

# Critical infarct size to induce ventricular remodeling, cardiac dysfunction and heart failure in rats

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Ventricular remodeling, decreased left ventricular systolic function, and heart failure have been associated with poor long-term outcomes after myocardial infarction [1,2]. Thus, it is critical to know the pathophysiological alterations involved in these processes for myocardial infarction management.

A rat coronary artery ligation leads to a wide range of infarct size, cardiac remodeling and left ventricular dysfunction. In addition, it is accepted that the coronary occlusion consequences are closely related to infarction size, which is a powerful determinant of survival [3], ventricular remodeling [4], and cardiac systolic function [5]. In order to determine the association between infarct size and outcomes after coronary occlusion, the animals were divided into three groups (small, moderate or large infarct size), but this division was not homogeneous among the studies because the animals were randomly placed in the groups. Consequently, the infarct size that is necessary to determine morphological, functional, and clinical alterations after the coronary occlusion, still needs to be determined. The present study was carried out to determine the critical infarct size to induce ventricular remodeling, cardiac dysfunction, and heart failure in a rat model.

Myocardial infarction was induced following the method described previously [6]. Six months after surgery, Sham (n=67) and infarcted animals (n=166) were subjected to transthoracic echocardiography, and euthanized the next day.

The echocardiographic exams and morphometric analysis were performed as described previously [6,7]. The left ventricular remodeling was defined as the infarcted values of the left ventricular end-diastolic cavity areas with a difference of >2 and standard deviations above Sham group mean. The systolic dysfunction was defined as the infarcted values of posterior wall shortening velocity (PWSV) with a difference of <2 and standard deviations under Sham

group mean. The diastolic dysfunction was defined as the infarcted values of E/A ratio with a difference of >2 and standard deviations above Sham group mean.

The variables utilized to determine heart failure included pleuropericardial effusion, left atrial thrombi, ascites, or right ventricular hypertrophy (right ventricle weight-to-body weight ratio >0.8 mg/g) [8].

An infarct size cut-off value was derived from the receiver-operating characteristic (ROC) curve according to the infarct size value to maximize sensitivity, specificity, and predictivity values in order to anticipate remodeling, cardiac dysfunctions, and heart failure. The bias between histology and echocardiogram in the infarct size assessment was evaluated by Bland & Altman.

After a six-month follow-up, the infarct size in the infarcted animals ranged from 18.5% to 57%, with an average of  $40 \pm 9\%$  assessed by histology, and  $42 \pm 9\%$  assessed by echocardiogram, and bias between the methods at  $-1.15\%$ .

The infarct size cut-off values were 36% (area under ROC curves (AUC): 0.70; 95% CI: 0.61-0.78;  $p=0.017$ ) for ventricular remodeling; 38% (AUC: 0.72; 95% CI: 0.64-0.80;  $p=0.001$ ) for PWSV; 44% (AUC: 0.72; 95% CI: 0.64-0.79;  $p<0.001$ ) for E/A ratio and 40% (AUC: 0.65; 95% CI: 0.56-0.73;  $p=0.004$ ) for heart failure.

Among the infarcted rats, 93% presented ventricular remodeling; 86% showed systolic dysfunction; 47% presented diastolic dysfunction; and 66% had heart failure.

The knowledge of the critical infarct size necessary to induce remodeling, cardiac dysfunction, and heart failure in this model can be extremely useful, since infarct size can be early estimated after the coronary occlusion by non invasive methods, such as echocardiogram. In the present study, the infarct size was determined by histology, even though this measurement had low bias when compared to infarct size measured by echocardiography.

In conclusion, the identification of the critical infarct size to induce ventricular remodeling, cardiac dysfunction, and heart failure might be particularly useful in longitudinal studies about therapeutic and pathophysiological aspects following coronary occlusion. Our data indicate that the minimum infarct size to cause these morphological, functional and clinical abnormalities is 36%, 38%, and 40%, respectively.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology (Shewan and Coats 2010;144:1-2).

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## Assessment of the effects of the A3872G polymorphism on the C-reactive protein gene in patients with diabetes mellitus type 2

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Several studies have shown that C-reactive protein (CRP) contributes to the pathogenesis of diabetes mellitus type 2 (DM2) and predicts its incidence [1]. Further evidence suggests that CRP is not just an acute phase protein, but rather a mediator of atherogenesis, serving as a marker of acute coronary syndromes, as well as predicting events after interventional procedures [2,3]. Genetic polymorphisms are nowadays considered to be potential regulators of several biomarkers in patients with or without coronary artery disease (CAD) [4] and specifically CRP gene polymorphisms have been proposed to modulate CRP levels and probably the risk for CAD events in healthy individuals. There is also susceptibility for a role of CRP gene polymorphisms in the development of DM2 by increasing CRP levels [5]. However, the results regarding a specific CRP gene polymorphism such as the adenine/guanine 3872 (A3872G) on CRP levels seem to be conflicting. In the present study we examined the impact of the A3872G polymorphism in CRP gene, on the risk for DM or CAD, on CRP levels, glucose levels, haemoglobin A1c (HbA1c) and the duration of diabetes in patients with documented diabetes mellitus.

The study population consisted of 427 patients with DM2 (documented for CAD or not) and 227 healthy controls. Subjects were characterized as patients with DM2 if fasting plasma glucose levels were  $\geq 126$  mg/dl, or 2-hour post glucose loading plasma levels  $\geq 200$  mg/d according to the American Diabetes Association [6]. Risk factors were evaluated as described previously [4,7]. Coronary artery disease was evaluated by coronary angiography

(stenosis  $> 50\%$ ) or positive exercise test, while the absence of CAD was considered in those patients presenting with normal electrocardiogram, absence of symptoms, negative exercise test and/or normal coronary angiography. Exclusion criteria were any acute or chronic inflammatory disease, malignancies, recent acute coronary event during the last 2 months, renal or liver failure. The study was approved by the Institutional Ethics Committees, and an informed consent was given by all the participants.

Venous blood samples were centrifuged at 3500 rpm at 4 °C for 15 min, and plasma or serum was collected and stored at  $-80$  °C until assayed. Serum concentrations of total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and were determined by using colorimetric enzymatic method in a Technicon automatic analyzer RA-1000 (Dade-Behring Marburg GmbH, Marburg, Germany). Serum CRP levels were measured by particle-enhanced immunonephelometry (N Latex; Dade-Behring Marburg GmbH). Moreover glucose levels were measured with standard techniques and HbA1c by an automated analyzer using the HPLC technique (HA8160, ADAMS A1c, ARKRAY, Inc. Kyoto, Japan). Genomic DNA was extracted from 2 to 5 ml of whole blood using standard methods (QIAamp DNA blood kit; Qiagen, West Sussex, UK). For the detection of A3872G (rs1205) polymorphism on CRP gene, we used primer pairs to amplify a part of the gene by Polymerase Chain Reaction (PCR) with the following flanking intronic primers: rs1205F: 5'cacaagagtggacgtgaa-3' and rs1205R:5'cttatagacctggcagc-3'. The resulting product (630 bps) was digested by HpyCH4III restriction endonuclease (3 h at 37 °C and resolution by electrophoresis at 1% agarose gel). Digested fragments were visualized after ethidium bromide staining under ultraviolet light.

Allele frequencies were tested with chi-square test, if conformed to Hardy-Weinberg equilibrium proportions. Our priori power analysis had shown that this population (654 cases) is capable to show significant differences between the study parameters such as the duration and the risk of diabetes. More specifically, the sample size of our study was calculated according to the applied priori power analysis based on mean values of duration/years (Mann-Whitney) collected from pilot data. Accordingly a total sample size of  $\sim 425$  participants (divided into two unequal groups with a proportion of 1/5 approximately), was adequate to reveal an effect size of 0.35, achieving the desirable statistical power of  $> 80\%$ , at a prefixed 0.05 type I error. Furthermore, (as regards the risk of DM2) the power analysis with a sample size of 321 subjects was adequate to reveal an effect size of 0.3, achieving the desirable statistical power

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