Low serum levels of CCL2 are associated with worse prognosis in patients with Acute Coronary Syndrome: 2-year survival analysis

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ABSTRACT

Inflammation is very important in Acute Coronary Syndrome (ACS) as well as in cardiac remodeling after an acute myocardial infarction (MI). Our study examined the prognostic value of Chemokine (C-C motif) ligand 2 (CCL2) in patients with ACS in the ERICO (Strategy of Registry of Acute Coronary Syndrome) study. We evaluated serum samples from 803 patients. The prognostic value of CCL2 was evaluated at the 2-year follow-up, according to cutoff points established by the median. Kaplan-Meier curves and Cox regression were used for analysis of all-cause mortality, cardiovascular mortality, and a combined outcome of fatal myocardial infarction or new non-fatal MI. There were 115 deaths from all causes, 78 deaths due to cardiovascular causes and 67 events in combined outcomes. CCL2 levels below the median (≤100.9 pg/mL) were associated with increased risk of MI death or new non-fatal MI, even after model adjustment. Low serum levels of CCL2 shows a significant association with fatal or new non-fatal MI.

1. Introduction

Acute Coronary Syndrome (ACS) is primarily caused by the formation of a thrombus or by sudden hemorrhage from an atherosclerotic plaque that results in partial or complete occlusion of the coronary artery, interrupting the blood supply to the heart muscle [1,2]. ACS is the most severe manifestation of Coronary Artery Disease (CAD), due to its high prevalence and worse prognosis [3]. Advances in prevention strategies and acute treatments have promoted a reduction in mortality from ACS. Risk scores have been elaborated, and several biomarkers have been studied to aid clinical decision making [4,5].

Inflammation contributes to many of the characteristics of the plaques involved in the pathophysiology of ACS, including regulation of the thrombogenic potential of atherosclerotic plaques [2,6,7]. It is known that macrophages are cells of great importance in the pathophysiology of ACS, acting at all stages of atherosclerosis and in the complications arising from it [6,8]. These cells also play an essential role in cardiac repair, being defects in this process associated with complications after infarction [9]. The Chemokine (C-C motif) ligand 2 (CCL2) is one of the main factors involved in the initiation of inflammation [10].

CCL2 is secreted by fibroblasts, endothelial cells, smooth muscle cells, monocytes, T cells and other cell types that mediate the influx of cells into the sites of inflammation [10,11]. It promotes monocyte chemotaxis and migration toward the inflammatory area and induces smooth muscle cell proliferation and metalloproteinase synthesis [12]. It has been reported that increases of CCL2 in human serum occur within the first 24 h after AMI [10].

Recruitment and activation of monocytes/macrophages in the infarcted myocardium are essential for the processes that occur after AMI.
Activated macrophages promote the release of cytokines and protei-
nases, which may induce left ventricular inflammation and remodeling
[13]. The studies confirm that monocytes/macrophages dominate the
cellular infiltrate within the first two weeks after infarction and partic-
ipate in the healing of infarcted tissue [14-17]. Studies with deletion
of CCL2 or its receptor (CCR2) point to the central role of this che-
mokine and its receptor in the recruitment of monocytes to the in-
farction [18,19].

Despite the importance of CCL2 in ACS, studies evaluating the
prognostic value of the molecule are scarce [20]. Our objective is to
evaluate the role of CCL2 as a predictor of the prognosis of patients
included in the ERICO (Strategy of Registry of Acute Coronary Syn-
drome) study in a 2-year follow-up.

2. Material and methods

2.1. Study design

The “ERICO” (Strategy of Registry of Acute Coronary Syndrome)
study was previously described [21,22]. Briefly, it is a prospective co-
hort study that included from February 2009 to December 2013 in-
dividuals admitted to treatment for ACS at the Hospital of the Uni-
versity of São Paulo (HU-USP), located in the District of Butantan, São
Paulo, Brazil. This study was in accordance with the Declaration of
Helsinki and was approved by the Research Ethics Committee (CEPT-HU
/ USP 866/08). All patients signed the Informed Consent Form.

Acute myocardial infarction (AMI) was defined by the presence of
symptoms consistent with myocardial ischemia within 24 h of hospital
admission and troponin I level above the 99th percentile value with a
coefficient of variation < 10%. ST-segment elevation myocardial in-
farction (STEMI) was defined by the criteria for AMI, in addition to (a)
the presence of persistent ST segment elevation ≥1 mm in two con-
tiguous electrocardiographic leads (lead ECG) or (b) new (or supra-
sedly new) left bundle branch block (LBBB). Non-ST-segment elevation
myocardial infarction (NSTEMI) was defined by the criteria for AMI
plus the absence of persistent ST segment elevation ≥1 mm in two con-
tiguous electrocardiographic leads and of new or supposedly new
LBBB. Unstable angina (UA) was defined as the presence of symptoms
compatible with myocardial ischemia in the last 24 h, absence of AMI
diagnosis and at least one of the following five criteria: (a) history of
previous Coronary Artery Disease; (b) positive stratification of invasive
or non-invasive ischemic heart disease; (c) dynamic or evolutionary
ECG changes; (D) troponin I > 0.4 ng/mL or (e) agreement on UA
diagnosis between two independent physicians.

Trained interviewers collected data during the hospital phase re-
lated to sociodemographic characteristics, cardiovascular risk factors
and medication (including anti-inflammatory and immunosuppress
agents), as previously described [22]. Data on smoking, hypertension,
diabetes, and dyslipidemia were reported by the patients or proxies
[21]. Blood samples were collected within 24 h of admission. LDL
cholesterol was calculated using the Friedewald equation [23]. Ana-
lyses of troponin I, plasma glucose, triglycerides, and total and HDL
cholesterol were performed at the University Hospital, University of São
Paulo (HU-USP). Patients were reevaluated 30 days after the acute
event with new laboratory tests. After six months and annually, all
individuals were contacted by telephone to update vital status in-
formation and fatal and nonfatal cardiovascular outcomes. Whenever a
participant reported a potential new MI event, new investigation pro-
cedures were initiated to confirm it and underwent adjudication by
research doctors.

2.2. Measurement of CCL2

Serum samples collected at admission were sent to the Laboratory of
Atherosclerosis and Nutritional Biochemistry (LABiN) of the Federal
University of Minas Gerais (UFMG) and kept frozen until analysis. The
concentration of CCL2 on admission was evaluated by Enzyme-Linked
Immunosorbent Assay (ELISA), following the kit (CCL2: 7–1000 pg/mL,
88-7399-88, eBioscience Inc., San Diego, CA, USA) instructions.

2.3. Outcomes

Events were analyzed in a follow-up of up to 2 years. Study out-
comes were: all-cause mortality, cardiovascular mortality, and the
combined outcome of fatal MI and new non-fatal MI. The strategy for
collecting and classifying mortality data, including searching for official
death records, was detailed in a previous report [21]. In summary,
participants were included in death from cardiovascular causes if the
cause of death was identified in the 10th version of the “International
Classification of Diseases” (ICD-10), chapter IX, “Circulatory system
diseases” or classified with code R57.0 of ICD-10, “Cardiogenic shock.”
In cases where it was not possible to determine the cause of death,
the data were censored for all outcomes, except for death from all causes.

2.4. Statistical analysis

A total of 1085 patients were included in the study (Fig. 1). How-
ever, 282 of them were excluded for not meeting the criteria for the
present study (data of serum CCL2 and 2-year follow-up). Thus, 803
patients were included in the statistical analysis.

The Kolmogorov-Smirnov test was used to evaluate normality. Since
CCL2 levels presented asymmetric distribution (Fig. 2), the median

Fig. 1. Patient flow diagram. BMI: Body Mass Index. CCL2: Chemokine (C-C
motif) ligand 2. ERICO: Strategy of Registry of Acute Coronary Syndrome.

Fig. 2. Distribution of CCL2 serum level (803 patients). Bars represent the
number of cases in each range. CCL2: Chemokine (C-C motif) ligand 2.
concentration was used to define the cutoff point, separating cases from less than or equal to the median (called "low" group) or higher than the median (called "high" group).

The chi-square and Mann-Whitney tests were used to compare groups. Kaplan-Meier curves and the log-rank test were used to evaluate the difference between low and high groups. Risk estimates (Hazard Ratios with their respective 95% confidence intervals) for the events were calculated using Cox regression. Three models were constructed, including the following variables in addition to CCL2: Model 1 – age, gender and type of ACS; Model 2 – variables of Model 1 + diabetes and medication at admission (aspirin, statins, fibrates, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids and immunosuppressant); Model 3 – variables of Model 2 + smoking, hypertension and dyslipidemia. A two-tailed p-value < 0.05 was considered significant.

The software programs SPSS (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY: IBM Corp.) and GraphPad Prism (version 5.01 for Windows, San Diego, California: GraphPad Software) were used to carry out the analyses.

3. Results

As previously mentioned, 803 patients were included in this study. The median age was 63 years, 58.5% of patients were men, and 41.5% had NSTEMI. High frequencies of hypertension (75.5%), diabetes (37.9%) and dyslipidemia (47.1%) were found. The median BMI was 26.8 kg/m2. We observed high plasma glucose (6.3 mmol/L), low HDL (37.9%) and dyslipidemia (47.1%) were found. The median age was 63 years, 58.5% of patients were men, and 41.5% had NSTEMI. High frequencies of hypertension (75.5%), diabetes mellitus. NSTEMI was also the predominant type of ACS (64.2%) in the group that presented death due to AMI or new non-fatal AMI (Table 1).

It was found that the patients who presented events in the combined outcome of death due to AMI or new non-fatal AMI were older than the patients who did not present events and presented more frequently with diabetes mellitus. NSTEMI was also the predominant type of ACS (64.2%) in the group that presented death due to AMI or new non-fatal AMI (Table 1).

Regarding CCL2 levels, no differences were observed between groups presenting or not “all-cause” event and cardiovascular events (Fig. 3A and B). However, when the combined outcome was considered, the CCL2 concentration was lower in the group that presented this event (Fig. 3C) compared to the group without fatal or new MI. We then grouped 803 patients according to the levels of CCL2. By evaluating the characteristics of the sample (Table 2), lower levels of total cholesterol and LDL cholesterol were observed in the low CCL2 group. The Kaplan-Meier curves showed a lower rate of survival in patients presenting the combined outcome of fatal MI or new non-fatal MI in the low CCL2 group (p = 0.015, Fig. 4C). CCL2 was not associated with all-cause mortality and cardiovascular mortality (Fig. 4A and B).

The analysis of the Hazard Ratios (Table 3) showed an increase in the frequency of patients who reported never having smoked was also higher in the group that presented this event than in the survivors (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All-cause mortality</th>
<th>Cardiovascular mortality</th>
<th>Death by AMI or new non-fatal AMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes* (n = 115)</td>
<td>No (n = 688)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76 (67–82)</td>
<td>61 (53–70)</td>
<td>&lt; 0.001</td>
<td>76 (66–82)</td>
</tr>
<tr>
<td>24.9 (22.3–29.6)</td>
<td>26.9 (24.1–30.1)</td>
<td>0.005</td>
<td>25.6 (23.1–30.4)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>65 (56.5)</td>
<td>0.637</td>
</tr>
<tr>
<td>50 (43.5)</td>
<td>405 (58.9)</td>
<td></td>
<td>34 (43.6)</td>
</tr>
<tr>
<td>ACS type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>70 (60.9)</td>
<td>263 (38.2)</td>
<td>52 (66.7)</td>
</tr>
<tr>
<td>STEMI</td>
<td>26 (22.6)</td>
<td>198 (28.8)</td>
<td>14 (17.9)</td>
</tr>
<tr>
<td>UA</td>
<td>19 (16.5)</td>
<td>227 (33.0)</td>
<td>12 (15.4)</td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual smoker</td>
<td>14 (13.6)</td>
<td>212 (32.1)</td>
<td>12 (17.4)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>41 (39.8)</td>
<td>239 (36.2)</td>
<td>26 (37.7)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>48 (46.6)</td>
<td>209 (31.7)</td>
<td>31 (44.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>98 (87.5)</td>
<td>598 (74.9)</td>
<td>67 (88.2)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>58 (51.8)</td>
<td>246 (36.9)</td>
<td>40 (51.9)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>50 (49.5)</td>
<td>328 (54.0)</td>
<td>34 (50.7)</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.8 (6.1–9.7)</td>
<td>6.8 (5.7–8.9)</td>
<td>0.087</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.4 (1.0–1.7)</td>
<td>1.5 (1.1–2.1)</td>
<td>1.222</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>3.9 (3.1–4.8)</td>
<td>4.5 (3.7–5.3)</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.8 (0.7–1.2)</td>
<td>0.9 (0.8–1.2)</td>
<td>0.010</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>2.3 (1.7–2.9)</td>
<td>2.7 (2.0–3.4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Yes and No represent presence (yes) or absence (no) of (events for each outcome. Values are median (interquartile interval) or n (%). ACS: Acute Coronary Syndrome. NSTEMI: non-ST-segment elevation Myocardial Infarction. STEMI: ST-segment elevation Myocardial Infarction. UA: Unstable Angina. BMI: Body Mass Index in kg/m2. Data of glucose, triglycerides, total cholesterol, HDL (High density lipoprotein) and LDL (Low density lipoprotein) are presented as mmol/L. Mann-Whitney test or chi-square test.
**Table 2**

General characteristics in the hospital phase according to CCL2 groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CCL2 Low (n = 803)</th>
<th>CCL2 High (n = 402)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (54–74)</td>
<td>63 (54–73)</td>
<td>0.804</td>
</tr>
<tr>
<td>BMI</td>
<td>26.4 (23.5–30.1)</td>
<td>27.1 (24.2–30.1)</td>
<td>0.078</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.967</td>
</tr>
<tr>
<td>Male</td>
<td>235 (58.5)</td>
<td>235 (58.6)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>167 (41.5)</td>
<td>166 (41.4)</td>
<td></td>
</tr>
<tr>
<td>ACS type</td>
<td></td>
<td></td>
<td>0.486</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>166 (41.3)</td>
<td>167 (41.6)</td>
<td></td>
</tr>
<tr>
<td>STEMI</td>
<td>106 (26.4)</td>
<td>118 (29.4)</td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>130 (32.3)</td>
<td>116 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Smoking habits</td>
<td></td>
<td></td>
<td>0.459</td>
</tr>
<tr>
<td>Current</td>
<td>109 (28.2)</td>
<td>117 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>150 (38.4)</td>
<td>130 (34.6)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>128 (33.1)</td>
<td>129 (34.3)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>314 (79.1)</td>
<td>292 (74.3)</td>
<td>0.111</td>
</tr>
<tr>
<td>Diabetes</td>
<td>149 (37.9)</td>
<td>155 (40.2)</td>
<td>0.521</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>178 (50.1)</td>
<td>200 (56.7)</td>
<td>0.082</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.8 (5.6–9.0)</td>
<td>7.1 (5.9–9.1)</td>
<td>0.585</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.5 (1.1–2.1)</td>
<td>1.4 (1.1–2.1)</td>
<td>0.874</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>4.3 (3.5–5.2)</td>
<td>4.5 (3.7–5.4)</td>
<td>0.035</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.9 (0.8–1.1)</td>
<td>0.9 (0.8–1.2)</td>
<td>0.952</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>2.5 (1.9–3.2)</td>
<td>2.7 (2.0–3.5)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Values are median (interquartile interval) or n (%). ACS: Acute Coronary Syndrome. BMI: Body Mass Index in kg/m2. CCL2: Chemokine (C-C motif) ligand 2. HDL: High density lipoprotein. LDL: Low density lipoprotein. NSTEMI: non-ST-segment elevation Myocardial Infarction. STEMI: ST-segment elevation Myocardial Infarction. UA: Unstable Angina. Data of glucose, triglycerides, total cholesterol, HDL (High density lipoprotein) and LDL (Low density lipoprotein) are presented as mmol/L. Mann-Whitney test or chi-square test.

the risk of death due to MI or new non-fatal MI in two years for the low CCL2 group in the crude models (HR: 1.84. 95% CI 1.12–3.03. p = 0.017) and remained significant in the adjusted models (HR: 1.81. 95% CI 1.10–2.99. p = 0.019; HR: 2.00. 95% CI 1.20–3.34. p = 0.008; and HR: 2.70. 95% CI 1.45–5.01. p = 0.002).

4. Discussion

In our study, we observed a worse prognosis at two years (higher risk of a new infarction) in patients with lower chemokine levels. It has been reported that CCL2 concentration is increased in human serum in the first 24 h after myocardial infarction as well as in patients who presented new cardiovascular events [10,24]. Moreover, Buyukkaya et al. [25] showed an association between higher CCL2 levels and worse prognosis in patients with ACS. However, corroborating our finding, Ding et al. [20] found that both high and low levels of CCL2 are associated with an increased risk of death from all causes and cardiovascular causes in patients with Coronary Artery Disease.

It is possible that the worse prognosis observed in our study in the "low CCL2" group is related to adverse cardiac remodeling due to inadequate (or reduced) monocyte recruitment to the lesion site. Defects in infarct healing may be directly involved in the development of potentially lethal complications, such as cardiac rupture and ventricular aneurysm formation [9]. Nahrendorf et al. [26] proposed that the outcome after myocardial infarction is related to the number of monocytes at the lesion site in the first two weeks. In case of insufficient monocyte recruitment, the healing process will be compromised due to inefficient clearance of cell debris. CCL2 plays an essential role in the initial macrophage recruitment, indirectly affecting the healing process [18].

CCL2 can influence cardiac repair after infarction in different ways. It has been reported that CCL2 promotes the protection of cardiomyocytes against hypoxia-induced death [27] and induces the neovascularization and cardiac accumulation of myofibroblasts, preventing left ventricular dysfunction [13].

CCL2 knockout mice presented reduced TNF, TGF-β, IL-10 and IL-1β expression in a model of MI compared to control mice, associated with a reduction in macrophage infiltration and the delayed phagocytic removal of dead cardiomyocytes [18]. Despite the attenuation of macrophage infiltration, the infarct lesion area was similar between the CCL2 knockout and wild-type mice. The data suggest that the clinical consequences of CCL2 inhibition in the early phase of myocardial scarring are potentially unfavorable, since a delay in the phagocytosis of injured cardiomyocytes may increase the arrhythmicogenic potential or favor a predisposition to mechanical complications, such as heart rupture and ventricular aneurysms.

Ding et al. [20] suggest that insufficient amounts of CCL2 and pro-inflammatory monocytes could delay the healing process. Nonetheless, the permanence of pro-inflammatory monocytes in the injury area can impair the repair function of monocytes. Therefore, there is probably an optimum CCL2 concentration that promotes the adequate recruitment of monocytes to the injury site. The effect of pro-inflammatory cytokines and chemokines cannot be classified as beneficial or harmful. Their effects are likely dependent on factors such as the post-MI period and the composition of the cellular microenvironment.

Moreover, an experimental study showed that during the healing process of the infarction there is a positive regulation of CCL2 expression, whereas in the chronic phase there is a reduction in chemokine expression [18]. Another hypothesis is that patients in our study and patients with lower levels of CCL2 have more severe injuries, which is corroborated by the recent study by Ritter et al. [28] which...
cardiac remodeling, once insuspected. The worse prognosis observed in the low CCL2 group could be related to adverse events. However, other important risk factors are increased in this group such as the higher percentage of hypertension and diabetes.

Interestingly, we observed a higher number of patients who reported never having smoked in the groups of patients that presented prolonged hypertension had lower levels (and similar to those found in our study) of CCL2 than patients without the disease. These authors, like us, believe that this chemokine can be differently regulated according to the degree of organ damage.

There is no consensus on the CCL2 concentration in patients with ACS. Values ranging from 697 pg/mL to 105 pg/mL were described in the literature. [20,25,28–31]. The chemokine concentration in healthy individuals also presents wide variation, from 261 pg/mL to 809 pg/mL. [32–35]. The differences among the CCL2 values found in the literature and in our study are in part due to the diversity of methods (flow cytometry, multiplex assays, different ELISA kits) of CCL2 detection and the period of blood collection (1–14 days after the event).

Interestingly, we observed a higher number of patients who reported never having smoked in the groups of patients that presented events. However, other important risk factors are increased in this group such as the higher percentage of hypertension and diabetes [36,37].

Our study limitations include the unicentric characteristic, the absence of a control group as well as data from the pre-event period, which would allow us to determine variation in the marker concentration after ACS.

In summary, this work presents new information about the prognostic value of CCL2 (mainly about low levels) in ACS, suggesting that not only the high levels may be important in the prognosis of these patients, but also the lower levels. We hypothesize that the worse prognosis observed in the low CCL2 group could be related to adverse cardiac remodeling, once insufficient amounts of CCL2 and pro-inflammatory monocytes could delay the healing process.

5. Conclusions

Lower levels of CCL2 were associated with death by AMI or new non-fatal AMI, increasing the risk of those outcomes. Our results suggest that CCL2 may be useful in predicting which patients are more likely to have fatal or recurrent MI.

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Conflict of interest

The authors report no potential conflict of interest.

References