Homocysteine and inflammatory biomarkers plasma levels, and severity of acute coronary syndrome

Homocystéine et marqueurs inflammatoires, et sévérité du syndrome coronaire aigu

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Abstract. Recently, homocysteine and new inflammatory biomarkers are demonstrated to be involved in cardiovascular diseases. These risk factors are not well studied in acute coronary syndrome. We investigated the distribution of homocysteine and inflammatory markers in patients with acute coronary syndrome and evaluated the association between these parameters and severity of the disease. One hundred and twenty-two patients with acute coronary syndrome were recruited in the cardiac intensive unit care of military hospital of Tunis. Classic risk factors, lipid parameters, total homocysteine, HsCRP, IL-6 and TNF-α were determined for all participants. We investigated the distribution of these parameters according to the number of diseased vessels in patients with acute coronary syndrome. Patients with three affected vessels showed significant elevated homocysteine, HsCRP, IL-6, TNF-α, total cholesterol, LDL-cholesterol and Lp (a) compared to those with one and those with two affected vessels. Homocysteine (OR = 1.14; 95%IC: 1.04-1.25; P = 0.006), TNF-α (OR = 1.27; 95%IC: 1.13-1.44; P = 10^-3), HsCRP (OR = 1.09; 95%IC:1.03-1.16; P = 0.005) and IL-6 (OR = 1.15; 95%IC: 1.06-1.25; P = 0.001) were significant predictors of severity of the disease. We conclude that homocysteine and inflammatory biomarkers appear to enhance the degree of affected arteries and so the severity of coronary artery disease.

Key words: acute coronary syndrome, homocysteine, inflammatory markers, lipid profile

Résumé. L’homocystéine, ainsi que de nouveaux biomarqueurs inflammatoires sont liés à la survenue des maladies cardiovasculaires. Ces marqueurs ne sont pas bien étudiés dans le syndrome coronaire aigu. Nous avons étudié la distribution des concentrations plasmatiques de l’homocystéine et des marqueurs inflammatoires chez des patients avec syndrome coronaire aigu, et avons évalué l’association entre ces paramètres et le degré de sévérité de la maladie. Les patients avec syndrome coronaire aigu, au nombre de 122, ont été recrutés de l’unité de soins intensifs de cardiologie. Les facteurs de risque classiques, les paramètres lipidiques, l’homocystéine, la CRPus, l’IL-6 et le TNF-α ont été déterminés pour tous les patients. La distribution de ces paramètres est déterminée en fonction du nombre des artères affectées. Les patients avec trois artères affectées montrent des concentrations significativement élevées d’homocystéine, CRPus, IL-6, cholestérol total, LDL-cholestérol et Lp (a) comparés à ceux avec une ou deux artères affectées. L’homocystéine (OR = 1.14 ; 95 %IC : 1.04-1.25 ; p = 0.006), le TNF-α (OR = 1.27 ; 95 %IC : 1.13-1.44 ; p = 10^-3), la CRPus (OR = 1.09 ; 95 %IC : 1.03-1.16 ; p = 0.005) et l’IL6 (OR = 1.15 ; 95 %IC : 1.06-1.25 ; p = 0.001) sont des prédicteurs significatifs de la sévérité de la maladie. En conclusion, l’homocystéine et les biomarqueurs inflammatoires peuvent augmenter le degré de la sévérité des maladies coronariennes.

Mots clés : syndrome coronaire aigu, homocystéine, marqueurs inflammatoires, profil lipidique

Cardiovascular diseases represent the essential cause of morbidity and mortality in the world. Many factors are demonstrated to be responsible for these diseases. Elevated homocysteine (Hcy) level is considered as a risk factor for the development of atherosclerosis. It has been suggested that Hcy enhances inflammatory response that is recognized for their role in atherosclerotic disease [1, 2]. Recent studies suggest that markers of inflammation may also reflect different aspects of the atherothrombotic process and have a potential role for the prediction of risk for developing coronary artery disease (CAD) [3, 4]. In fact, cytokines released from the inflammatory cells may reflect the inflammatory process in atherosclerotic plaques. Importantly, biomarkers of different processes may be combined to enhance risk stratification above that of any single marker [5]. Dyslipidemia is a well-established risk factor for the development of coronary artery disease [6]. C-reactive protein (CRP) is another proinflammatory factor that was implicated in the pathogenesis of CAD and may have prognostic value in patients with acute coronary syndromes [7].

The aims of our study are to investigate the distribution of homocysteine and inflammatory markers blood levels in patients with acute coronary syndrome and to evaluate the association between these parameters and the severity of the disease.

**Patients and methods**

**Population study**

Patients hospitalized with acute coronary syndrome (ACS) were recruited from the coronary care unit of department of cardiology of military hospital of Tunis. The group is composed by 122 patients (77 men and 45 women) with mean age 64 ± 10 years. ACS was defined either as an unstable angina or as an acute myocardial infarction, which was diagnosed based on the presence of chest pain and typical ischemic electrocardiographic changes. Stable angina was defined as the basis of the presence of typical and stable chest pain during effort, a positive treadmill exercise test, and obstructive coronary lesions as determined by coronary angiography. The extent of CAD was assessed by the number of diseased coronary vessels. We subdivided study group according to number of affected arteries from one to three. Physical and clinical data were noted in patient’s files and include body mass index (BMI) calculated as weight (kg) / height (m²), cigarette smoking, alcohol consumption, systolic and diastolic blood pressure and family history of cardiovascular disease. Seventy-two patients have hypercholesterolemia defined as increased levels in one or more lipid parameters (total cholesterol [TC] > 5.2 mmol/L; LDL-cholesterol [LDLc] > 3.40 mmol/L; TG > 1.70 mmol/L; HLD-cholesterol [HDLc] < 1.05 mmol/L) and use of hypolipemiant drugs. Hyperhomocysteinemia was defined as tHcy > 15 μmol/L. None of patients had infectious disease, renal or hepatic failure or was administrated any vitamin supplementation during a study period. All subjects accepted to participate to the study and they signified consent provided by the local committee of the study.

**Biological assays**

Blood samples were collected from subjects after 12 h of fast. TC, triglycerides (TG) and HDLc levels were measured by using a colorimetric enzymatic method in a Technicon automatic analyzer (RA-1000, Dade Behring, Germany). LDLc was calculated by Friedwald formula: LDLc = TC - (HDLc + TG/2.18) for TG < 4.5 mmol/L. High sensitive C reactive protein (HsCRP) levels were determined by immunonephelometric method on BNII Nephelometer Analyser (Dade Behring, Marbourg, Germany) and expressed in mg/L. Interleukin 6 (IL-6) and tumor necrosis factor (TNF-α) were determined by immunometric sequential chimiluminescent test on Immulite 1000 (Immulite DPC, Los Angeles, USA) and expressed in pg/mL. Blood samples were collected from subjects after 12 h of fast. TC, triglycerides (TG) and HDLc levels were measured by using a colorimetric enzymatic method in a Technicon automatic analyzer (RA-1000, Dade Behring, Germany). LDLc was calculated by Friedwald formula: LDLc = TC - (HDLc + TG/2.18) for TG < 4.5 mmol/L. High sensitive C reactive protein (HsCRP) levels were determined by immunonephelometric method on BNII Nephelometer Analyser (Dade Behring, Marbourg, Germany) and expressed in mg/L. Interleukin 6 (IL-6) and tumor necrosis factor (TNF-α) were determined by immunometric sequential chimiluminescent test on Immulite 1000 (Immulite DPC, Los Angeles, USA) and expressed in pg/mL. Vitamin B12 and folates were measured by the analyzer Immulite 2000 (Immulite DPC, Los Angeles, USA) based on a competitive immunoassay; levels expressed in μmol/L. Vitamin B12 and folates were measured by the analyzer Immulite 2000 (Immulite DPC, Los Angeles, USA) and expressed in pg/mL and folates in ng/mL.

**Statistical analysis**

Statistical analysis was carried out using SPSS for Windows 11.5 software (SPSS Inc., Chicago, IL, USA). Values were reported as means and standard deviation, or percents. The difference between groups was compared by independent samples t test for continuous variables and by Chi² tests for categorical variables. To evaluate the association between studied factors and number of affected arteries, the means were compared using ANOVA test. Statistical significance was set at P < 0.05. Multivariate analysis was also performed. To assess predictor factors of events (the probability to develop a new affected arteries), logistic regression was performed by backward elimination [9] and models verified with Hosmer-Lemeshow test and adjusted odds ratios were calculated. The dependent variable was the...
number of affected arteries dichotomized in one and two or more than two affected arteries and the independent variables were the significant variables in the univariate analysis.

Results

Baseline characteristics for patients showed elevated frequencies in hypertension, dyslipidemia, type 2 diabetes and cigarette smoking (59, 53, 48 and 58% respectively). Of 122 patients three of them were alcohol users (2.5%) and 25% had a familial history of cardiovascular disease.

Distribution of studied anthropometric risk factors according to the number of affected arteries was showed in Table 1. No statistically significant differences were found between patients with one (n = 50), two (n = 33) or three (n = 39) affected vessels. This result attenuates the effect of such anthropometric risks on the distribution of biological factors according to affected arteries.

Comparison of biological factors showed statistically significant differences between patients with one affected artery and those with three, in tHcy (P < 0.001), folated (P < 0.001), IL-6 (P < 0.001), TNF-α (P < 0.001), HsCRP (P < 0.01), TC (P < 0.01), LDL (P < 0.05) and Lp (a) (P < 0.01). A similar result was found for patients with two arteries compared to those with three arteries who represent elevated levels (Table 2).

As no difference was found between patients with one and those with two affected arteries, we regrouped these
patients with one and two affected arteries. For biological markers, we found statistically significant elevated levels of tHcy (<i>P</i> < 0.001), IL-6 (<i>P</i> < 0.001), TNF-α (<i>P</i> < 0.001), HsCRP (<i>P</i> < 0.001), TC (<i>P</i> < 0.01), LDLc (<i>P</i> < 0.05) and Lp (a) (<i>P</i> < 0.001) but low folates (<i>P</i> < 0.01) were independent risk factors for recurrent ACS in a free form [15]. In our study, we found that Hcy concentration at 17.8 μmol/L can predict development of new affected artery in patients with ACS. Facila <i>et al.</i> [16] showed that moderately raised plasma tHcy concentration measured at admission is a strong predictor of all cause mortality in patients admitted with non-ST segment elevation ACS. The mechanism by which tHcy may promote atherosclerosis is unclear, but implication of inflammatory markers was recently involved. Recently, tHcy was demonstrated to contribute to the initiation and progression of vascular disease by activating monocytes, resulting in the secretion of cytokines that amplify the inflammatory response [17]. The demonstration of a relationship between tHcy, inflammation and autoimmunity intriguingly expands the spectrum of the possible pathogenic implications for hyperhomocysteinemia in the course of arteries disease [18]. In our study, inflammatory markers were elevated in patients with multi-affected arteries. After the determination of new cut-off, we found that only tHcy, IL-6, TNF-α and HsCRP had independently effects on the severity of the disease. In the literature, enhanced inflammation may be associated with homocysteine-related cardiovascular disease, possibly involving IL-6-related mechanisms [19]. High circulating concentrations of IL-6 are independent correlates of hyperhomocysteinemia and may explain, at least in part, the association between tHcy and atherosclerosis [20]. Evidence has been obtained for the presence of TNF-α and IL-6 in atherosclerotic lesions [21, 22]. In fact, in response to infection or tissue inflammation, IL-6 and TNF-α stimulate the production of CRP. IL-6 is a regulator of CRP and has a key role in initiation of inflammation. CRP attracts monocytes, activates complement and induces the decrease of endothelial function [23]. High sensitive CRP [24] and IL-6 are considered new biomarkers of CVD and increased IL-6 and HsCRP levels are strongly associated

### Table 3. Comparison of biological parameters after regrouping patients with one and two affected arteries.

<table>
<thead>
<tr>
<th>Number of diseased vessels</th>
<th>One or two arteries (&lt;i&gt;n&lt;/i&gt; = 83)</th>
<th>Three arteries (&lt;i&gt;n&lt;/i&gt; = 39)</th>
<th>&lt;i&gt;P&lt;/i&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (μmol/L)</td>
<td>15.0 ± 5.2</td>
<td>23.6 ± 10.4</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fol (ng/mL)</td>
<td>7.1 ± 3.2</td>
<td>5.2 ± 2.6</td>
<td>0.002</td>
</tr>
<tr>
<td>VitB12 (pg/mL)</td>
<td>382 ± 229</td>
<td>318 ± 222</td>
<td>NS</td>
</tr>
<tr>
<td>IL6 (pg/mL)</td>
<td>9.2 ± 5.9</td>
<td>16.5 ± 10.1</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>8.5 ± 4.3</td>
<td>17.0 ± 8.0</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>HsCRP (g/L)</td>
<td>12.8 ± 8</td>
<td>20.12 ± 12</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.3 ± 1.3</td>
<td>5.15 ± 1.15</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>0.97 ± 0.29</td>
<td>0.88 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.78 ± 1.15</td>
<td>3.35 ± 1.18</td>
<td>0.012</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.52 ± 1.1</td>
<td>1.31 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA (g/L)</td>
<td>1.23 ± 0.24</td>
<td>1.23 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.00 ± 0.31</td>
<td>0.97 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Lp (a) (g/L)</td>
<td>0.31 ± 0.27</td>
<td>0.49 ± 0.17</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 4. Adjusted odds ratios for significant risk factors.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio</th>
<th>IC 95%</th>
<th>&lt;i&gt;P&lt;/i&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy (μmol/L)</td>
<td>1.14</td>
<td>1.04-1.25</td>
<td>0.006</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>1.27</td>
<td>1.13-1.44</td>
<td>10&lt;sup&gt;−3&lt;/sup&gt;</td>
</tr>
<tr>
<td>HsCRP (g/L)</td>
<td>1.09</td>
<td>1.03-1.16</td>
<td>0.005</td>
</tr>
<tr>
<td>IL6 (pg/mL)</td>
<td>1.15</td>
<td>1.06-1.25</td>
<td>0.001</td>
</tr>
</tbody>
</table>

This study showed that patients with multi-affected arteries presented significantly elevated mean levels of studied risk factors and that Hcy, TNF-α, HsCRP and IL-6 were independent predictor factors for developing new affected arteries in acute coronary syndrome. Several epidemiological studies have identified moderately raised concentrations of tHcy as a potentially modifiable risk factor for coronary heart disease [10-12] and may contribute to the development of atherosclerosis [13]. One of the major causes of hyperhomocysteinemia is deficiency in vitamin B12 and folates, important cofactors in Hcy metabolism. In our study, patients with multi-affected arteries have lower folates and vitamin B12 levels. Elevated Hcy level was demonstrated to be predictor of next cardiac event in patients with ACS [14] and represents an independent risk for recurrent ACS in a free form [15]. In our study, we found that Hcy concentration at 17.8 μmol/L can predict development of new affected artery in patients with ACS.
with the inflammatory system and the course of clinical and hemodynamically significant CAD [25]. HsCRP concentrations are significantly correlated to clinical symptoms in patients having acute coronary syndrome and by this way help for risk stratification [26]. Consistent findings showed that plasma tHcy is associated with CRP in the Framingham Heart Study [27] and in the Physician’s Health Study [24]. Importantly, elevated IL-6 levels are observed in subject with elevated they levels but without hypercholesterolemia [17]. Vascular endothelium damaged by Hcy [28] and cells of the immune system stimulated by Hcy are most likely a source of these cytokines [29]. Additionally, adipose tissue, mainly visceral, is a site of regular expression and production of such cytokines as TNF-α and IL-6. Plasma tHcy is a determinant of TNF-α in hypertension and that obesity or a history of vascular events aggravates inflammation in patients with hypertension. A positive correlation between tHcy and TNF-α suggests a mechanism underlying the pro-atherogenic properties of tHcy [29]. As demonstrated in many studies, lipid profile have also important role in atherosclerosis. The interaction between lipid and inflammation processes defines the principal pathogenesis. In fact, lesions have a large lipid core with signs of active inflammation and macrophage accumulation at the site of plaque rupture [30]. In the present study, we found elevated LDL but lower HDL levels in patients with multi-affected arteries. Korhonen et al. [30] demonstrated that high levels of LDL are associated with an increased risk of CAD, and the more vessels obstructed, the higher the serum LDL level. Epidemiological surveys have observed that elevated levels of LDLc are associated with increased risk of coronary heart disease [31]. In fact, an increased proportion of small, dense LDL particles may be more susceptible to oxidative modification [32, 33]. Although HDLc levels did not represent a significant protective effect in our study, it must be implicated in the screening of patients at higher risk as we found any that 59% of patients had lower HDLc levels. Although ApoB levels is demonstrated to be superior to LDLc as an index of lipid related risk of vascular disease [34] and the ApoB/A ratio is strong predictor of coronary events in middle aged men and women [35], we did not found variation in ApoA and ApoB levels according to affected arteries. Significant elevated Lp(a) levels were observed; in fact, Lp(a) concentrations appeared linked to CHD across a wide spectrum of epidemiologic studies [36].

Conclusion

Homocysteine and inflammatory markers appear to play an important role in the initiation of ACS. Despite the impressive gains in understanding atherosclerosis, many questions persist about the use of these markers in clinical practice. Investigation of the relationship between the different markers will be informative in a large number of cases but we need a well-designed evaluation of these markers.

Acknowledgments. This work was supported by grant from the Ministry of Defense Military hospital of Tunis Department of Biochemistry. We would like to thank all participants to the study.

Conflicts of interest: none.

References


