

# Cystatin C and glomerular filtration rate in the cardiac surgery with cardiopulmonary bypass

*Cistatina C e taxa de filtração glomerular em cirurgia cardíaca com circulação extracorpórea*

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## Abstract

**Objective:** The aim of this study was to compare cystatin C versus creatinine as a marker for acute kidney injury in patients submitted to cardiac surgery with cardiopulmonary bypass.

**Methods:** Fifty consecutive patients submitted to coronary artery bypass grafting were studied. Renal function was evaluated by serum cystatin C and creatinine. Blood samples were obtained from each patient at three time points: before operation, and on the first and fifth postoperative days. Glomerular filtration rate (GFR) was calculated by Cockcroft-Gault (CG), Modification of Diet in Renal Disease (MDRD), and Larsson (Cys-GFR) formulas.

**Results:** Creatinine and Glomerular filtration rate by Cockcroft-Gault and Modification of Diet in Renal Disease formulas did not show statistical difference between study times. After renal injury from surgery, there was an increase in cystatin C on the 1<sup>st</sup> and 5<sup>th</sup> day after surgery, being

significantly different on the 5<sup>th</sup> postoperative ( $P<0.01$ ). The Glomerular filtration rate by Larson formula was higher in the preoperative time ( $105.2 \pm 41.0$  ml/min) than in the 5<sup>th</sup> postoperative day ( $89.5 \pm 31.5$  ml/min;  $P<0.012$ ).

**Conclusion:** The cystatin C and the Cys-GFR showed significant changes after cardiac surgery when compared with the creatinine and respective Glomerular filtration rate calculated by the Cockcroft-Gault and Modification of Diet in Renal Disease formulas.

**Descriptors:** Cystatins. Cardiopulmonary Bypass. Creatinine.

## Resumo

**Objetivo:** Avaliar a cistatina C como marcador de função renal em pacientes submetidos à cirurgia de cardíaca com circulação extracorpórea, comparando com a dosagem sérica de creatinina.

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**Métodos:** Foram analisados 50 pacientes consecutivos submetidos à cirurgia de revascularização do miocárdio. A função renal foi avaliada com a dosagem sérica de cistatina C e de creatinina no pré-operatório, no primeiro e no quinto dia de pós-operatório. Foram utilizadas as fórmulas de Cockcroft-Gault (CG) e Modification of Diet in Renal Disease (MDRD) para calcular a taxa de filtração glomerular estimada (TFG) através da creatinina, e a fórmula de Larsson para a TFG estimada através da cistatina C (TFG-Cis).

**Resultados:** A creatinina e o TFG através das fórmulas de CG e MDRD não mostraram diferença significativa nos momentos estudados. Após a agressão renal pela cirurgia, houve um aumento da cistatina C no 1º e 5º pós-operatório,

sendo que no 5º pós-operatório com diferença estatisticamente significativa ( $P < 0,01$ ). Houve uma queda da TFG estimada pela cistatina C de  $105,2 \pm 41,0$  ml/min, no pré-operatório, para  $89,5 \pm 31,5$  ml/min no 5º dia pós-operatório ( $P < 0,012$ ).

**Conclusão:** A cistatina C e a TFG-Cis apresentaram mudanças significativas no pós-operatório de cirurgia de revascularização do miocárdio quando comparadas a creatinina e a respectiva TFG estimada pelas fórmulas de Cockcroft-Gault e MDRD.

**Descritores:** Cistatinas. Circulação extracorpórea. Creatinina.

## INTRODUCTION

Acute renal injury (ARI) represents a serious and frequent complication in patients submitted to cardiac surgery, being associated to the increase in time of hospital internment, costs, morbidity and mortality. Its incidence varies from 3.5% to 31% [1-3]. The need for postoperative dialysis is required in 1% to 5% of the cases [1,2]. The presence of ARI increases the rate of mortality from 0.4% and 4.4% to 1.3% and 22.3%, reaching 25% to 88.9% when the dialytic therapy becomes necessary [3]. The pathophysiology of the renal injury is of multifactorial cause. The main mechanisms responsible include the low cardiac output, hypoperfusion and renal ischemia, the loss of the pulsatile flow during extracorporeal circulation (ECC), hypothermia, embolism or microthrombo-renalovascular and the generalized inflammatory response induced by the ECC [4]. These factors can result in tubular and glomerular injury, especially in patients that present previous co-morbidities such as diabetes mellitus, high blood pressure, elderliness, left ventricular dysfunction and complication of the renal function prior to surgery. In an attempt to alleviate ARI in the postoperative of cardiac surgeries, it becomes necessary the implementation of earlier and new detection methods. One of the best evaluation indexes of the renal function is the creatinine clearance in the urine sample collected after 24 hours, however, it is a slow method that requires meticulous and sequential urine collection for a specific period of time, which is usually, clinically unfeasible. Consequently, serum creatinine and estimated creatinine clearance are the evaluation methods for kidney injury largely applied. Although, serum creatinine is considered specific, but not very sensitive, for its level does not increase significantly until the glomerular filtration rate suffers a reduction less than 50% of the normal values [5]. The concentration of serum creatinine is significantly influenced by several factors such as muscular mass, age, gender, diet, tubular secretion alterations, as well as the

interference of drugs and endogen substances and their effects [6]. On the other hand, the concentration of serum cystatin C is not influenced by these factors [7]. Cystatin C is a plasmatic protein of low molecular weight (13 kDa), member of the group of competitive inhibitors of the lysosomal proteinase cysteine. Its functions are involved in the extra-cellular proteolysis, modulation of the immune system and, antiviral and antibacterial activity. It is considered an endogenous marker of the renal function due to its stable production throughout all the nucleate cells [8]. It is filtrated through the basal membrane of the glomeruli and it is almost immediately reabsorbed and degraded by the proximal tubules, being the glomerular filtration its only elimination outlet [9]. For this reason, the dosage of serum cystatin C has been used to estimate the glomerular filtration rate in several experiments and clinical assays. The present study aims to evaluate the behavior of cystatin C as a marker of the renal function in patients submitted to myocardial revascularization surgery (MRS) with ECC, a clinical example of renal injury in patients with risk factors for kidney injury. We compared cystatin C to a more usually applied method, the serum creatinine dosage. Also, we evaluated the GFR estimated by the respective markers.

## METHODS

### Patients

There were analyzed 50 patients consecutively submitted to MRS with ECC, from January to November, 2007. The patients were operated at the Hospital das Clinicas - Universidade Estadual Paulista (UNESP). Patients of both genders, all age groups, submitted to MRS with ECC, participated in the study by signing an agreement term. It was adopted as a criterion of exclusion patients submitted to cardiac surgery without the use of ECC, reoperations, preoperative renal insufficiency with the need of dialysis, patients that presented other cardiac

pathologies (valvar, congenital, corrected or not during the same surgical procedure).

### Anesthesia

The induction of general anesthesia was performed with midazolam (0.05 to 0.1 mg.kg<sup>-1</sup>) and etomidate (0.3 mg.kg<sup>-1</sup>). The maintenance was performed with sufentanil citrate (0.3 to 0.6 µg/kg/h) and isoflurane (1 MAC = minimal alveolar concentration). As muscle relaxant it was used vecuronium bromide (0.08 mg.kg<sup>-1</sup>).

In the operation room it was used a multiparameter device (Dixtal DX2010) for continuous monitoring of the electrocardiogram (derivations DII and V5), pulse oximetry, capnography and mean blood pressure (MBP). The temperature was monitored with nasal-pharyngeal thermometer (Ag-2000 Braile Biomedica Ltda.). The MBP was obtained by dissection of the radial artery. Central venous access was provided by deep venous punch (subclavian vena or internal jugular) and introduction of double lumen catheter. All patients were submitted to vesical probing for diuresis control. It was used a thermometer in the oral-pharynx cavity for monitoring temperature.

### Extracorporeal circulation and surgical technique

The patients were anticoagulated with sodium heparine with dosage of 4mg/Kg for maintenance of the activated coagulation time (ACT) higher than 480 seconds. During the ECC the patients were submitted to non pulsatile arterial flow keeping the MBP between 60 and 80 mmHg. The temperature of the patient was kept between 35 and 37°C. The myocardial protection was performed with intermittent anterograde hypercalemic blood cardioplegia, repeated every 15 minutes or less. The distal anastomoses were performed during the aortic clamping time. The proximal anastomoses were performed with an open heart and partial clamping (tangential) of the aorta. It was performed complete revascularization in all patients. In all patients it was used the internal thoracic artery as first choice grafting, being that the right internal thoracic artery, the radial artery and the saphena were also used. In the process of interruption of the ECC, the arterial flow was gradually reduced, observing the maintenance of MBP above 65 mmHG. After completing the myocardial revascularization and having all pulmonary and cardiac functions restored, the cannulas removed and the heparinization reversed with protamin hydrochloride to maintain the ACT around 120 seconds. It was applied dobutamin regularly with initial dosage of 3 to 5 µg.Kg<sup>-1</sup>.min<sup>-1</sup> after finishing ECC. Noradrenalin was used when MBP was lower than 60 mmHg.

### Laboratorial dosage

Blood samples were collected in the preoperative in the first postoperative day (after 24 hours of the patient's arrival

in the ICU) and in the fifth postoperative day. The dosage of serum cystatin C was done by nefelometry using the device BN II (Dade Behring). The dosage of serum albumin, creatinin and urea were analyzed by the equipment Vitros 950 (Johnson & Johnson). The GFR was estimated through the following formulas largely used in literature [10-13]:

1. Cockcroft-Gault (CG) =  $\frac{[(140 - \text{age}) \times \text{weight (Kg)}]}{\text{plasmatic creatinin} \times 72} \times (0,85 \text{ in case of female patient})$ ;

2. MDRD =  $170 \times (\text{plasmatic creatinin})^{-0,999} \times (\text{age})^{-0,176} \times (0,762 \text{ in case of female patient}) \times (1,18 \text{ in case of black patient}) \times (\text{urea})^{-0,17} \times (\text{albumin})^{+0,318}$ ;

3. Formula of Larsson (Cys-GFR) =  $77,24 \times (\text{cystatin C})^{1,2623}$ ; The markers of the renal function were divided by the value of serum albumin in order to minimize the dilution effect of patients in the postoperative, once the patients submitted to ECC are usually hemodiluted in the postoperative and, consequently, it occurs a dilution of the markers present in the blood. The serum albumin for being a protein of heavy molecular weight would represent the hemodilution state of the patient. These ratios could be represented as follows: cystatin C/ albumin (Cyst/Alb) and serum creatinine (SCr/Alb).

### Statistical analysis

For the statistical analysis it was used the program SAS 9.2.

The data of the continuous variables are represented in the form of mean ± standard deviation.

Ordinal variables are represented in the frequency form (%).

For the comparison of the periods it was used the repeated measurement analysis, followed by the method of Turkey for the comparison of the means.

For the study of the correlation between variables it was used the correlation coefficient of Pearson.

The value of  $P < 0.05$  was considered statistically significant.

### RESULTS

The general characteristics of the patients studied are displayed in Table 1. The times of aortic clamping, extracorporeal circulation and surgery are represented in Table 2. The number of coronary arteries revascularized ranged from 1 to 4, with mean of  $2.7 \pm 0.8$ .

The Table 3 indicates the mean values and respective standard deviations of the variables in the three times of study. Creatinin and cystatin C were analyzed in the preoperative in the first and fifth postoperative day. The GFR estimated by the creatinine was calculated by the formulas of Cockcroft-Gault and MDRD. The GFR estimated by the cystatin C was calculated by the formula of Larsson. The table also shows the value of serum albumin, the

ratios creatinine/albumin (SCr/Alb) and cystatin C/albumin (Cyst/Alb).

It was performed the correlation coefficient between the variables cystatin C and creatinin for the moments studied (Figures 1, 2 and 3).

Two patients died in the postoperative (4%). The first patient presented high blood pressure, diabetes and dislipidemy, acute myocardial infarction in the 1<sup>st</sup> PO day and, cardiogenic shock, requiring vasoactive drugs and

intra-aortic balloon, dying in the 16<sup>th</sup> PO day. This patient presented values of cystatin C in the preoperative, 1<sup>st</sup> PO and 5<sup>th</sup> PO days respectively of 0.8; 1.02 and 1.12 mg/l, and creatinin values of 0.6; 0.8 and 0.8 mg/dl. The other patient presented high blood pressure, diabetes, smoking addiction, and progressed in the postoperative with ARI and respiratory insufficiency, dying in the 14<sup>th</sup> PO day. This patient presented values of cystatin C in the preoperative, 1<sup>st</sup> PO and 5<sup>th</sup> PO respectively of 1.02; 2.42 and 1.61 mg/l and creatinin values of 1.1; 1.2 and 1.3 mg/l, reaching 3.0 mg/l in the 10<sup>th</sup> PO day.

The average time in the ICU was 57.0 ± 58.5 hours and the time of postoperative internment was 8 ± 2.7 days.

Table 1. General Characteristics of the Patients Studied

Age (years)	62.9 ± 10.2
Gender (male/female)	32 (64%) / 18 (36%)
Ethnicity (Caucasian)	46 (92 %)
HBP	42 (84 %)
DM	17 (34 %)
Dislipidemy	33 (66 %)
Smoking addiction	30 (60 %)
BMI	26.1 ± 3.8
FDDLVL (mm)	49.3 ± 5.5
Ejection Fraction (%)	62.9 ± 5.5

FDDLVL: final diastolic diameter of the left ventricle, DM: diabetes mellitus, HBP: high blood pressure, BMI: body mass index

Table 2. Intra-operative Data

Time (min)	Minimum	Maximum	Mean	Standard Deviation
Aortic clamp.	20	119	56.94	± 22.15
ECC	32	170	93.04	± 34.18
Surgery	180	280	233.30	± 16.09

ECC: extracorporeal circulation, Aortic clamp.: aortic clamping

Table 3. Mean and standard deviation of the values of creatinine, cystatin C and glomerular filtration rate referring the preoperative, first and fifth postoperative

	Preop.	1st PO	5st PO	P
Creatinine(mg/dl)	0.98±0.25	0.97±0.37	1.01±0.32	0.50
Cystatin C(mg/l)	0.87±0.28b	0.93±0.40ab	0.98±0.31a	0.01
GFR	76.7±25.5	85.9±48.5	77.9±34.3	0.09
MDRD	55.7±18.0	54.6±31.9	52.6±20.0	0.58
Cys-GFR	105.2±41.0a	106.4±57.4a	89.5±31.5b	0.012
Albumin (g/dl)	3.5±0.6a	2.5±0.3c	3.1±0.4b	0.000
Cyst / Alb	0.26±0.10c	0.38±0.17a	0.32±0.11b	0.000
SCr / Alb	0.29±0.09c	0.40±0.15a	0.33±0.11b	0.000

Small letters compare mean periods. Alb: albumina serum, Cyst: cystatin C, SCr: serum creatinine, PO: postoperative, Preop: preoperative, GFR: glomerular filtration rate estimated by the formula of Cochrcroft-Gault, Cys-GFR: glomerular filtration rate calculated by the formula of Larsson, MDRD: glomerular filtration rate estimated by the formula MDRD

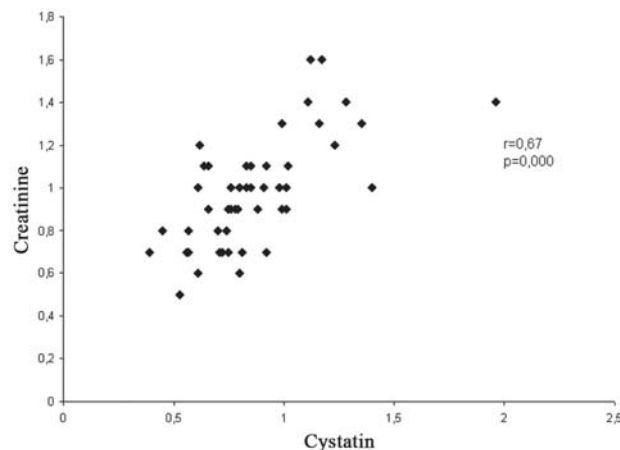


Fig. 1 - Correlation between cystatin C and creatinine in the preoperative. r: correlation coefficient; p: level of significance

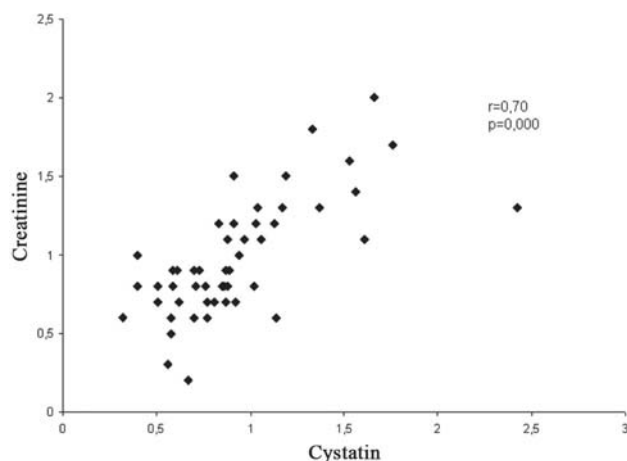


Fig. 2 – Correlation between cystatin C and creatinine in the first postoperative. r: correlation coefficient; p: level of significance

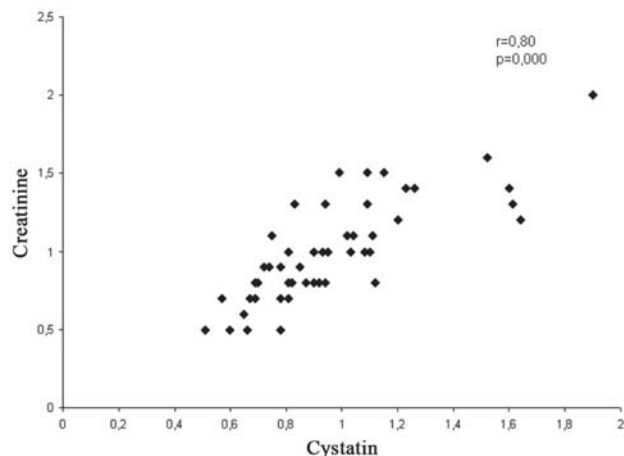


Fig. 3 - Correlation between cystatin C and creatinine in the fifth postoperative. *r*: correlation coefficient; *p*: level of significance

## DISCUSSION

Extracorporeal circulation introduced by John Gibbon Jr., in 1953, is essential for the performance of many cardiac surgeries [14]. Its technological development provided significant benefits with lower complication rates. However, ECC triggers an important systemic inflammatory reaction with interstitial fluid accumulation, leukocytosis and organic dysfunction [15]. The following substances are generated during ECC: hemoglobin in the plasma, elastase, endothelin, free radicals including superoxides, hydrogen peroxide and hydroxyl radicals. All these substances injure the membranes of the kidneys tubular cells. In addition, intrinsic variables of ECC such as hypothermia, intraoperative pH, tubes and drains, low blood pressure, non-pulsatile arterial flow and hemodilution are risk factors for ARI in cardiovascular surgeries [16].

Due to the acquired knowledge that the estimated clearance of creatinine has its value limited for detection of ARI, new researches have developed means to estimate the rhythm of glomerular filtration, such as through the dosage of serum cystatin C, a fast and precise method that can be applied regularly.

In a meta-analysis in 2002, Dhannidharka et al. [17] published that cystatin C is better than serum creatinin as a marker of the renal function. On the other hand, in 2007, Zahran et al. [18] performed a literature review with 43 studies of renal transplantation and patients with primary renal disease; and found a large number of researches in favor of cystatin C to stimulate GFR, but still there are many studies that show that there are no advantages of cystatin C over cretinine. However, there are few studies of cystatin

C as a marker of the renal function in patients submitted to cardiac surgery.

Analyzing our results, we could find a moderate correlation between cystatin C and cretinine in the moments studied (Figures 1, 2 and 3). Although, the dosage of serum creatinine and GFR (calculated through the same creatinine by the formulas of Cockcroft-Gault and MDRD) did not show significant statistical difference in the moments evaluated. However, after the kidney injury by the procedure and by ECC, there was an increase in the mean value of cystatin C in the 1<sup>st</sup> and 5<sup>th</sup> postoperative days (PO), being that in the 5<sup>th</sup> PO with a significant statistical difference compared to the preoperative collection ( $P = 0.01$ ). The effect was also observed by the decrease of GFR, for it dropped from  $105.2 \pm 41.0$  ml/min in the preoperative to  $89.5 \pm 31.5$  ml/min in the 5<sup>th</sup> PO ( $P = 0.012$ ). Unfortunately it was not possible to correlate these alterations with the clinical evolution of the patients, for in this sample the patients presented low level of complications and the time of hospital internment was relatively short and it did vary a little.

In spite of the mean increase of cystatin C having been of 13% and the reduction of Cys-GFR being around 15%, lower than the criteria used for the diagnostic of acute renal injury (> 50%), the study shows that cystatin C and Cys-GFR presented higher alteration than creatinine and GFR estimated by the creatinine in the moments studied.

Momeni et al. [19] recently performed a study aiming to compare cystatin C and creatinine during ECC in the first 72 hours of postoperative in patients submitted to MRS. During the observed period there was no significant increase of cystatin and creatinine. The values of clearance of these two substances also did not show statistical difference.

Our results were different from the Momeni study. Probably due to the observation time of this author having been shorter, only in the first 72 hours of postoperative. In this period the patients are still under the considerable effect of hemodilution and, as a result, the increase in the levels of serum cystatin is not sufficient for these levels to become significant, for cystatin is also hemodiluted. An evidence of hemodilution in the PO was the drop of serum albumin concentration, a protein of heavy molecular weight. In the study of Momeni the mean value of the serum proteins exceeded  $7.1 \pm 0.33$  g/dl (preoperative) to  $4.1 \pm 0.49$  g/dl (PO), and in our study the values varied from  $3.5 \pm 0.6$  g/dl to  $2.5 \pm 0.3$  g/dl ( $P = 0,000$ ).

Patients submitted to cardiac surgery with ECC remain hemodiluted in the postoperative [20]. Many anesthetic drugs (especially volatile agents) cause peripheral vasodilatation and, in order to maintain the pressure levels, vasoconstrictors and crystalloid solutions are applied. The drop of volemia due to bleeding in the surgical field is

preserved by hemoderivative infusion and also crystalloids. The initial volume that fills the circuit of ECC also hemodilutes the patient [21,22]. The surgery leads to an increase in catabolic hormones and cytokins, where the main effect is the increase in secretion of the antidiuretic hormone (ADH) and aldosterone causing a decrease in the excretion of water [23,24].

The markers of the renal function also suffer interference of hemodilution and become underestimated. Lassnigg et al. [25], in an article published in 2008, performed a multicentric work with 3,123 patients where it was observed that the minimal increase of serum creatinine in 48 hours of postoperative is associated with an increase in mortality, even proposing a review in the ARI concepts in the postoperative of this group of patients.

Albumin was used, then, as a relation for serum dosages of cystatin and creatinine to minimize the hemodilution effect. Therefore, the results of dosages applied in the blood were also presented by the ratios cystatin C/ albumin (Cyst/ Alb) and creatinine/albumin (SCr/Alb). Through this it could be observed a statistically significant increase

( $P = 0,000$ ) of the mean values of cystatin and creatinine in the first and fifth PO. This finding could be another evidence of supremacy of cystatin over creatinine, for its increase suppresses the hemodilution and only the cystatin remains statistically significant when compared independently to the mean values of the markers in the postoperative.

Within the limitations of our work, we know that there is a need for further multicentric studies with a larger number of samples in order to acquire further information regarding the markers of renal function. With the present study, we also hope to offer evidence in order to supplement the researches and the literature on cystatin C, a substance used in other situations where the alteration of renal function is present.

## CONCLUSION

Hemodilution, present in the initial period of postoperative of patients submitted to surgery with ECC, makes possible for the serum markers of the renal function to be underestimated, that is, also hemodiluted.

Even though it is a method broadly applied, the serum creatinine dosage, as well as the GFR calculated by the formula CG and MDRD (through creatinine) do not present significant alterations in the renal function in the first and fifth postoperative day.

Cystatin C and Cys-GFR presented significant changes in the postoperative of myocardial revascularization surgery with ECC when compared to creatinine and the respectively GFR estimated by the formula of Cockcroft-Gault and MDRD.

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