Assessment of Three Inflammatory Markers of Cardiovascular Diseases with a Special Accent on C-Reactive Protein

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Abstract

Background: Elevated levels of CRP, myoglobin and creatine kinase are always associated with pathological changes and hence their values give useful information for exact diagnosis and therapy. They are helpful in monitoring the inflammatory processes and associated diseases.

Aim: The aim of this study was to determine the usefulness and practical value of application of CRP detection by comparing it with the results obtained for the classical enzymes – markers of myocardial damage, myoglobin and creatine kinase isoenzyme MB (CK-MB) in pts with acute coronary syndrome (ACS), pts with chronic coronary artery disease (CAD) and in healthy individuals.

Material and Methods: Sera were taken from a total of 152 individuals (78.9% males, 21.1% females, mean age 61.87 ± 10.32 years). The subjects were divided in three groups: 63 pts with ACS; 52 pts CAD and a group of 36 conditionally healthy individuals. Analysis of patients’ sera for presence of markers for myocardial damage: myoglobin, CK-MB along with determination of CRP level was done on the Immulite system, DPC (Diagnostic Products Corporation), Los Angeles, USA.

Results: Comparison of examined biomarker’s values in pts divided according to diagnosis showed statistically significant higher levels in patients with ACS vs. others. As for biomarker’s cut-off values, out of all CK-MB \( \geq 5.7 \) ng/mL was found in 34 (53.1%) pts with ACS with significant difference among the groups in favor of its higher values in pts with ACS (p=0.0001). Out of all, myoglobin \( \geq 25 \) ng/mL was found in 54 (84.4%) pts with ACS without significant difference among the groups. As for CRP, value of \( \geq 3 \) mg/L was found in 39 (60.9%) pts with ACS and there was significant difference among the groups in favor of higher values in pts with ACS (p=0.001). There was significant positive correlation among the levels of examined three biomarkers: CK-MB in correlation to myoglobin (r=0.460; p=0.0001) and to CRP level (r=0.204; p=0.009), as well as myoglobin to CRP level (r=0.218; p=0.005).

Conclusion: We could conclude that determination of CRP levels is a valid test for detection of acute coronary artery disease in addition to the classical, standard markers for myocardial damage.

Introduction

Atherosclerosis has many characteristics of a chronic infection. It is a well-known fact that there is inflammatory process in all stages of atherosclerosis. Inflammatory cells, macrophages and T lymphocytes play a key role in its pathogenesis. Patients with acute coronary syndrome have increased levels of C-reactive protein, amyloid A, interleukin 6, as well as myocardial markers such as myoglobin and creatine kinase. Major characteristics of chronic infections are increased values of cytokines and activation of macrophages, which is a response of the organism to the tissue damage.
Basic Science

Certain examinations are employed in determination and assessment of the infection, tissue damage and identification of the risk for cardiovascular diseases. Elevated levels of CRP, myoglobin and creatine kinase are always associated with pathological changes and hence their values give useful information for exact diagnosis and therapy. They are helpful in monitoring the inflammatory processes and associated diseases.

Inflammation is a recognized key pathogenic mechanism in atherosclerosis. Clinical and epidemiological investigations have shown results for CRP measured with high sensitivity CRP (hsCRP) to be a test of choice for determination of the inflammatory markers in atherosclerosis. The result obtained has a prognostic value, especially in patients with acute coronary syndrome and is a strong independent predictor of future coronary events [1-4].

Myoglobin, the smallest of all markers, diffuses rapidly in the vascular system and quickly gives indication for a possible acute myocardial infarction (AMI). Myoglobin levels increase within 0.5 -2 hours from the onset of chest pain, reach peak values at 5-12 hours. Normal circulatory concentration might be detected after 16-36 hours [5-7].

CK-MB is one of the most important myocardial markers, and it is an established marker in confirmation of AMI. In AMI plasma concentration of CK-MB is increased within 3-8 hours of onset of chest pain, the peak is reached within 9-30 hours and return to baseline levels after 48-72 hours [8-10].

Currently, it is of critical importance to reduce the time between the onset of chest pain and hospital admission. What is of great importance is determining the most sensitive and usefull marker, helpful in finding the best, most effective treatment of the patients with ACS.

The aim of this study was to determine the usefulness and practical value of application of CRP detection by comparing it with the results obtained for the classical enzymes – markers of myocardial damage, myoglobin and CK-MB.

Material and Methods

Sera were taken from a total of 152 individuals, 120 (78.9%) males, and 32 (21.1%) females. The subjects were divided in three groups: the group of 63 patients with acute coronary syndrome (ACS) comprising patients with the following diagnosis: unstable angina pectoris; acute myocardial infarction (AMI) with and without ST segment elevation; 52 patients with chronic coronary artery disease (CAD) and a group of 36 conditionally healthy individuals- control group. The mean age (± SD) of the subjects was 61.87 ± 10.32 years. Diagnosis of the patients was made at the University Clinic of Cardiology in agreement with the protocol given in the evidence-based guidelines.

Analysis of patients’ sera for presence of markers for myocardial damage: myoglobin, CK- MB along with determination of CRP level was done on the Immulite system, DPC (Diagnostic Products Corporation), Los Angeles, USA. The entire process is automated in the Immulite system. Bound antibodies are quantified with dioxetane substrate that produces light. Light emission is detected by a photomultiplying tube, and the report for each sample is generated on the computer of the system.

Interpretation of the results

Levels of CRP in relation to cardiovascular events are interpreted as follows: level < 1mg/L is a low risk for cardiovascular disease; level 1-3 mg/L is a medium risk for cardiovascular disease; level >3 mg/L is a high risk for anticipation of cardiovascular disease. If the obtained value is > 25 ng/mL, it is considered to be an elevated level of myoglobin. If the level is > 5.7 ng/mL, it is considered to be an increased value of the enzyme CK-MB.

Statistical analysis

All data are expressed as mean ± standard deviation (SD) and percentages. Comparison between numeric variables was performed using unpaired T-test or Pearson Chi-square test as well as for more than two groups analysis of variance (ANOVA) was used followed by post-hoc multiple comparison analysis. Correlation of selected variables was estimated using Spearman correlation coefficient. For all test, a p value <0.05 is considered statistically significant. All statistical analysis was performed using statistical software SPSS, version 15.

Results

Analysis of the levels of examined biomarkers and its comparison in pts divided according to the diagnosis (Table 1) revealed significant difference concerning the CK-MB and myoglobin levels in ACS pts in
comparison to healthy individuals (p=0.0001; p=0.001, respectively) and those with CAD (p=0.0001). There was no significant difference between healthy individuals and CAD pts (p=0.963; p=0.994, respectively). As for CRP level, significant difference was also found between pts with ACS and healthy individuals (p=0.006) as well as those with CAD (p=0.024). There was no significant difference between healthy individuals and CAD pts (p=0.464).

Table 1. Levels of biomarkers according to diagnosis and its comparison.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>0 (n=36)</th>
<th>1 (n=64)</th>
<th>2 (n=52)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>2.05 ± 1.70</td>
<td>8.98 ± 146.65</td>
<td>3.00 ± 2.62</td>
<td>0.0001</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>36.56 ± 23.69</td>
<td>131.01 ± 210.55</td>
<td>36.79 ± 23.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>CRP</td>
<td>3.04 ± 3.98</td>
<td>10.07 ± 16.09</td>
<td>4.95 ± 9.51</td>
<td>0.010</td>
</tr>
</tbody>
</table>

0= healthy individuals; 1=ACS; 2=CAD.

Out of all, CK-MB ≥ 5.7 ng/mL was found in 40 (26.32%) examined individuals: 34 (53.1%) with ACS, 5 (0.1%) with chronic CAD and in 1 (0.03%) controls (Figure 1). There was significant difference among the groups in favor of higher values of CK-MB in patients with ACS (p=0.0001).

Out of all myoglobin ≥ 25 ng/mL was found in 118 (77.63%) examined individuals: 54 (84.4%) with ACS, 40 (76.9%) with chronic CAD and in 24 (66.7%) controls. We could not find significant difference among the groups.

Out of all CRP ≥ 3 mg/L was found in 67 (44.07%) examined individuals: 39 (60.9%) with ACS, 18 (34.6%) with chronic CAD and in 10 (2.8%) controls (Figure 2). There was significant difference among the groups in favor of higher values of CRP in patients with ACS (p=0.001).

Table 2: Comparison of CK-MB and CRP levels in examined individuals according to the myoglobin cut-off value.

<table>
<thead>
<tr>
<th>Myoglobin</th>
<th>&lt; 25 ng/mL</th>
<th>≥ 25 ng/mL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>2.09 ± 1.10</td>
<td>49.60 ± 115.92</td>
<td>0.018</td>
</tr>
<tr>
<td>CRP</td>
<td>7.47 ± 19.11</td>
<td>6.42 ± 9.59</td>
<td>NS (0.661)</td>
</tr>
</tbody>
</table>

Comparison of CK-MB, myoglobin and CRP levels in patients divided according to its cut-off values revealed (Tables 2 and 3) significant higher levels for CK-MB (p=0.018) in patients with myoglobin ≥ 25 ng/mL in comparison with those with < 25 ng/mL as well as significant higher levels for myoglobin (p=0.0001) in patients with CK-MB ≥ 5.7 ng/mL in comparison with
those with < 5.7 ng/mL. As for CRP level (Table 4), significant higher levels of CK-MB was found in the group of pts with CRP levels <1 mg/L in comparison to those with CRP > 3 mg/L (p=0.004). The difference was low comparing CK-MB levels in patients with CRP < 1 mg/L and CRP 1-3 mg/L (p=0.055) and there was lack of significant difference (p=0.493) between pts with CRP 1-3 mg/L and CRP >3 mg/L. Significant difference was present concerning the comparison of myoglobin levels (Table 4) in patients with CRP <1 mg/L and those with CRP >3 mg/L (p=005). There was no significant difference in the myoglobin levels in patients with CRP < 1 mg/L and CRP 1-3 mg/L, (p=0.233) as well as those with CRP 1-3 mg/L and CRP >3 mg/L (p=0.493).

Table 4: Comparison of CK-MB and myoglobin levels in patients according to the CRP cut-off values.

<table>
<thead>
<tr>
<th>CRP</th>
<th>&lt; 1 mg/L (n=47)</th>
<th>1-3 mg/L (n=38)</th>
<th>&gt;3 mg/L (n=67)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td>3.09 ± 2.76</td>
<td>45.99 ± 134.51</td>
<td>60.17 ± 114.77</td>
<td>0.013</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>33.40 ± 15.92</td>
<td>70.52 ± 106.75</td>
<td>109.92 ± 197.45</td>
<td>0.019</td>
</tr>
</tbody>
</table>

There was significant positive correlation among the values of examined three biomarkers: CK-MB in correlation to myoglobin (r=0.460; p=0.0001) and to CRP value (r=0.204; p=0.009), as well as myoglobin to CRP value (r=0.218; p=0.005).

Discussion

The present study showed, that the level of all three examined biomarkers are significantly higher in pts with acute coronary syndrome (ACS) in comparison to those with chronic coronary artery disease (CAD) and healthy individuals with a lack of significance between later, giving possibility of making difference between pts with acute and chronic disease. Regarding its cut-off values we could not find difference of CRP level between lowest and highest levels of CK-MB and myoglobin, although significant correlation among biomarkers was observed, thus emphasizing the importance of determination of this marker along with the other markers of myocardial damage.

Over the last decade new knowledge has emerged about atherosclerosis and its onset and development based on the growing evidence about the important role of the inflammatory cells such as the effect of monocytes on the walls of arteries [11, 12]. Inflammation is considered to be one of the major factors that contribute to pathogenesis of atherosclerosis. One of the principal issues set by the cardiologists is whether inflammatory markers can be used for prediction of the clinical course in cardiology patients. In 1982, de Beer et al. [14] showed that subjects with myocardial infarction developed elevated values of CRP.

In 1992, Berk et al. [15] observed significantly different values of CRP in patients with unstable angina pectoris when compared with values found in subjects with stable angina pectoris. Two years later, Luizzo et al. [16] demonstrated that elevation of CRP and serum amyloid A on hospital admission of patients predicted a bad outcome in patients with unstable angina pectoris. In 1977, Rodker et al. [17] showed that CRP values might be a sign for prediction of the risk for myocardial infarction or cerebrovascular insult in apparently healthy individuals [18].

CRP was initially isolated as a protein, which binds for C-polysaccharide of pneumococcal cell wall [19, 21]. Its value can be increased 100 times within 24, 48 hours during inflammatory processes. It has been proved that CRP is bound to damaged tissues, to nuclear antigens, to lipoproteins and to apoptotic cells. It also participates in the complement activation and tissue damage [19, 20]. CRP is found in atherosclerotic plaques, but not in the normal wall of the blood vessels [21, 22] and CRP deposits in the early atherosclerotic lesions might have monocytes as well. It has been shown that CRP can induce expression of adhesion molecules and chemokines in human endothelial cells [23-25]. There is evidence that CRP acts synergistically with lipopolysaccharides by activation of endothelial cells. CRP could also act in synergism with lipopolysaccharides in initiation of production of tissue factor by the monocytes [26]. Having this in mind, CRP is not only a marker of inflammation, but its amplifier, too.

Chew et al. [25] in their study advises the clinical doctors and general practitioners to pay attention to atherosclerosis with its characteristics as an inflammatory disorder as well as to take into consideration the clinical status of the patient prior to undertaking any intervention. Special approach is also recommended for patients with elevated serum values of CRP [1, 3-5, 7, 9].

Limitation of the study: This study does not have a large examined group. There are large variations in biomarker levels in ACS patients which is expected because patients with and without ST-segment elevations have been incorporated giving possibilities of underestimation and/or overestimation of the results.

Conclusion: Clinical and laboratory investigations...
shows that determination of CRP levels as inflammatory marker of atherosclerosis in addition to the classical, standard markers for myocardial damage is a valid test for detection of coronary artery disease. The results have a considerable value, especially in patients with acute coronary syndrome.

References


