Evaluation of IL-6 (-174 G/C) Polymorphism in Acute Coronary Syndrome in the Northeast of Brazil

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Abstract

Background: Acute coronary syndrome (ACS) is a leading cause of morbidity and mortality worldwide. It is a multifactorial disease caused by obstruction of the coronary arteries by atheromatous plaques and leads to heart ischemia. Several studies suggest that some genetic polymorphisms change the cytokines levels and influence ACS development.

Objective: In this study, we evaluated the IL-6 (-174G/C) polymorphism, serum levels of cytokine and its relationship with ACS and the thrombolysis in myocardial infarction (TIMI) risk score.

Materials and Methods: A sample of 200 patients with ACS [TIMI risk – Low (70); Intermediate (89); High (41)] in Brazilian population was used. Genotyping was carried out by polymerase chain reaction, followed by DNA sequencing.

Results: There was no significant differences in genotype (p = 0.53) and allele (p = 0.32) distributions between ACS patient and without ACS patients groups on IL-6 allelic polymorphism and between the three different TIMI risk score (p > 0.05). Moreover IL-6 polymorphism did not affect the cytokine levels and these levels were not related to TIMI score.

Conclusions: With these results, we suggest that the IL-6 (-174 G/C) polymorphism, until now, is not related to ACS and did not change the levels of the cytokine in studied population. Further studies with different populations should be done to verify those results. It is important to emphasize that, since ACS is a multifactorial disease, other risk factors and other pro-inflammatory cytokines should be assessed to better understand this pathology. (Int J Cardiovasc Sci. 2016;29(4):288-294)

Keywords: Acute Coronary Syndrome; Myocardial Ischemia; Polymorphism, Genetic; Interleukin-6.

Introduction

Acute coronary syndrome (ACS) is a cardiovascular disease of great importance in the world due to its high mortality, resulting in 30% of all deaths worldwide. ACS includes acute myocardial infarction (AMI) and unstable angina (UA) caused by obstruction of the coronary arteries by atheromatous plaques leading to myocardial ischemia. ACS patients can be classified according to the risk of death by TIMI score. This classification selects patients at low, intermediate or high risk of death, according to clinical, electrocardiographic abnormalities and myocardial injury biomarkers.

This disease has a multifactorial phenotype determined by genetic factors and influenced by risk factors such as...
as age, gender, smoking, diabetes, arterial hypertension and dyslipidemia.\(^5\)

In fact, there is evidence that genetic markers may be related to the susceptibility, expression and outcome of ACS, including therapeutic response.\(^6\) The single nucleotide polymorphism (SNP) in certain gene regions has been associated with some diseases.\(^5,6\)

SNP -174 G/C (rs1800795) in promoter region of interleukin-6 (IL-6) gene has been associated with ACS.\(^9,10\) Some authors argue there is an increased transcriptional activity in G allele presence and high levels of serum IL-6 with consequent exacerbation of inflammatory response.\(^9,11\) For others, the variant allele C is responsible for increasing IL-6 serum levels,\(^10,12\) and CC genotype associated with cardiovascular disease.\(^12\)

Because of the controversies found in the relationship between IL-6 gene and ACS, studies about these polymorphisms are an important tool in primary prevention of this disease. Therefore, the aim of this study was to evaluate the association between IL-6 polymorphism and risk of ACS.

### Materials and methods

#### Population study

In a prospective, analytical and cross-sectional study comparing groups, 200 patients (mean age 61.8 ± 10.3) with ACS admitted to Real Hospital Português (RHP), in Recife - Pernambuco, Brazil from 2012 to 2015 were recruited. Patients on anti-inflammatory drugs treatment, with recent trauma, infectious process or cancer were excluded from this study.

The second group consisted of 50 patients (mean age 58 ± 15.9) admitted to the RHP but without ACS diagnostic.

Risk factors and TIMI score data were collected. Ethics Committee of RHP approved the study (CAAE 03187512.2.0000.5202), according to Declaration of Helsinki. All participants signed informed consent forms. Adjustment for ethnicity was not performed, since previous studies about Brazilian populations showed that skin color or self-referred ethnic origin are not accurate biomarkers of ancestry in Brazil.\(^13\)

#### Genotyping

After DNA extraction with “illustra genomicPrep blood Mini Spin kit”, amplification of IL-6 gene was performed by polymerase chain reaction (PCR) with Platinum Taq DNA polymerase (Invitrogen Life Technologies).

The following primer pairs were used: Forward 5’AGC CTC AAT GAC GAC AGC ATC3’ and Reverse 5’GTC ACT GGA TGA GAT GGC TCA TT3’. Amplification conditions were: initial denaturation (94°C for 2 minutes), then 35 cycles consisting of denaturation (94°C, 1 minute), annealing (65°C, 1 minute), extension (68°C, 1 minute) and a final extension of 68°C for 5 minutes. As a negative control, reagents without DNA were used. The fragments were visualized on 1% agarose gel and submitted to DNA sequencing at Núcleo de Plataformas Tecnológicas from Centro de Pesquisas Aggeu Magalhães (CPqAM), using the ABI 3100 Genetic Analyzer (Applied Biosystems, USA) to display the alleles.

#### Serum levels of IL-6

The IL-6 cytokine was measured in patients serum by enzyme linked immunosorbent assay (ELISA; Quantikine kit R&D Systems, Minneapolis, MN) according to manual instructions (minimum detectable < 0.70 pg/mL).

#### Statistical analysis

Chi-square test (χ\(^2\)) was used to verify the Hardy-Weinberg equilibrium. Differences in genotype and allele frequencies between groups were compared using Williams G test. Odds ratios (ORs) and 95% confidence interval (CI) were also calculated. A Multinomial logistic regression was used, with low risk TIMI as a reference, to compare genotype and allelic distribution among the TIMI score. To compare IL-6 plasma levels between groups, Kruskall Wallis test was used. BioEstat software version 5.3 (Belem, PA, Brazil)\(^14\) was used. Data were considered statistically significant when p value < 0.05.

#### Results and discussion

In this study, mean age was 67.5 (± 14.2) and 60 (± 12.2) years old for women and men, respectively. According to Magee et al. (2012), mean age considered risk for ACS is from 60 years for men and 70 years for women, which is in agreement with our results. For Overbaugh,\(^16\) however, the average age of risk is from 45 years for men and 55 for women.

Most patients were male: 56.0% in without ACS group and 76.5% in ACS group (p = 0.006) (Table 1).
These results confirm the data reported in the literature\(^5,\)\(^17\) that suggests that males are more likely to develop cardiovascular disease. The higher prevalence of men in the ACS group, as found here and in a study by Lemos et al. (2010)\(^5\) (63.8%), may be due to the fact that women tend to take better care of their health, and men are more frequently exposed to some risk factors for ACS, such as smoking and obesity.\(^15\) In addition, the female estrogen hormone seems to confer greater protection against atherosclerosis.\(^17\)

While smokers, diabetics and dyslipidemic individuals represented 10.0%, 26.0% and 15.2% of the group without ACS, respectively, these values were higher in the ACS group (30.5%, 45.5% and 63.0%, respectively), with significant differences between groups (\(p = 0.01, p = 0.02\) and \(p < 0.0001\), respectively) (Table 1).

A study conducted by Piegas et al. (2013),\(^18\) with 2,693 Brazilian patients with ACS, a profile of 26.5% of smokers, 27.8% of diabetics and 45.7% of dyslipidemic was found. Although they are considered classic risk factors for cardiovascular disease, it is noted in this study that most patients with ACS were non-smokers and non-diabetics, indicating that other factors are involved in this disease.

The importance of dyslipidemia, presented in 63% of ACS patients in this study, is due to the fact that cholesterol can be deposited in the vascular wall and is a potential inflammatory and atherogenic molecule.\(^19\)

Most individuals with ACS and without ACS have arterial hypertension: 78.5% and 69.6%, respectively (\(p = 0.27\)). Also in the study by Piegas et al. (2013),\(^18\) hypertension was found in 69.8% of patients. This risk factor, even alone, is a powerful predictor of cardiovascular disease.\(^20\) A study performed by Rhéaume et al. (2014),\(^21\) that followed 9,580 men and 12,250 European women for 11 years, reported that hypertension patients had approximately 3.0 times greater risk of coronary heart disease.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACS ((n = 200))</th>
<th>Without ACS ((n = 50))</th>
<th>OR</th>
<th>95% CI</th>
<th>(p)</th>
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<td>Gender</td>
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<td>22</td>
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<td>139</td>
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<td>Yes</td>
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<td>5</td>
<td>3.6</td>
<td>1.35-9.55</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>91</td>
<td>12</td>
<td>2.36</td>
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<td>14</td>
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<tr>
<td>Yes</td>
<td>157</td>
<td>32</td>
<td>1.59</td>
<td>0.78-3.25</td>
<td>0.27</td>
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<td><em>Dyslipidemia</em></td>
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<td>Yes</td>
<td>126</td>
<td>7</td>
<td>9.36</td>
<td>3.98-21.98</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

\(n\): number of studied patients; OR: odds ratio; CI: confidence interval; \(p < 0.05\); *Four patients with no information were excluded from statistics analysis.
The studied population was in Hardy-Weinberg equilibrium (p > 0.05). The genotype frequencies of ACS patients were: 109 (54.4%) GG, 80 (40.0%) GC and 11 (5.5%) CC. The C allele frequency was 25.5%. The group of patients without ACS had the following distribution: 23 (46.0%) GG and GC, 4 (8.0%) CC and 31.0% of C allele. Genotype frequencies of C allele carriers (GC + CC) did not differ from GG (p = 0.35) patients (Table 2).

Similarly, Ghazouani et al. (2001), studying 418 patients, found no association between IL-6 genotypes and the risk of coronary artery disease (CAD) in Tunisian patients. In contrast, Satti et al. (2013), in a study with 36 Pakistani patients and Tonet et al. (2008) with 175 patients from Central West region of Brazil, found that there is an association between the IL-6 polymorphism and the risk of CAD development, demonstrating the importance of study in specific populations. Even though these two studies present risk association, the low sample size used suggests more studies to confirm the results.

The median IL-6 serum levels produced by ACS patients did not differ between GG and C carriers patients (18.91 pg/ml, 12.56 pg/ml, respectively; p = 0.21 - Kruskal-Wallis test). Similar results were found by Nauck et al. (2002) in a study conducted with 942 German patients. This could possibly be explained by localized tissue-specific IL-6 production whereas systemic IL-6 concentrations remain unaffected. While Satti et al. (2013) found that the CC genotype was associated with high IL-6 serum levels in Pakistani patients and that this genotype was associated with an increased risk of CAD, Tonet et al. (2008) found association between this disease and G allele in Brazilian patients.

From the 200 ACS patients in our study, 70 were low TIMI risk, 89 intermediate risk and 41 high risk. The genotype frequencies of low-risk patients were: 45 (64.3%) GG, 21 (30.0%) GC, 4 (5.7%) CC and 20.7% of C allele. For intermediate risk patients, the frequencies were: 42 (47%) GG, 41 (46.1%) GC, 6 (6.7%) CC and 29.8% of C allele. Finally, the high-risk group, 22 (53.6%) presented the GC genotype, 18 (44.0%) GC, 1 (2.4%) CC genotype and 24.4% of C allele.

When the low risk group was used as reference in a multinomial logistic regression, carriers of C allele (GC + CC) were more frequent in the intermediate risk group (52.8%) than in the low risk group (35.7%) (p = 0.046) (Table 3). Despite that, serum median levels of IL-6 in low, intermediate and high TIMI risk groups were similar (13.5 pg/mL, 16.20 pg/mL and 18.65 pg/mL, respectively; p = 0.83 - Kruskall-Wallis test) and were not influenced by genotype.

Moreover, no association was found between IL-6 serum levels and ACS, but carriers of C allele patients appeared more frequently in intermediate TIMI risk group than in low group. Wypasek et al. (2010) showed that CAD patients carrying C allele may produce high serum levels of C-reactive protein, an important liver protein that also influences the inflammatory process and may increase the relative risk of CAD.

In fact, since this disease is multifactorial, other variables such as risk factors and the study of more

<p>| Genotype frequencies of patients with acute coronary syndrome (ACS) and without ACS |
|-----------------------------------------------|----------------------|----------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>ACS (n = 200)</th>
<th>Without ACS (n = 50)</th>
<th>p*</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<tr>
<td>IL6 (rs1800795)</td>
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<tr>
<td>GG</td>
<td>109</td>
<td>54.5</td>
<td>23</td>
<td>46.0</td>
<td>0.53</td>
<td>1</td>
</tr>
<tr>
<td>GC/CC</td>
<td>91</td>
<td>45.5</td>
<td>27</td>
<td>54.0</td>
<td>0.35</td>
<td>0.71</td>
</tr>
<tr>
<td>Alleles</td>
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</tr>
<tr>
<td>G</td>
<td>298</td>
<td>74.5</td>
<td>69</td>
<td>69.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>102</td>
<td>25.5</td>
<td>31</td>
<td>31.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: number of studied patients; p: Williams G-test; OR: odds ratio; CI: confidence interval; p < 0.05.
pro-inflammatory cytokines at the same time are important for the understanding of this pathology. Because of all the setbacks involving cytokine polymorphisms and development of diseases, it notable that the populations need to be evaluated individually. It is important to emphasize that no isolated polymorphisms marker gives the diagnosis or exclusion of ACS, and their presence must always be considered within the clinical context.

This is the first study that verifies whether there is an association between IL-6 genotype distribution and the TIMI risk. Thus, further studies are needed to allow a comparison between the results.

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Author contributions

Conception and design of the research: Carvalho VCV, Werkhauser RP, Montenegro ST, Silva CGR, Morais CNL, Montenegro SML. Analysis and interpretation of the data: Carvalho VCV, Silva LCA, Werkhauser RP, Montenegro ST, Silva CGR, Gomes AV, Morais CNL, Montenegro SML. Statistical analysis: Carvalho VCV, Werkhauser RP, Gomes AV, Montenegro SML. Obtaining financing: Werkhauser RP, Silva CGR, Morais CNL, Montenegro SML. Writing of the manuscript: Carvalho VCV, Silva CGR, Montenegro SML. Critical revision of the manuscript for intellectual content: Carvalho VCV, Werkhauser RP, Montenegro ST, Silva CGR, Gomes AV, Morais CNL, Montenegro SML.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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