A diagnosis of a renal injury by early biomarkers in patients exposed to cardiopulmonary bypass during cardiac surgery

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ABSTRACT

We prospectively studied renal function in 158 patients scheduled for elective cardiac surgery with the use of cardiopulmonary bypass (CPB).

The patients involved in this study had normal renal function as well as normal function of the left ventricle. The results of the study showed a statistically significant increase of early markers of renal injury Alpha-1-Microglobulin (A1M) and Neutrophil Gelatinase-Associated Lipocalin (NGAL), which were being traced in the patients' urine 5 hours and 24 hours after CPB. In contrast with the aforementioned early markers, the so-called "classical" markers of renal injury – serum urea and creatinine – did not show a statistical significance of value increase after CPB. Using early factors of renal injury A1M and NGAL, the study managed to show slight, subclinical injuries of the proximal renal tubules after CPB and cardiac surgeries. The value of these factors lies in their early and precise detection of renal injury, which is a significant clinical parameter for monitoring renal function, especially after cardiac surgery with the use of CPB.

Key words: renal injury, cardiopulmonary bypass, cardiac surgery, markers of tubular renal injury, alpha-1-microglobulin, neutrophil gelatinase associated lipocalin

Introduction

A great number of routine cardiac surgeries are carried out with the use of a system for cardiopulmonary bypass (CPB). The system replaces the function of heart and lungs during a surgery. The technique of the system is prosperous and is adjusted to a great extent to the physiological functions of our organism. In spite of this, CPB has unwanted effects upon physiological functions of our organism. (1-5), including an effects upon possible renal injury. (6-10) The frequency of acute renal failure after cardiac surgeries with the use of CPB is between 1 and 5%. The frequency of subclinical renal injuries is greater, but cannot be proved by routine traditional tests for the time being. These traditional tests are the so-called tests for diagnosis of renal injury, serum urea and creatinine, creatinine clearance and the analysis of the urine's sediment. (11,12) Initial tubule-interstitial changes are revealed with difficulty with the use of these traditional tests, although the changes can be revealed in greater renal injuries. Subclinical renal injuries, especially of renal tubules, can be proved by early markers. (13-15) Two of the aforementioned markers are highly specific early markers of proximal renal tubules injuries: Alpha-1-Microglobulin (A1M) and Neutrophil Gelatinase-Associated Lipocalin (NGAL), which are determined in urine. (16-19)

This paper primarily studied unwanted effects of CPB upon renal injury – that is, the function of proximal renal tubules. To define renal injury, early markers of renal tubules' injuries in urine (NGAL and AIM) were determined. In order to compare them, the traditional tests of urea and creatinine were determined in serum.

Materials and methods

Under approval by the Ethical Committee of the Medical Faculty Zagreb, and the Ethical Committee of the Clinical Hospital Zagreb, 158 patients scheduled for elective cardiac surgery with CPB were studied prospectively (with previously informed consent) between November 2012 to June 2013.
The selected patients had normal renal function and normal left ventricular

ejection fraction, and had not been exposed recently to nephrotoxic drugs (including radiographic contrast media, antibiotics etc.). Emergency surgery, hemodynamic instability, use of inotropes and associated diseases, including diabetes mellitus, were considered as causes for exclusion. The patients' ages ranged from 18 and 83 years, with a mean of 63 years $\pm$ SD 13.6.

Patients were pre-medicated with 0.1 mg/kg morphine sulphate. Anaesthesia was induced with fentanyl 20-40 μg/kg, ethomidat 10-20 mg and rocuronium 0.1 mg/kg and was maintained with sevoflurane in oxygen, as well as additional sufentanyl and rocuronium. Monitoring was completed after induction and included invasive arterial pressure, a thermodilution pulmonary artery catheter in some cases, a urinary bladder catheter and a transesophageal echo. Non-pulsatile CPB was performed with a roller pump and membrane oxygenator ("Medtronic Affinity" and "Euroset Admiral") primed with 1600 ml of prime solution. The pump flow was 2.2 L x min^{-1} x m^{-2}. Moderate hypothermia (26-28 °C) was used in all patients. Cardioplegic solution was intermittently infused. Alpha stat Ph. management was used for acid-base management.

The main arterial pressure during the bypass was allowed to vary between 55-100 mm Hg (mean 73.29 mmHg $\pm$ SD 7.31). Norepinephrine or nitroglycerine was used to correct deviations beyond this range. Inotropes were not used intra-operatively or early post-operatively in any of the study patients. Blood and urine samples were obtained at the following times: 1) baseline measurements: blood samples for urea and creatinine measurements taken between 1 to 2 days before the operation; urine samples for NGAL and A1M were taken after induction of anaesthesia. 2) 5 hours post-operatively: urine samples for NGAL and A1M. 3) 24 hours post-operatively: urine samples for NGAL and A1M and blood samples for urea and creatinine. 4) 48 hours post-operatively: blood samples for urea and creatinine. Urine was collected using a Foley catheter.

Until the determination of the quantity of A1M in the laboratory, all the urine samples were marked in plastic test tubes and were kept in a refrigerator at a temperature of -18 °C. A series of 50 samples were determined in the laboratory. Until the determination of the quantity of NGAL in the laboratory all the urine samples were marked in

plastic test tubes and centrifugated. After being centrifugated, which lasted 5 minutes at >400 RCF (Relative centrifugal Force), the supernatant was taken and was separated into marked plastic test tubes which had been, after marking, kept in a refrigerator at a temperature of -70 °C. A series of 80 samples were determined in the laboratory. Serum samples were immediately determined in the laboratory after being taken and it was not necessary to keep them. Urea concentrations were measured by a Kinetic UV test with urease (Olympus AU 2700, Japan). Serum urea values were expressed in mmol/L and the normal value is 2,.8-8.3 mmol/L. Creatinine concentrations were measured by continuous photometry with alcal picrate (Olympus AU 2700, Japan). Serum creatinine values were expressed in μmol/L and the normal value was 79-125 μmol/L for male patients and 63-107 μmol/L for female patients. Urine concentration of NGAL was measured by the CMIA – Chemiluminescent Microparticle Immunoassay (Abbot Diagnostic, Architect 1000 SR, Wiesbaden, Germany). NGAL values were expressed in ng/mL and the normal value was below 132 ng/mL. Urine concentration of A1M was measured by

Table 1. Descriptive statistics of renal injury markers in urine and serum in a total sample

| | N | Mean | SD | Min | Max | Percentile | | |
						25.	Median	75.
A1M before CPB	150	19.74	14.00	5.56	92.76	8.54	17.63	25.72
A1M 5 h after CPB	150	28.25	37.70	5.56	219.00	5.56	14.60	32.48
A1M 24 h after CPB	150	35.75	25.35	5.56	146.00	20.65	31.40	45.30
NGAL before CPB	66	15.87	18.38	1.70	145.70	7.47	11.35	18.18
NGAL 5 h after CPB	66	102.04	342.57	0.00	2305.50	2.68	6.20	15.35
NGAL 24 h after CPB	66	35.26	29.31	1.00	115.00	14.28	23.45	46.60
Urea before CPB	158	6.31	1.83	2.70	12.10	4.90	6.15	7.50
Urea 24 h after CPB	158	5.36	2.19	1.40	12.00	3.70	5.00	6.55
Urea 48 h after CPB	158	5.91	2.64	0.80	16.00	4.00	5.55	7.03
Kreatinin before CPB	158	102.49	20.30	57.00	169.00	89.00	103.00	113.25
Kreatinin 24 h after CPB	158	109.42	25.87	67.00	225.00	90.00	105.00	124.00
Kreatinin 48 h after CPB	158	109.18	30.82	63.00	272.00	87.75	105.00	123.00

A1M, alpha-1-microglobulin; CBP, cardiopulmonary bypass; NGAL, neutrophil gelatinase-associated lipocalin

Table 2. A1M and NGAL levels after the end of extracorporeal circulation depending on time dynamics: Friedman's test

	N	Percentile			Mean rank	Values of testing	P
		25.	Median	75.			
A1M before CPB	150	8.54	17.63	25.72	1.66		
A1M 5 h after CPB	150	5.56	14.60	32.48	1.70	100.64	<0.001
A1M 24 h after CPB	150	20.65	31.40	45.30	2.64		
NGAL before CPB	66	7.47	11.35	18.18	1.85		
NGAL 5 h after CPB	66	2.68	6.20	15.35	1.50	46.03	<0.001
NGAL 24 h after CPB	66	14.28	23.45	46.60	2.65		

A1M, alpha-1-microglobulin; CBP, cardiopulmonary bypass; NGAL, neutrophil gelatinase-associated lipocalin

the imunonefelometric method (Behring Werke, Marburg, Germany). A1M values were expressed in mg/L and the normal value was <15 mg/L.

The data are presented in the tables and graphically. Using Kolmogorov-Smirnovljev's test, an analysis of the normality of data distribution was carried out. According to the obtained data, corresponding non-parameter tests were used in a further analysis. Differences in dependent measures were analysed by Friedman's test. The level of significance was 0.05. The STATISTICA programme support (version 10.0) was used in the analysis (www.statsoft.com).

Results

Table 1 shows the descriptive statistics of renal injury markers. A1M was determined in all patients and NGAL was determined in 66 patients. Urea and creatinine were determined in 8 patients more in contrast with A1M because in these patients A1M samples were incorrectly taken.

Table 1 shows A1M and NGAL increase after ECC in relation to the initial values before surgery (that is, before CPB). The so-called "classic" renal injury markers (serum urea and creatinine) do not show any dynamics of increase.

Using Friedman's test, the statistical significance of A1M and NGAL levels were determined, depending on time dynamics (table 2).

Table 2 shows the statistical significance (P <0.001) in both studied markers. Using the same test, urea and creatinine values do not show a statistically significant difference depending on time dynamics (for creatinine P = 0.034). By studying certain A1M values one can notice that they are slightly above normal values, thus confirming a slight renal injury. NGAL values are not higher than normal values. However, one can notice their increase, so we can talk about a slight subclinical renal injury.

Discussion

All the patients in the study had normal renal function before a surgery and CPB.

Not a single patient developed acute renal failure. By traditional "classic" renal injury markers – urea and creatinine – the study did not show renal injury caused by CPB. Using early factors of renal injury, A1M and NGAL, the study showed slight subclinical renal tubular injury after CPB and cardiac surgeries. The definition of acute kidney injury (AKI) is difficult. Results of many studies that investigate biomarkers of renal injury are difficult to extend because of important differences in AKI definitions, measurement methods, timing, population and several possible confounding factors. (20) For example, A1M and NGAL are markers of tissue damage and proximal tubular stress, but not of kidney function of glomerular

filtration rate like creatinine and urine output. AKI in cardiac surgery patients is multifactorial including factors such hypovolemia, hypotension, chronic kidney disease and drug toxicity, that occur at differential timing before, during and after surgery. (21) Today, a large number of clinical studies and reviews indicate that A1M and NGAL should be considered as reliable diagnostic and prognostic biomarkers for kidney injury. Reference ranges, adjusted for age, gender and ethnicity, as well as reliable cut-off values calculated on large patient populations, for ruling in and out AKI, are still lacking. (22) Ideally, the performance of the biomarkers should be tested in addition to the clinical and laboratory evaluations available in current clinical practice. There is a consensus that most of the emerging kidney injury biomarkers may be able to detect injury earlier than serum creatinine and urea. Biomarker performance is measured by assessing the diagnostic or predictive performance based on the actual standard, serum creatinine. (23)

Conclusion

The value of the early factors of renal tubular injury that were studied lies in their early detection of renal injury, which is a new additional clinical parameter for monitoring renal function, especially after cardiac surgeries with the use of CPB.

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