<td>7011.5 ±1985.2</td>
<td>7211.9 ±2148.4</td>
</tr>
<tr>
<td>Elastase (µg/L)</td>
<td>5.0 – 40.0</td>
<td>68.4 ±18.1</td>
<td>62.5 ±17.2</td>
<td>55.8 ±26.1</td>
</tr>
</tbody>
</table>

Abbreviations: Ht hematocrit, WBV whole blood viscosity; nat. native; cor. Corrected for a 45% hematocrit. PV plasma viscosity; Fib fibrinogen; EAI erythrocyte aggregation index, PAI plasminogen activator inhibitor type 1; AT III antithrombin III, p significant level, nd no dimension, sd standard deviation, N.S. non significant; * Variation during the time of the study (discharge, 6 and 12 months); ** p<0.05; *** p<0.01.
Prolonged disturbances of haemostatic, haemorheologic and inflammatory profiles in MI

PMN elastase correlates directly with PAI 1 \((r = 0.73, p < 0.05)\) (Figs. 5-7).

Relation between the clinical profile and the discharge haemostatic, hemorheologic and inflammatory profiles

Patients with previous ischemic heart disease had lower \((p < 0.01)\) protein C \((98.6 \pm 18.0\% \text{ vs. } 84.0 \pm 27.0\%)\) and higher \((p = 0.018)\) elastase activity \((66.0 \pm 18.0 \mu g/L \text{ vs. } 80.0 \pm 12.0 \mu g/L)\). Those with arterial hypertension have higher \((p < 0.05)\) plasma viscosity \((1.36 \pm 0.11 \text{ mPa.s vs. } 1.46 \pm 0.12 \text{ mPa.s})\). Patients with smoking habits have lower \((p<0.05)\) plasma viscosity \((1.41 \pm 0.12 \text{ mPa.s vs. } 1.35 \pm 0.10 \text{ mPa.s})\). The other cardiovascular risk factors had no relationship with the laboratorial determinations.

Patients in Killip-Kimball class above I have lower \((p < 0.01)\) erythrocyte aggregation index \((14.5 \pm 5.7 \text{ vs. } 11.5 \pm 2.5)\) and higher \((p < 0.05)\) plasma viscosity \((1.37 \pm 0.11 \text{ mPa.s vs. } 1.43 \pm 0.11 \text{ mPa.s})\). Patients with an ejection fraction below 40% have higher \((p < 0.01)\) plasma viscosity \((1.36 \pm 0.11 \text{ mPa.s vs. } 1.46 \pm 0.12 \text{ mPa.s})\). Five patients with left ventricle ejection fraction below 35% have higher \((p < 0.01)\) plasma viscosity \((1.37 \pm 0.15 \text{ mPa.s vs. } 1.51 \pm 0.15 \text{ mPa.s})\) and higher \((p < 0.05)\) whole blood viscosity at high shear stress native \((7.40 \pm 0.50 \text{ mPa.s vs. } 7.78 \pm 0.18 \text{ mPa.s})\) and with haematocrit 45% correction \((7.18 \pm 0.27 \text{ mPa.s vs. } 7.69 \pm 0.27 \text{ mPa.s})\). There is a negative significant correlation between plasma viscosity and the ejection fraction \((r = 0.35, p < 0.01)\).

Thrombolysis did not significantly affect the analytical profile. Nevertheless, those patients with reperfusion clinical criteria (in relation to all other patients independently if submitted to thrombolysis) had a higher \((p = 0.01)\) erythrocyte aggregation index \((12.8 \pm 4.6 \text{ vs. } 16.2 \pm 6.1)\) and higher \((p < 0.05)\) PAI 1 \((4.0 \pm 2.0 \text{ vs. } 5.1 \pm 1.7)\).
Figure 5: Relationship between plasminogen activator inhibitor 1 (PAI 1), elastase and the erythrocyte aggregation index (EAI) at hospital discharge in patients with or without cardiovascular events in the following 12 months. In patients who did not suffer an event only the relation EAI-PAI was determined (and much weaker). In the figure it is represented in the trend line and the value of the R square (Rsq). See text for more details.
Seven patients experienced a cardiovascular event. Those who had an event had higher (p<0.05) PAI 1 (4.47 ± 1.84 vs. 6.34 ± 1.56) and higher (p < 0.01) leukocyte count (8237.0 ± 1900 vs. 10835.0 ± 4300). Those patients with protein C activity above the upper quartile had no cardiovascular event.

**Discussion**

The main finding of the present work is that transmural MI survivors have haemostatic, inflammatory and hemorheologic profiles dynamic variations after hospital discharge. Some tend to normalize while others tend to aggravate or return to the already previously abnormal profile.

The inflammatory profile expressed by the determination of the leukocyte count and polymorphonuclear (PMN) elastase decreased over time (Fig. 4). In the first 6 months, this variation is more pronounced. The leukocyte count expresses only an absolute value, however it is an indirect index of a highly inflammatory state (actual or previous). It is well known that elastase expresses leukocyte activation. There was a decrease of the absolute leukocyte count and the leukocyte activation.
(expressed by the PMN elastase concentration) observed during the first year. The subgroup of patients with the leukocyte count above the upper quartile was at a higher risk for a cardiovascular event. This observation is similar to that of other authors (52). In the subgroup of patients who did not have an event, there was a positive relationship between plasma viscosity and the leukocyte count at 6 and 12 months, but not at discharge. At 6 and 12 months (Fig. 7), a proinflammatory state (higher leukocyte count and elastase) is associated with increasing plasma viscosity and with the disarrangement of the fibrinolytic system (increasing PAI-1 at 12 months).

Elevated plasma levels of several inflammatory mediators, along with the leukocyte count, are well known markers of cardiovascular events in patients with ACS and stable angina. Those include P-selectin, interleukine-6, tumor necrosis factor α, soluble intercellular adhesion molecule -1 (61, 62), C reactive protein (CRP). Many of these molecules regulate the attachment and transendothelial migration of leukocytes. CRP was considered an epiphenomenon of an inflammatory reaction, however, nowadays there is a lot of evidence to show a more direct proinflammatory effect of this protein (53). CRP is a strong mortality predictor after AMI (54, 55), unstable angina (54, 56), in patients with multiple coronary events (58), in first degree relatives (57), predictor of thrombolysis efficacy (59 and sudden death (60). Statins, decrease CRP levels by modulating the chronic inflammatory state of these patients (63, 64), however the therapeutic target remains unknown. These mediators along with the leukocyte activation probably will tend to interfere with the normal functions at the microcirculation level. Leukocytes are less deformable than erythrocytes and have a more rigid membrane which might cause local disturbances in the already damaged blood flow (38-40). There is a relationship between CRP levels and the erythrocyte aggregation parameters (65). CRP evolution after an AMI is unknown, nevertheless its prognostic value in stable patients, reflects a chronic systemic inflammatory state. Unfortunately, from 1994 to 1996 we were unable to determine CRP plasma levels in our laboratory. We propose that CRP could be the bridge between the haemostatic and hemorheological long-term modifications in this group of transmural MI survivors. However, it calls for speculation.

The determination of the PAI-1, protein C activity and antithrombin III characterized the haemostatic profile.

PAI-1 is a proteinase inhibitor (41), key regulator of the fibrinolytic system (42), and an important protective mechanism against thrombosis. High risk for myocardial infarction and sudden death (14, 43, 44) associates with high levels of PAI-1 (fibrinolytic disarrangement).

Protein C is a vitamin K dependent coagulation inhibitor that: (1) up regulates the activity of the coagulation factors V and VIII, associating with venous thrombosis (45), cerebrovascular disease (46) and more recently to arterial thrombosis (47); (2) Protein C anti inflammatory properties have been associated (low level) to a worst prognosis in septic shock (48, 49). These anti thrombotic, pro fibrinolytic and anti-inflammatory properties of protein C might have an important role in preventing microvascular thrombosis (22).

In our work, PAI-1 (Fig. 3) decreased significantly during the first 6 months, and then increased in the 12-month determination. In contrast, protein C (Fig. 3) activity decreased during the 12 months. Protein C activity should accompany PAI-1 activity in order to compensate any disarrangement of the fibrinolytic system. During the 6 months, protein C activity decreased with the decreasing PAI-1, however during and after the 6th month there was a pro-coagulant tendency with a high PAI-1 level and low protein C activity.

Those patients who had cardiovascular events had higher PAI 1 at hospital discharge, and those with protein C concentration above the upper quartile had no events. Therefore, PAI-1 is associated to future cardiovascular events (death, non fatal MI, unstable angina) and protein C activity had a protective effect against the cardiovascular events when within the normal range.

In order to characterize the hemorheologic profile, the determination of whole blood viscosity at high and low shear stress native and 45% haematocrit correction, and its main determinants (fibrinogen, plasma viscosity, erythrocyte aggregation, haematocrit) were performed.

Plasma viscosity (Fig. 1) and fibrinogen concentration (Fig. 1) decreased over the 12-month period while haematocrit and the erythrocyte aggregation index (Fig. 2) increased significantly. Whole blood viscosity did not vary significantly. The interaction between fibrinogen-plasma viscosity on the one hand and haematocrit erythrocyte aggregation on the other is explained by blood viscosity static behavior. The increase of the haematocrit and erythrocyte aggregation compensates the decreasing fibrinogen and plasma viscosity. During the first year the erythrocyte membrane of MI, survivors tends to rigidify (12), which could explain the increase in erythrocyte aggregation.

The determined variations in the hemorheologic profile are accompanied by similar variations in the inflammatory profile (50). In vitro, fibrinogen is a major determinant of plasma viscosity, increasing the erythrocyte aggregation index, and decreasing the erythrocyte aggregation index (51). The positive correlation between fibrinogen and plasma viscosity was determined in patients with and without events. Nevertheless, in the subgroup of patients without cardiovascular events there was a negative relation between the erythrocyte aggregation and fibrinogen or plasma viscosity, which was not expected. On the other hand, the subgroup of patients with fibrinogen below 370 mg/DL (normal range) had higher erythrocyte aggregation and higher whole blood viscosity (independent of the haematocrit). Therefore, there is an interfering mechanism, which could relate to circulating substances (endogenous or exogenous) with the
ability to interfere with the erythrocyte membrane and its ability to interact.

In the subgroup of patients who had a cardiovascular event during the first year, there was a positive relationship between PAI-1, elastase and the erythrocyte aggregation (Fig. 5). Elastase concentration, PAI-1 level and the erythrocyte aggregation were all associated directly. On the other hand, higher PAI-1 concentration associates with higher erythrocyte aggregation. Therefore, a proinflammatory state associates or relates to a thrombogenic state and with erythrocyte hyperaggregation. These associations were not determined in the subgroup of patients without cardiovascular events. Hence, patients at higher risk for a cardiovascular event have an association between the three profiles (hemorheologic, inflammatory and haemostatic). The correlation between the red blood cell aggregation and the inflammatory state, characterized with CRP and fibrinogen has been described (65). In the present work, we confirmed in the patients with events the relationship between inflammation and the erythrocyte aggregation. The inverse relation between fibrinogen and the erythrocyte aggregation should be further investigated.

Patients with clinical signs of heart failure had lower erythrocyte aggregation and higher plasma viscosity. The latter inversely relates with the left ventricle ejection fraction (Fig. 6). Thus, lower left ventricle ejection fraction associates with higher plasma viscosity. This abnormality in this particularly sensitive subgroup of patients could associate with microcirculatory flow stasis with the consequent compromise of the teicidal perfusion and tissue hypoxia.

In the present study, the patient’s clinical profile related to the laboratory determinations in a hospital setting. Those with a history of ischemic heart disease had higher plasma viscosity, higher elastase and lower protein C activity. Arterial hypertension associates with higher plasma viscosity and fibrinogen. Smoking is associated with higher plasma viscosity. These abnormalities could relate with microcirculatory flow abnormalities and the possible perpetuation of an inflammatory and prothrombotic state because of low teicidal perfusion pressure in blood stasis and increased local haematocrit.

Thrombolytic treatment with streptokinase did not affect the patient’s laboratory profile; nevertheless, those who had reperfusion (in relation to all other patients) had higher erythrocyte aggregation and higher PAI-1, which contradicts other studies (28), and relates perhaps to time sampling. This effect could relate to the thrombolytic itself, which calls for speculation.

In conclusion, after discharge and during the first 12 months, survivors of a transmural myocardial infarction experience dynamic changes in the haemostatic, hemorheologic and inflammatory profile with interrelations between them and associations with the clinical profile. These associations and profile modifications over time might help to explain the clinical evolution of survivors of transmural acute myocardial infarction.

References


