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Cystatin C in cardiac surgery

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Abstract : Cystatin C has recently been proposed as an alternative marker of glomerular filtration rate. The study compares cystatin C and creatinine concentrations during cardiopulmonary bypass and the first 72 hours postoperatively in patients undergoing coronary artery bypass graft. Forty-nine patients with normal preoperative renal and cardiac function were scheduled for coronary artery bypass graft. Blood was sampled for creatinine and cystatin C measurements at 7 time points till 72 hours postoperatively. Glomerular filtration rate was estimated from calculated clearance using the Cockroft and Gault formula for creatinine and Larsson equation for cystatin C.

The baseline values of both markers were within the normal range. Their concentrations were comparable during the whole study period. This was also the case for the calculated creatinine and cystatin C clearance .

In patients with normal preoperative renal function undergoing coronary artery bypass graft, measured creatinine concentration remains a cheap and easy way of estimating renal function.

Key words : Cardiopulmonary bypass ; coronary artery bypass graft ; cystatin C ; creatinine ; renal dysfunction.

Despite new advances in cardiopulmonary bypass (CPB) technology, surgical and anesthetic techniques, acute renal dysfunction following cardiac surgery remains a frequent complication. The definition of acute renal dysfunction varies between studies, contributing to the variation of reported incidences from 7 to 30% (1). One to 4% of patients are reported to develop renal dysfunction requiring renal replacement therapy, a condition associated with a substantial risk for postoperative death. In order to reduce post-CPB acute renal dysfunction, adequate means for early detection of this complication are necessary.

The gold standard for evaluating glomerular filtration rate (GFR) is measured creatinine clearance on 24 h-urine collection samples. However, the method is cumbersome, costly and slow. Therefore, serum creatinine (Cr) concentration is used as a rough measure of overall renal function and to evaluate glomerular filtration rate (GFR) in clinical practice. Calculated creatinine clearance (Cr-Cl) using the Cockroft and Gault equation, adjusted for body surface area (BSA) has been proposed as a better estimate of GFR (2) and is widely used. The formula provides an acceptable estimate of Cr-Cl in most stable cardiac surgical patients (3). However, it has several disadvantages. Therefore, a stable, convenient and clinically reliable marker of GFR remains desirable.

Cystatin C could be a good candidate. Indeed, cystatin C (cys C) is a nonglycosylated, low molecular weight (13 kDa), cationic protein produced by all nucleated cells at a constant production rate. Its low molecular weight allows it to be freely filtered by the glomerular membrane, without tubular secretion. It is then reabsorbed and almost totally catabolised in the proximal tube (4-6).

In contrast with Cr, plasma levels of cys C are independent of age, muscle mass, weight and height. Its measurements can be made and interpreted from a single random sample. Furthermore there's a conversion formula (Larsson equation) (7) transforming cys C expressed as mg/L to GFR expressed as ml/min. This formula has shown a significantly stronger correlation between cys C (mg/L) and iohexol clearance than between creati-

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nine and iohexol clearance. It would provide clinicians reliable and readily available GFR data based on a single measurement of cys C concentrations. To compare the superiority of cys C to creatinine as a marker of GFR during coronary artery bypass graft (CABG) with extracorporeal circulation, we studied a population of patients with normal preoperative renal and cardiac function.

METHODS

1. *Patients*

After local ethics committee approval and informed written consent, 50 patients undergoing coronary artery bypass grafting were selected. Patients with baseline creatinine clearance ≤ 60 ml/min and impaired left ventricular function (LVEF ≤ 35%) were not included. Baseline Cr clearance was calculated from preoperative creatinine concentration the day before surgery, using the Cockroft and Gault equations adjusted for 1,73 m^2 of body surface area (2, 3). Those who underwent redo, emergent surgery or combined surgical procedures (eg. valvular replacement associated to CABG) were also excluded. Other exclusion criteria were the use of concomitant nephrotoxic agents eg. Vancomycin and radiocontrast agents taken within 2 weeks before surgery. The use of dopamine nor furosemide was allowed.

2. *Anesthesia*

Anesthesia was induced with sufentanil, midazolam, etomidate and pancuronium bromide.

It was then continued with either propofol, Sevoflurane or Isoflurane. All patients received Cefamandole as antibiotic prophylaxis.

When necessary vasopressors (norepinephrine or phenylephrine) were given to maintain mean arterial blood pressure between 50 and 70 mmHg before bypass, and above 40 mmHg during bypass. Intravascular volume replacement therapy was performed with crystalloids, gelatins, salvaged blood from cell-saver devices and packed red blood cells if needed. The transfusion trigger was a hemoglobin concentration of 65 g/L on bypass, and 75 g/L off bypass unless signs of poor tolerance developed. No dextrans or hydroxyethyl starch solutions were used. Intravascular volume was increased until a central venous pressure of 7-12 mmHg was obtained.

3. *Cardiopulmonary bypass and surgical techniques*

Heparinization was accomplished to maintain the kaolin activated clotting time above 400 sec (HR-ACT, Medtronic, Inc., Minneapolis, USA) before venous cannulation and during the whole CPB time. A standard Phosphorylcholine Inert Surface CPB circuit (Dideco, Sorin Group Company, Salugia, Italy) was primed with 1000 ml of Ringer Acetate Solution (Plasmalyte A®, Baxter, S.A.Lessines, Belgium), 500 ml of Gelatin (Gelofusine®, Fresenius Kabi, Belgium), 50 mg of heparin, and 0.5 g/kg of mannitol.

Patients were kept normothermic during CPB at a standardized continuous non-pulsatile flow of 2.2 – 2.4 l/m^2/min.

Myocardial protection was achieved by the induction of electromechanical arrest with warm blood enriched with potassium chloride and magnesium sulfate. The anterograde cardioplegia solution was administered every 15 min or earlier during at least 2 minutes, whenever electrical activity was seen (8, 9).

Distal anastomoses were completed during a single period of aortic cross-clamping. Proximal anastomoses were completed on the beating heart using an aortic partial occlusion clamp. Total revascularisation was achieved for all patients.

4. *Sample collection*

Blood was sampled for Cr and cys C at 7 time points : prior to the induction of anesthesia (T0), 1 hour after the start of CPB (T1), after heparin reversal by protamine sulfate (T2), 6 (T3), 24 (T4), 48 (T5) and 72 hours (T6) after the admission of the patient in the ICU.

All Cr samples were analyzed immediately after collection. Cys C samples were kept at 7°C and analyzed as a batch for each patient.

5. *Laboratory methods*

Plasma cys C concentrations were determined by immunonephelometric method (N latex Cystatin C) on the Dade-Behring BNII analyser. Samples were mixed with polystyrene particles coated with antibodies specific to human cys C. All samples were stored for a period shorter than 7 days at 7°C. Lipemic samples were clarified by centrifugation (10 min at approximately 15,000 g) prior to testing. Samples were automatically diluted 1:100 with N diluent. The diluted samples were measured within

4 h. Cr concentrations were obtained routinely on the Beckman LX20 analyzer.

6. *Statistical methods*

Statistical analysis was carried out using STATISTICA package (Statsoft, 1997 Editors) and Excel 1997 (Microsoft Inc.). All data were controlled for normal distribution. Sample size of the study was calculated based on the measurement of cys C as the primary outcome variable. A minimum detected difference of 0.2 ng/ml was considered clinically significant.

For a power of 0.9 and alpha = 0.01, a sample size of 48 patients was calculated to be appropriate.

Multivariate analysis of variance (MANOVA) was used for the cys C concentration to compare the values at each time point with the preinduction values. This was also done for the calculated clearances using Larsson formula and Cockroft-Gault formula. Tukey post-hoc-test was performed to look for statistical difference. Significant differences were considered when p value was < 0.01.

RESULTS

Pre- and postoperative characteristics of patients are described in tables 1 and 2. One patient was excluded after the induction of anesthesia on the basis of perioperative transoesophageal echocardiography. Thirty-five patients received tranexamic acid (30 mg/kg) and 14 patients received high-dose aprotinin regimen (2×10^6 KIU pre CPB, 2×10^6 KIU in prime, and 500,00 KIU/h during surgery) because they had not discontinued aspirin for at least 5 days before surgery (10). Nine patients (18,4%) required inotropic support (dobutamine, epinephrine or amrinone) to be weaned off bypass, and twenty patients (41%) needed vasopressor support (norepinephrine). None of the patients needed ultrafiltration during CPB. None of our patients required renal replacement therapy. One patient died on the fifth postoperative day because of acute respiratry distress.

Table 3 indicates the mean values of observed Cr and cys C with their standard deviations at the different time points. The mean values of calculated Cr-Cl and calculated cys C clearance are shown in table 4. The baseline values of creatinine and cys C were within the normal range. The measured Cr and cys C concentrations were comparable during the whole study period in comparison with the preinduction values. This was also the case for the calculated creatinine clearance by means of Cockroft- Gault equation and calculated cys C clearance using the Larsson formula.

Table 1

Clinical Data of the 49 patients enrolled in the study

Variable	Mean ± SD	Median	Minimum/maximum
Age (Y)	60 ± 9	60	40/78
Weight (kg)	81,5 ± 14,3	80,0	50/118
Height (cm)	169 ± 10	170	144/191
BMI (kg/cm²)	28,2 ± 4,4	28,1	18.5/39.7
Ejection fraction (%)	62,8 ± 9,7	63,0	40/82
Cr Clearance(ml/min)	113 ± 31	114.2	65/253
CPB time (min)	99 ± 27	100	23/165
Cross-clamp time (min)	73 ± 22	77	20/120
N° systems involved (*)	2,3 ± 0,75	2	1/4
N° of proximal grafts	0,78 ± 0,80	1	0/3
N° of distal grafts	3,6 ± 0,99	4	1/5
N° of arterial conduits	2,06 ± 0,63	2	1/3
Initial HCT	41,3 ± 3,6	42,0	31/50
Lowest HCT	26,2 ± 4,4	27,0	15/38
Initial plasma proteins (g/dL)	7,15 ± 0,33	7,2	6.5/7.8
Post-CPB plasma proteins (g/dL)	4,19 ± 0,49	4,1	3.4/5.3
Reexploration for bleeding	2		
N° of units PRB transfused (n° of patients transfused = 18)	0,87 ± 1,39	0	0/6

(*) – neurologic – endocrine – epithelial
– respiratory – gastro-intestinal
– cardiovascular – orthopedic / muscular

Y : years ; min : minutes ; HCT : hematocrit ; PRB : packed red blood cells.

Table 2

Outcome Data

Variable	Mean ± SD	Median	Minimum/maximum
LOS in ICU (hours)	41 ± 16	41	20/93
Postoperative LOS (days)	10 ± 3	8	6/63
Dialysis			0
Death			1

LOS : length of stay.

Table 3

Mean values of Cr (mg/dL) and cys C (mg/L) with their standard deviations.
Significant differences were considered when p value was < 0.01

	T0	T1	T2	T3	T4	T5	T6
Cr values	0.86 ± 0.18	0.94 ± 0.20	0.97 ± 0.21	1.03 ± 0.23	1.00 ± 0.27	0.98 ± 0.35	0.94 ± 0.28
cys C values	0.93 ± 0.17	0.88 ± 0.19	0.88 ± 0.18	0.97 ± 0.26	0.92 ± 0.30	0.93 ± 0.29	0.94 ± 0.27

* P < 0.01

T0 : preinduction ; T1 : 1 hour into CPB ; T2 : post protamine injection ; T3 : 6 hours postoperatively ; T4 : 24 hours postoperatively ; T5 : 48 hours postoperatively ; T6 : 72 hours postoperatively.

DISCUSSION

Renal dysfunction following cardiac surgery is multifactorial. It is most commonly attributed to perioperative low cardiac output and/or to renal damages as a consequence of CPB.

Free plasma hemoglobin, elastase and endothelin, free radicals including superoxide, hydrogen peroxide and the hydroxyl radicals can be generated during CPB, and can induce injury to the renal brush border membrane (11). Inadequate flow and or low systemic perfusion pressure during CPB are associated with sympathetic and hormonal mediation of vasomotor activity which may adversely influence postoperative cardiac and renal function (12).

Up to date the serum Cr concentration and the calculated Cr-Cl are tests used routinely to detect any renal dysfunction. However, they are influenced by many external and internal factors. Creatinine is influenced by age, gender, physical activity, muscle mass and diet (13). Plasma Cr concentration shows a poor correlation with GFR when renal function changes rapidly. Furthermore, a small and non-constant amount is excreted by the organic cation secretory system in the proximal tubule. Therefore the amount of Cr found in the urine exceeds the amount expected from filtration alone by 10% (14). Cr-Cl has also several disadvantages. It may underestimate GFR and thus overestimate risk in obese patients and in patients with very low plasma Cr levels. On the other hand it underestimates risk in hemodynamically unstable patients with acute renal injury, since plasma Cr level may not have the time to reach its peak (15).

Cys C could appear to be a better marker. In a meta-analysis, DHARNIDHARKA *et al.* (16) have demonstreated that cys C is superior to serum creatinine as a marker of kidney function. To our knowledge, 2 studies in cardiac surgery have already been done with cys C as a marker of GFR (17, 18). In contrast with the 2 other studies, we decided to compare the two renal function markers in a population of patients with normal preoperative renal and cardiac function undergoing CABG with CPB. Our results differ from those of ABU-OMAR *et al.* (17), where the serum Cr and cys C concentrations peaked at day 2 postoperatively. Our results show no significant increase of the measured cys C and Cr at any time. We further decided to look at calculated clearance of creatinine and cys C to estimate the GFR during the study period. To do so, we calculated the Cr-Cl using the Cockroft- Gault equation and cys C clearance by means of Larsson equation. This equation transforms cys C expressed as mg/L to GFR expressed as ml/min. For the Dade Behring calibration of the cys C method (mg/L) used in this study, the following formula was used : $Y = 77{,}24\ X^{-1{,}2623}$. Again, the values of the two clearances were comparable during the whole study period.

There are several limitations to our study. The tubular function has not been studied. As already mentioned cys C is almost completely catabolised by proximal tubular cells. The non-metabolised cys C fraction is eliminated in urine and may represent a useful marker of tubular injury or renal tubular dysfunction (19). Alterations of tubular function

Table 4

Mean values of calculated creatinine clearance (Cr-Cl) and cystatin C clearance (cys C-Cl) in ml/min. Cr-Cl was calculated using the Cockroft-Gault equation and cys C-Cl using the Larsson equation. Significant differences were considered when p value was < 0.01

	T0	T1	T2	T3	T4	T5	T6
Cr-Cl	147 ± 44	135 ± 35	132 ± 34	125 ± 40	131 ± 43	137 ± 47	143 ± 46
cys C-Cl	88 ± 19	96 ± 25	95 ± 22	88 ± 25	96 ± 30	93 ± 28	90 ± 26

* P < 0.01

T0 : preinduction ; T1 : 1 hour into CPB ; T2 : post protamine injection ; T3 : 6 hours postoperatively ; T4 : 24 hours postoperatively ; T5 : 48 hours postoperatively ; T6 : 72 hours postoperatively.

can also be evaluated by assessing other low molecular weight proteins and enzymes. Urinary levels of α_1-microglobulin, β_2-microglobulin and N-acetyl-D-glucosaminidase are other sensitive markers of renal tubular injury. However, there is no evidence that perioperative increases of these markers are associated with postoperative morbidity or mortality (20).

Antifibrinolytics (e.g. ε–aminocaproic acid, tranexamic acid, and aprotinin) often used in cardiac surgery cause microglobulinuria independent of renal injury (21). The renal metabolism of albumin and other low molecular weight proteins (e.g. lysozyme, ribonuclease, retinol binding protein) are also influenced by antifibrinolytic therapy, and similar concerns about their use as markers of proximal tubular insult apply. MERCIERI *et al.* (18) have already demonstrated that high-dose aprotinin without other nephrotoxic agents administered to patients undergoing CABG with CPB, does not alter cys C metabolism.

The anesthesiologist was free to choose the anaesthesia technique. The so called fluoride-related toxicity of sevoflurane has never been observed, neither in clinical or in animal studies, nor after prolonged administration in patients with preexisting renal disease. The only proven direct toxic effect of any anaesthetic agent is the fluoride-related toxicity of methoxyflurane (22, 23).

Finally in our study, we only enrolled a population of patients with normal preoperative renal function.

So far, in patients with normal preoperative renal function undergoing CABG with CPB, cys C seems comparable to plasma creatinine as an indicator of glomerular function. Our results in contrast with those of ABU-OMAR *et al.* did not show any changes in the perioperative period. This could be the result of different surgical and bypass techniques. Considering the on-pump group of Abu-Omar, we used different bypass technique and consequently different organ protection was achieved. Different bypass temperature and/or myocardial protection techniques could alter the urinary homeostasis in different ways. Important factors that contribute to postoperative renal dysfunction are CPB duration, CPB perfusion flow and mean arterial blood pressure during bypass (24).

The other difference between the 2 studies, is that all our patients had normal preoperative renal and cardiac function. Patients with impaired cardiac function are at increased risk for perioperative and postoperative low cardiac output. Intra- and postoperative low cardiac output have clearly been shown as independent predictors for postoperative renal replacement therapy (25, 26).

In summary, creatinine measurement is still a cheap, easy and reliable way of estimating renal function in patients with normal preoperative renal function undergoing cardiac surgery with CPB.

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