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Preoperative Endogenous Ouabain Predicts Acute Kidney Injury in Cardiac Surgery Patients

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Abstract

Objectives—Acute kidney injury is a frequent complication of cardiac surgery and increases morbidity and mortality. As preoperative biomarkers predicting the development of acute kidney injury are not available, we have tested the hypothesis that preoperative plasma levels of endogenous ouabain may function as this type of biomarker.

Rationale and Design—Endogenous ouabain is an adrenal stress hormone associated with adverse cardiovascular outcomes. Its involvement in acute kidney injury is unknown. With studies in patients and animal settings, including isolated podocytes, we tested the above mentioned hypothesis.

Patients—Preoperative endogenous ouabain was measured in 407 patients admitted for elective cardiac surgery and in a validation population of 219 other patients. We also studied the effect of prolonged elevations of circulating exogenous ouabain on renal parameters in rats and the influence of ouabain on podocyte proteins both “in vivo” and “in vitro.”

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Main Results—In the first group of patients, acute kidney injury (2.8%, 8.3%, 20.3%, $p < 0.001$) and ICU stay (1.4 ± 0.38 , 1.7 ± 0.41 , 2.4 ± 0.59 days, $p = 0.014$) increased with each incremental preoperative endogenous ouabain tertile. In a linear regression analysis, the circulating endogenous ouabain value before surgery was the strongest predictor of acute kidney injury. In the validation cohort, acute kidney injury (0%, 5.9%, 8.2%, $p < 0.0001$) and ICU stay (1.2 ± 0.09 , 1.4 ± 0.23 , 2.2 ± 0.77 days, $p = 0.003$) increased with the preoperative endogenous ouabain tertile. Values for preoperative endogenous ouabain significantly improved (area under curve: 0.85) risk prediction over the clinical score alone as measured by integrate discrimination improvement and net reclassification improvement. Finally, in the rat model, elevated circulating ouabain reduced creatinine clearance (-18% , $p < 0.05$), increased urinary protein excretion ($+54\%$, $p < 0.05$), and reduced expression of podocyte nephrin (-29% , $p < 0.01$). This last finding was replicated ex vivo by incubating podocyte primary cell cultures with low-dose ouabain.

Conclusions—Preoperative plasma endogenous ouabain levels are powerful biomarkers of acute kidney injury and postoperative complications and may be a direct cause of podocyte damage.

Keywords

critically ill; Na pump inhibitor; Na, K-ATPase; renal damage

Approximately 2 million cardiac surgeries are performed around the world each year. Acute kidney injury (AKI) is a frequent complication of cardiac surgery (1). Patients who double their serum creatinine or need acute dialysis have a two- to five-fold higher risk of death (2, 3), and patients who recover their kidney function remain at increased risk of chronic kidney disease (CKD) and premature death (4, 5). A number of novel plasma and urinary early postoperative biomarkers have recently been proposed to assess the risk of postoperative AKI on the assumption that early detection of renal damage may limit its progression to overt renal failure with appropriate therapeutic interventions (6). However, there are neither preoperative biomarkers nor robust validated risk models that predict AKI not requiring dialysis. Preoperative biomarkers offer the potential to target and develop therapeutic intervention aimed at blocking the initial sequence of events that lead to AKI (7).

Certainly AKI is multifaceted in nature with derangements at various intrarenal sites (glomerular and tubular) and is triggered by a variety of factors including reduced cardiac output, systemic hypotension, and activation of neuroendocrine reflexes (8, 9). Endogenous ouabain (EO) is a neuroendocrine hormone that may contribute to the development of AKI in critically ill patients. Numerous human and intact animal studies, as well as adrenocortical cell culture studies, indicate that EO is synthesized in the adrenal cortex (10–13). EO may be considered a stress hormone secreted by the adrenal gland since a) normal rats after exposure to acute swim stress have significant increases of EO in plasma and adrenal glands (14); b) marked and rapid increases of plasma EO in humans during physical exercise have been reported (15); c) about 50% of humans with untreated essential hypertension have significantly elevated plasma EO that correlates directly with blood pressure (BP [16–18]); d) increased circulating EO has been related to cardiomyopathy (19) and decreased renal function (20, 21); and e) different types of endogenous glycosides, including EO, are elevated in a large proportion of critically ill patients. The occurrence of these substances is associated with increased morbidity and hospital mortality rates (22).

EO modulates the activity of the Na,K-ATPase and induces signal transduction via sodium-calcium exchange and the Src-dependent pathway (23). EO exerts a biphasic effect on Na,K-ATPase, either stimulating or inhibiting its activity at low (subnanomolar) or high (nanomolar) concentrations, respectively (24, 25).

The accumulating clinical and experimental data show that prolonged exposure to elevated levels of EO in vivo induces underlying kidney damage that is not clinically obvious. We propose that when individuals with elevated preoperative EO and with or without underlying kidney damage are exposed to the acute stress of surgery, further elevations of circulating EO occur and contribute directly to AKI.

This hypothesis was tested in three settings:

1. By measuring EO levels in patients before cardiac surgery and correlating these levels to the outcome after surgery including AKI;
2. By infusing ouabain in rats and measuring BP, renal function, and podocyte proteins;
3. By measuring podocyte proteins in primary culture incubated with and without ouabain.

Therefore, the rationale underlying this approach was that high levels of EO may favor the development of AKI and do so by direct actions that lead to damage of podocytes.

Methods

Study Population

We prospectively enrolled adults undergoing cardiac surgery (coronary artery bypass graft or valve surgery). We excluded patients with evidence of AKI before surgery, prior kidney transplantation, preoperative serum creatinine level greater than 4.5 mg/dL (> 177 mmol/L), or end-stage renal disease (estimated glomerular filtration rate [eGFR] < 30 mL/min). Participants with multiple surgeries could only be enrolled in the study once. All participants provided written informed consent, and each institution's research ethics board approved the study.

First Cardiac Surgery Cohort—Four hundred and seven patients undergoing elective cardiac surgery were enrolled in the study. This was a prospective observational study conducted at a single center from December 2009 to June 2010.

Sample Collection—We collected urine and plasma specimens preoperatively and daily for up to five postoperative days. The first postoperative samples were collected soon after admission to the hospital. In addition to routine preoperative assessments, blood samples were obtained for plasma EO determinations within 24 hrs from admission to the clinic and for the first 24 hrs postoperatively. Samples were stored at -80°C until analysis. EO was extracted from plasma and measured by using a specific radioimmunoassay (see Supplemental Methods, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>, for details) (25).

Data Collection—An investigator blinded to the plasma biomarker concentration collected data from patient chart notes and the computerized data system. Disease severity was scored according to European System for Cardiac Operative Risk Evaluation (EUROSCORE [26]) and age, creatinine, and left ventricular ejection fraction (ACEF [27]) clinical scores (sex, age, creatinine, and left ventricular ejection fraction). eGFR was calculated using the standard value CKD–epidemiology collaboration equations (28).

Validation Cardiac Surgery Cohort

Two hundred and nineteen patients undergoing elective cardiac surgery were enrolled in the study after signing informed consent. This was a prospective observational validation study conducted from January 2011 to June 2011.

Outcome Definitions

The primary outcome was the development of severe AKI, defined by receipt of acute dialysis during the entire hospital stay or a doubling in serum creatinine from the baseline preoperative value (consistent with the Acute Kidney Injury Network (AKIN [29]) stage 2 or 3). All of the preoperative creatinine values were measured within 2 months before surgery. Additional clinical outcomes were in-hospital mortality, length of in-hospital and ICU stay, and necessity of renal replacement therapy (RRT).

Preoperative Risk Predictors

The clinical risk model of AKI considered the following variables: age, sex, preoperative ejection fraction (EF) basal eGFR, surgery type, hypertension, diabetes mellitus (DM), and redo-intervention. Even when some of these elements (like EF, history of hypertension, or DM) did not have a significant impact on the risk of AKI (logistic regression coefficients with a p value > 0.1), we preferred to keep all variables in the clinical model. On the basis of the value of their logistic regression coefficient, a severe AKI risk score (clinical model-acute kidney injury, CLIN-AKI) was developed and tested for accuracy using logistic regression analysis, Hosmer-Lemeshow statistics, and receiver operating characteristic (ROC) analysis. A further severe AKI risk score (preoperative endogenous ouabain added to clinical model-acute kidney injury, CLIN-EO-AKI) was developed by adding preoperative EO values to the CLIN-AKI score. Differences among areas under curve (AUCs) were tested with StAR (Statistical Analysis of ROC Curves). StAR is a server that computes ROC curves and several related statistics to assess the significance of their differences in performance.

Experimental Model

Ouabain-infused rats (OHR) were generated by subcutaneous ouabain infusion (Sprague-Dawley rats), of male rats (5–6-week old, weighing 120–130 g) with osmotic minipumps (Alzet, Charles River, Calco, Italy) containing a ouabain-saline solution that slowly released 15 $\mu\text{g/kg/day}$ ouabain (Sigma-Aldrich; $n = 7$) with osmotic minipumps for 8 weeks as previously described (17). The initial systolic blood pressure (SBP) of controls and OHR rats was comparable (average: 130–135 mm Hg). After 8 weeks of ouabain infusion, plasma levels were doubled, and SBP was significantly elevated in OHR rats (171 ± 1.3 mm Hg) vs. controls (150 ± 1.9 mm Hg). Heart rate was not different in either group (controls: 364 ± 4.3 , OHR: 374 ± 4.8 beats/min).

Statistical Analysis

The continuous data are expressed as mean $\pm$ SD. Dichotomous variables are presented as percentages. Whenever normality assumption was not met, nonparametric tests were applied. In particular to promote normality and linearity, we transformed EO (25) using the logarithmic scale. Geometric means and interquartile ranges (IQR) are presented. To compare continuous variables among EO tertiles we applied analysis of variance (ANOVA) test (along with Bonferroni post hoc analysis), where normality assumption was met. Otherwise, we applied Kruskal-Wallis tests. Chi-square analysis or Fisher's test, when appropriate, were used to compare discrete variables. Mann-Whitney test was used to compare EO levels among AKI groups. Pearson's correlation test and linear regression were applied to evaluate the relations among continuous variables. Spearman's correlation test

was applied for not normally distributed variables. Logistic regression analysis was used to estimate relative risk of AKI and hospital death in association with the EO tertiles and clinical variables and also to build the CLIN-AKI and CLIN-EO-AKI models.

The ROC curve was used to examine the performance of CLIN-AKI and CLIN-EO-AKI to predict AKI. The curve represents a plot of sensitivity vs. 1-specificity. The AUC (i.e., C-index) was calculated from the ROC curve. A statistically derived value, based on the Youden index (30), maximizing the sum of the sensitivity and specificity was used to define the optimal cutoff value. The differences between AUC (C-index) were tested by the StAR program in order to examine whether the addition of EO improved the discrimination of the model (31). The increased discriminative value of the scores was further examined by calculation of net reclassification improvement (NRI) and integrated discrimination improvement (IDI) indices described by Pencina et al (32). NRI and IDI are two new measures to quantify the degree of correct reclassification. NRI is the measure of net reclassification improvement gained with the CLIN-EO-AKI score compared with CLIN-AKI model between AKI and non-AKI patients. We used binary recode according to Youden index for CLIN-AKI (−0.04) and CLIN-EO-AKI score (6.65). IDI can be seen as continuous version of NRI with probability differences used instead of categories. Alternatively, it can be defined as a difference in discrimination slopes (Yates slopes [33]) between models.

A two-sided *p* value of less than 0.05 was considered to indicate statistical significance. All analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL).

Results

Cardiac Surgery Patients, First Group

Preoperative and postoperative patient characteristics are presented in the Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>. Cardiopulmonary bypass was used in 94.3% of 407 cardiac surgery patients. Custodial cardioplegia was used for 81% of patients, and in the remaining patients Buckberg cardioplegia was used (see Supplemental Table 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>). The prevalence of severe AKI was 10.3%. Furthermore, 33.3% of the patients that developed AKI needed RRT (3.4% of total). All of the patients who died (2.4%) had developed AKI.

Preoperative plasma EO levels, in patients with or without AKI were 238.3 (IQR: 172.7–336.5) and 150.9 (IQR: 103.5–223.0) pmol/L, respectively ($p < 0.001$, Mann-Whitney test). Preoperative EO values were highly correlated (correlation coefficient = 0.51, $p < 0.0001$) to postoperative values. Postoperative plasma creatinine was related to baseline EO concentrations (correlation coefficient = 0.22, $p < 0.0001$) and increased with each tertile of baseline EO ($p < 0.0001$, Kruskal-Wallis tests; Table 1). The relation between EO and plasma creatinine is shown in Figure 1A. Patients in the highest EO tertile (plasma EO > 207 pmol/L) displayed greater increases in plasma creatinine ($p < 0.0001$, Kruskal-Wallis tests). As shown in Figure 1, with each incrementing EO tertile, patients had increasing prevalence of AKI (Table 1; 2.8%, 8.3%, 20.3% chisquared $p < 0.0001$), ICU stay (Fig. 1B), and RRT (Fig. 1C), similar to those reported by other ICU studies (34). Multivariate logistical regression analysis showed that the highest preoperative baseline EO tertiles were associated with AKI after adjustments for covariates including age, basal eGFR, EF, hypertension, diabetes, surgery type, and redo-intervention (Table 2). Strikingly, neither EUROSCORE nor ACEF remained a significant predictor for AKI after correction for covariates. Following cardiac surgery, death occurred within 30 days in 6 (4.5%) of 133 patients in the third EO tertile group, while in 4 (1.5%) of 274 patients in the first and second EO tertile

group (Fisher's test, $p = 0.068$). The AUC of preoperative EO for the diagnosis of AKI was 0.73 (95% CI: 0.65–0.81; $p < 0.0001$).

Validation Cardiac Surgery Cohort

Two hundred and nineteen consecutive patients were included in the validation group, preoperative and postoperative clinical characteristics are shown in Supplemental Table 2 (Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>). The prevalence of severe AKI was 11.4% and 8% of patients that developed AKI needed RRT (0.9% of total). Preoperative plasma EO was higher (232.2 pmol/L, IQR: 198.5–279.7) in those patients that developed severe AKI than those without (169.0 pmol/L, IQR: 108.1–205.1, $p < 0.0001$, Median e Kruskal-Wallis test). As reported in Table 3, cardiac surgery patients grouped according to preoperative EO tertiles were older, with a lower EF, and increased CKD. Operative and postoperative parameters examined are presented in Table 3. The relation between EO and plasma creatinine is shown in Figure 2A. Patients in the highest EO tertile (plasma EO > 195 pmol/L) displayed greater increases in plasma creatinine (ANOVA, $p < 0.0001$, Bonferroni post hoc test: first vs. second and third tertile, $p < 0.0001$). With each incrementing EO tertile, patients had increasing values of plasma creatinine (Fig. 2A), length of hospital stay (Fig. 2D), and ICU stay (Fig. 2C). In this cohort, we also measured EO after 6 and 12 hrs after surgery. EO circulating levels increased ($p < 0.0001$) in the third tertile 6 and 12 hrs after surgery, while plasma levels decreased in the first and second tertiles, as shown in Figure 2B. Multivariate logistic regression analysis showed (Table 4) that the preoperative EO level was associated with AKI. The associations remained independently significant after adjustments for covariates (see below). The AUC of preoperative EO for the diagnosis of AKI was 0.80 (CI 95%: 0.74–0.87; $p < 0.0001$).

Cumulative Analysis AKI Prognosis

The AUC of preoperative EO in the 626 cardiac surgery patients for diagnosis of severe AKI (10.7%) was 0.75 (95 % CI: 0.69–0.81). CLIN-AKI has been obtained by adding the β value obtained by logistical regression with severe AKI (Supplemental Table 3, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>) including age, sex, surgery type and reintervention, preoperative EF, preoperative eGFR, and hypertension (AUC: 0.79;95% CI: 0.73–0.84). Even though many of these variables were significantly associated with AKI in our population, we preferred to keep these variables in our clinical model for clinical relevance. Adding the preoperative EO level to the clinical model increased the AUC to 0.85 (95% CI: 0.81–0.90); this model we term *CLIN-EO-AKI score*. Figure 3 reports the ROC curves of the two risk scores for AKI. When patients with preoperative eGFR lower than 60 mL were excluded, all analyses yielded similar results (Supplemental Table 4, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>). These results strongly support the primacy of EO in determining AKI.

Risk Prediction

To evaluate the improvement of risk prediction with the addition of preoperative EO to the clinical model, we determined the IDI and NRI (35) (Supplemental Methods Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>). We binary categorized all patients as being at low or high AKI risk for both clinical models alone (CLIN-AKI) or with preoperative EO (CLIN-EO-AKI score). Inclusion of EO provided improved risk prediction over the clinical models alone: IDI 0.07 ($p < 0.0001$) and NRI 0.07 as shown in Table 5.

Experimental Rat Model

To investigate the in vivo adverse consequences of chronic exposure to high plasma EO levels, we studied the effects of infused exogenous ouabain in otherwise normal rats. As

previously described (17, 24), the chronic infusion (8 weeks) of low doses of ouabain (15µg/kg/day) in rats significantly raised SBP (Fig. 4A). Here, we show that, compared with their matched controls, the ouabain-infused rats exhibited a significant reduction of creatinine clearance (−18%, $p < 0.02$, Fig. 4B).

Urinary protein excretion was also increased in the ouabain-infused rats (+54%, $p < 0.05$) compared with controls (Fig. 4C) and a similar result was obtained after normalization of proteinuria for urinary creatinine excretion (controls: 0.986 ± 0.076 , ouabain: 1.643 ± 0.179 , $p < 0.01$). The immunohistochemical analysis of podocyte protein expression, evaluated in rat kidney preparations, revealed a statistical significance reduction ($p < 0.05$, ANOVA) of staining for nephrin (Fig. 4D) but not for synaptopodin (Fig. 4D) in ouabain rats as compared with controls. These data were confirmed by Western blot analysis performed on rat renal microsomes (Fig. 4E). A direct effect of ouabain on podocyte nephrin was demonstrated in primary cultured podocytes from 10 day-old rats. Ouabain decreased nephrin expression measured by Western blot analysis (Fig. 4F).

Discussion

The main new results of these studies, obtained in two independent cohorts of patients, are the identification of a novel preoperative biomarker for the AKI that often follows cardiac surgery. Increased preoperative circulating concentrations of EO were associated with a longer ICU stay, a higher severity of illness, and an increased prevalence of severe AKI. These results were replicated also after the exclusion of patients with preoperative mild renal failure. To our knowledge, this is the first study investigating the relationship between plasma EO and AKI.

Similar findings were also obtained in a critically ill cohort of patients admitted to the ICU (ICU cohort). In all patients, increased EO concentrations were invariably associated with a higher severity of illness, as measured by ICU stay, and an increased prevalence of acute renal dysfunction. Stress-related activation of the hypothalamic-pituitary-adrenal axis could explain the increased EO concentrations observed in this study (Supplemental Results, Supplemental Tables 5 and 6, and Supplemental Fig. 1, Supplemental Digital Content 1, <http://links.lww.com/CCM/A572>).

A strength of our study was that the prospective complete specimen collection was performed under standardized conditions in consecutive patients undergoing cardiac surgery in two large cohorts of Caucasians. AKI was defined according to modern AKI staging systems and as a useful endpoint for future therapeutic trials AKIN II (26, 27). Furthermore, preoperative EO was strongly related to hard outcomes including ICU stay, RRT, and in-hospital mortality. Thus, we have built a simple clinical model able to predict the risk of postoperative AKI (CLIN-AKI, AUC: 0.79). Furthermore, we found that the addition of preoperative EO to the clinical model (CLIN-EO-AKI) improves the predictive ability (AUC from 0.79 to 0.85; $p < 0.01$) and significantly increases the discriminating power of the model to predict AKI and the proper reclassification of a proportion of the patients.

Thus, we find that the adrenal hormone EO, released under stress conditions (15, 36), is able to identify those patients who have a higher probability to develop postoperative AKI. Furthermore, the experimental studies in rats indicate that small sustained increases of plasma ouabain (i.e., two-fold the basal level and similar to those observed in humans) is itself sufficient to trigger the deterioration of renal function as measured by increased plasma creatinine, reduction of creatinine clearance, increased proteinuria, and decreased expression of podocyte nephrin; the latter being a structural protein involved in the formation of the glomerular filtration barrier (37). The ouabain-induced reduction of nephrin

is likely to underlie the changes of the glomerular filtration barrier permeability leading to protein leakage; this effect was also demonstrated in primary cultures of glomerular podocyte cells incubated with ouabain. Taken together, these data demonstrate a direct effect of elevated circulating ouabain on glomerular function and podocyte protein expression. The added stress of surgery appears to further unmask the renal damage attributed to EO.

The mechanism of the ouabain effect is likely mediated by changes in cell Ca^{2+} . According to Pulina et al (38), nanomolar doses of ouabain, through the modulation of the $\text{Na}_2\text{K-ATPase}$, $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 (NCX1), and C-type transient receptor potential (TRPC6) signaling cascade, favor an increase of intracellular Ca^{++} . In turn, high Ca^{++} levels can activate the Ca^{++} -dependent protease, calpain (39), favoring nephrin protein cleavage (40) or, via NFkB, may activate the expression of the transcriptional regulator, Snail, repressing nephrin expression (41). Preliminary data in cultured podocytes (not shown) indicate that ouabain (10^{-9} , 10^{-8} M) increases calpain protein expression.

The reduction of glomerular filtration rate (GFR) by EO/ouabain, as previously shown in patients (21), may also occur as a consequence of direct vascular effects including increased myogenic tone and reactivity of mesenteric arteries (42) and of contractile smooth muscle cells within glomerular podocytes (43). Indeed, podocytes, which are specialized cells that form a complex interdigitating network that envelopes the glomerular capillary, possess a stretch-sensitive, Ca^{++} -activated K^+ channel (BK channels) that responds to changes in the glomerular capillary pressure, possibly affecting the GFR (44). Since nephrin binds and regulates BK channel expression (45), any change in nephrin protein level, such as those documented here by ouabain, may affect BK channel expression and consequently the GFR.

The potential limitations of this study are those present in similar studies that attempt to assess the clinical usefulness of nonpostoperative biomarkers. For example, we dichotomized patients with and without AKI by using an arbitrary cutoff (AKIN II), this cutoff does not reflect the spectrum of renal damage, which may follow cardiac surgery.

The multifaceted nature of AKI is likely to require multiple biomarkers to gain a truly comprehensive assessment of all the potential mechanisms. For these reasons, the preoperative markers described here may only be at work in a subset of patients.

The major clinical need for AKI is to identify a therapeutic window during which to apply a putative intervention aimed at preventing or limiting renal damage. Our data suggest that the measurements of plasma EO before surgery identify patients/individuals that would be expected to benefit from inhibition of EO action. Indeed an inhibitor of EO has been described (46) and may be worth testing in these patients. Accordingly, preoperative plasma EO levels will, on one hand, improve the prediction of AKI and, on the other, may help in developing a therapeutic intervention to minimize AKI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Cardiac surgery patients. (A) Plasma creatinine, (b) ICU stay, and (C) renal replacement therapy (RRT) grouped by the tertile of endogenous ouabain (EO). All 406 patients were divided according to their preoperative plasma EO levels. Plasma EO tertiles—First tertile: < 118, second tertile: 118–207, and third tertile: > 207 pmol/L, respectively. Those with EO greater than 207 pmol/L had a higher RRT and ICU stay. All data represent after correction for confounders. ANOVA = analysis of variance.

Figure 2.

Validation cardiac surgery cohort. **(A)** Plasma creatinine, **(B)** time course of endogenous ouabain (EO), **(C)** ICU stay, and **(D)** length of hospital stay (LHS) grouped by the tertile of EO. Those with third tertile had higher ICU stay and LHS. The time course of EO circulating levels were increased ($p < 0.0001$) in the third tertile after 6 and 12 hrs after surgery, while decreased in the first and second tertile **(B)**. All data represent after correction for confounders. ANOVA = analysis of variance.

Figure 3.

Cumulative analysis receiver operating characteristic (ROC) curves. ROC curves show the diagnostic performance preoperative plasma endogenous ouabain (EO) levels (blue line), clinical model-acute kidney injury (green line), and preoperative EO added to clinical model-acute kidney injury (red line) in the 626 cardiac surgery patients. The area under curve (AUC) of preoperative plasma EO levels was 0.75 (95% CI: 0.70–0.81). Adding preoperative EO to the clinical model AUC increased the AUC from 0.79 (95% CI: 0.73–0.84) to 0.85 (95% CI: 0.81–0.90, $p < 0.001$). CLIN-AKI = clinical model-acute kidney injury; CLIN-EO-AKI = preoperative EO added to clinical model-acute kidney injury; EO-AKI = endogenous ouabain-acute kidney injury.

Figure 4.

Effects of ouabain on renal function and podocyte protein expression in rats and cultured podocytes. Ouabain (15 $\mu\text{g/kg/day}$) was subcutaneously infused into Sprague-Dawley rats (ouabain-infused rats [OHR]) for 8 weeks ($n = 7$). Normotensive control rats received subcutaneous saline ($n = 7$). (A) Indirect systolic blood pressure, (B) creatinine clearance, and (C) urinary protein excretion were measured in controls and OHR rats. (D) Immunofluorescence analysis of the podocyte proteins nephrin and synaptopodin in kidney sections from controls ($n = 3$) and OHR rats ($n = 3$) is seen, magnification 1000 $\times$. (E) Western blot analysis of nephrin and synaptopodin in renal microsomes from controls and OHR rats (10 μg protein/lane). The densitometric analysis, reported as arbitrary units, was normalized for actin content. (F) Glomerular podocyte cultures from neonatal Sprague-Dawley rats were incubated with 10^{-11} – 10^{-8} M ouabain for 4 days. Nephrin and synaptopodin were quantified by Western blot. The densitometric analysis, reported as arbitrary units, was normalized for actin content. Data are mean $\pm$ SEM of two separate experiments done in triplicate; * $p < 0.05$ and ** $p < 0.01$. SBP = systolic blood pressure; contr = control; WB = Western blot.

Table 1
Clinical Characteristics of 407 Cardiac Surgery Patients Divided According to
Preoperative Endogenous Ouabain Tertile Values

	First EO Tertile (n = 136)	Second EO Tertile (n = 134)	Third EO Tertile (n = 137)
Preoperative			
Sex (female/male)	56/80	44/90	58/79
Age (yrs)	61.3 ± 13.7	62.0 ± 13.9	61.8 ± 13.9
Body mass index (kg/m ²)	25.3 ± 3.51	24.8 ± 3.84	25.0 ± 4.51
Ejection fraction (%)	58.5 ± 9.2	56.7 ± 11.0	57.1 ± 10.3
European System for Cardiac Operative Risk Evaluation	4.38 ± 3.69	4.30 ± 4.03	5.39 ± 6.57
Age, creatinine, and left ventricular ejection fraction	1.08 ± 0.32	1.15 ± 0.39	1.13 ± 0.38
Plasma creatinine (mg/dL)	0.86 ± 0.17	0.87 ± 0.19	0.91 ± 0.21
Estimated glomerular filtration rate (ml/m 1.73m ²)	77.1 ± 18.1	76.2 ± 20.3	74.1 ± 21.4
Plasma EO (pmol/L)	82.3 (51–116)	159.7 (127–197)	312.6 (219–524)
Hypertension (%)	13.3	17.7	14.0
Chronic kidney disease (%)	0.7	1.7	2.0
Diabetes (%)	1.7	5.2	3.4
New York Heart Association I. II. III. IV (%)	28/47/23/2	22/52/24/1	22/53/22/3
Operative			
Surgery time (min)	232.0 ± 60.3	262.7 ± 88.5	276.9 ± 77.4
Cardiopulmonary bypass time (min)	89.8 ± 32.1	93.2 ± 39.7	98.4 ± 35.2
Aortic cross-clamp time (min)	68.7 ± 25.4	70.8 ± 28.3	73.3 ± 23.8
Mean arterial pressure (mm Hg)	70.8 ± 5.8	72.2 ± 6.4	72.5 ± 6.9
Inotropes (%)	14.1	11.9	16.9
Diuretics (%)	1.7	2.1	2.6
Inotropic arterial blood pressure (%)	1	1.4	1.9
Transfusion (%)	5	5.5	5
Postoperative			
Plasma creatinine (mg/dL)	1.06 ± 0.43	1.14 ± 0.56	1.36 ± 0.72 ^a
Plasma EO (pmol/L)	206.1 (108–410)	251.3 (150–409)	360.3 (203–712) ^a
Troponin T-peak	2.30 ± 4.46	1.93 ± 2.46	2.29 ± 2.51
ICU stay (d)	1.44 (1, 2)	1.67 (1, 2)	2.26 (1–4) ^b
Length of hospital stay (d)	8.82 (6–12)	8.94 (7–12)	10.3 (7–14) ^b
Acute kidney injury (%)	2.8	8.3	20.3 ^a
Renal replacement therapy (%)	0.5	0.7	2.2 ^a
In-hospital mortality (%)	1.5	1.5	4.4

EO = endogenous ouabain.

Patient preoperative, operative, and postoperative characteristics according to EO tertiles are shown in the top, middle, and bottom sections, respectively. Values are mean ± SEM. Plasma EO is the geometric mean (interquartile range).

^aBonferroni post hoc test < 0.05 vs. first and second EO tertile.

^bLog-rank test median (95% CI), $p < 0.01$.

Table 2
First Cardiac Surgery Group

Event Category	Logistic Regression	Linear Regression		
	Acute Kidney Injury	ICU Stay		
	Relative Risk (95% Confidence Interval)	<i>p</i>	<i>b^a</i>	<i>p</i>
Sex	0.74 (0.32–1.75)	n.s.	−0.116	0.03
Age (yrs)	1.04 (1.00–1.09)	0.07	−0.071	n.s.
Redo	3.34 (1.29–8.67)	0.01	0.094	n.s.
Hypertension	1.35 (0.59–3.07)	n.s.	0.009	n.s.
Diabetes	1.23 (0.44–3.50)	n.s.	−0.009	n.s.
Ejection fraction (%)	0.99 (0.44–3.50)	n.s.	−0.109	0.029
Estimated glomerular filtration rate (ml/min)	0.98 (0.95–1.02)	n.s.	−0.231	< 0.001
Surgery type ^b	3.05 (1.44–6.45)	0.004	0.200	< 0.001
European System for Cardiac Operative Risk Evaluation	1.05 (0.97–1.13)	n.s.	0.072	n.s.
Age, creatinine, and left ventricular ejection fraction	1.27 (0.81–5.10)	n.s.	0.028	n.s.
Endogenous ouabain (log pM)	11.01 (2.64–46.00)	0.001	0.112 ^c	0.017 ^c

Relative risk of acute kidney injury and ICU stay over the entire observational study population of 407 consecutive cardiac surgery patients.

^aStandardized coefficient.

^bCombined surgery vs. singular.

^cTertile.

Table 3
Second Cardiac Surgery Replica Group: Clinical Characteristics of 219 Patients Divided According to Preoperative Endogenous Ouabain Tertiles

	First EO Tertile (n = 73)	Second EO Tertile (n = 72)	Third EO Tertile (n = 74)
Preoperative			
Sex (female/male)	22/51	12/56	24/51
Age (yr)	58.7 ± 11.9	62.0 ± 11.6	64.7 ± 11.1 ^a
Body mass index (Kg/m ²)	25.4 ± 3.51	25.8 ± 3.84	25.1 ± 4.51
Ejection fraction (%)	58.2 ± 8.9	56.5 ± 7.8	53.4 ± 10.8 ^a
European System for Cardiac Operative Risk Evaluation	2.69 ± 2.51	3.29 ± 2.66	4.68 ± 5.12 ^a
Age, creatinine, and left ventricular ejection fraction	1.04 ± 0.35	1.14 ± 0.33	1.32 ± 0.52 ^a
Plasma creatinine (mg/dL)	0.84 ± 0.19	0.90 ± 0.19	0.91 ± 0.24 ^b
Estimated glomerular filtration rate (mL/m 1.73m ²)	92.1 ± 16.2	84.7 ± 16.8	81.7 ± 18.7 ^a
Plasma EO (pmol/L)	84.4 (48–124)	157.8 (132–186)	261.1 (202–418)
Hypertension (%)	35.6	35.1	30.7
Chronic kidney disease (%)	0.9	2.3	4.1 ^b
Diabetes (%)	2	5	5
New York Heart Association I. II. III. (%)	22/66/12	22/62/16	11/52/37 ^b
Operative			
Cardiopulmonary bypass time (min)	80.2 ± 24.0	97.8 ± 46.6	100.7 ± 37.8 ^a
Aortic cross-clamp time (min)	64.8 ± 19.3	77.8 ± 37.5	82.0 ± 33.4 ^a
Mean arterial pressure (mm Hg)	70.8 ± 5.8	72.2 ± 6.4	72.5 ± 6.9
Inotropes (%)	3.61	7.2	10.4 ^c
Diuretics (%)	7.7	12.2	14.4 ^c
Inotropic arterial blood pressure (%)	0	0	0.5 ^b
Transfusion (%)	5	4.5	5.5
Postoperative			
Plasma creatinine (mg/dL)	0.93 ± 0.27	1.21 ± 0.59	1.68 ± 1.1 ^a
Plasma EO (pmol/L)	154.7 (65–297)	233.0 (151–328)	311.0 (198–486) ^b
Troponin T-peak	0.83 ± 0.69	1.13 ± 1.13	1.61 ± 1.35 ^a
ICU stay (d)	1.2 (1–2)	1.4 (1–4)	2.2 (1–7) ^c
Length of hospital stay (d)	5.2 (4–7.6)	5.9 (4–9.5)	8.2 (4.6–19) ^c
Acute kidney injury (%)	0	6.8	27.4 ^b
Renal replacement therapy (%)	0.0	0.0	2.7 ^b
In-hospital mortality (%)	0.2	0.5	0.5 ^b

EO = endogenous ouabain.

Patient preoperative, operative, and postoperative characteristics according to EO tertiles are shown in the top, middle, and bottom sections, respectively. Values are mean $\pm$ sd. Plasma EO is the geometric mean (interquartile range).

^aBonferroni post hoc test < 0.01 .

^bBonferroni post hoc test < 0.05 vs. first and second EO tertile.

^cLog-rank test median (95% CI), $p < 0.01$.

Table 4
Second Cardiac Surgery Validation Group

Event Category	Logistic Regression		Linear Regression	
	Acute Kidney Injury		ICU Stay	
	Relative Risk (95% Confidence Interval)	<i>p</i>	<i>b^a</i>	<i>p</i>
Sex	0.28 (0.09–0.87)	0.027	−0.123	0.063
Age (yrs)	1.05 (0.98–1.12)	n.s.	0.011	n.s.
Redo	0.76 (1.14–4.10)	n.s.	0.245	< 0.001
Hypertension	0.80 (0.21–2.98)	n.s.	0.026	n.s.
Diabetes	2.61 (0.84–8.13)	0.097	−0.029	n.s.
Ejection fraction (%)	0.93 (0.89–0.98)	0.006	−0.093	n.s.
Estimated glomerular filtration rate (mL/min)	0.97(0.94–1.00)	n.s.	−0.092	n.s.
Surgery type ^b	0.00 (0– --)	n.s.	0.006	n.s.
European System for Cardiac Operative Risk Evaluation	1.05 (0.97–1.13)	n.s.	0.132	n.s.
Age, creatinine, and left ventricular ejection fraction	134.9 (0.56–32394)	0.079	0.181	n.s.
Endogenous ouabain (log pM)	84.49 (6.04–1182)	0.001	0.147 ^c	0.029 ^c

Relative risk of acute kidney injury and ICU stay over the entire replicate study population of 219 consecutive cardiac surgery patients.

^aStandardized coefficient.

^bCombined surgery vs. Singular.

^cTertile.

Table 5
Area Under the Curve, Net Reclassification Improvement, and Integrated Discrimination Improvement Analysis For Acute Kidney Injury in 620 Cardiac Surgery Patients

	Mean (SE) 95% CI	<i>p</i>
AUC endogenous ouabain (EO) (SE) 95% CI	0.74 (0.029) (0.70–0.81)	
Clinical model-acute kidney injury (CLIN-AKI) ^a (SE) 95% CI	0.79 (0.028) (0.73–0.84)	
Preoperative endogenous ouabain added to clinical model-acute kidney injury (CLIN-EO-AKI) (SE) 95% CI	0.85 (0.023) (0.81–0.90)	
AUC difference (95% CI)	–0.065 (–0.011–0.015)	< 0.01 ^b
Integrated Discrimination Improvement ^c (SE) 95% CI	0.07 (0.011) (0.05–0.9)	< 0.001
Net Reclassification Improvement ^c (SE) 95% CI	0.075 (0.011) (0.05–0.10)	n.s.

AUC = area under the curve; CI = confidence interval; EF = ejection fraction; eGFR = estimated glomerular filtration rate; n.s. = non significant.

^a Clinical model is comprised of age, sex, preoperative EF basal eGFR, surgery type, hypertension, diabetes, and redo-intervention.

^b *p* value AUC of CLIN-EO-AKI compared with CLIN-AKI model.

^c Integrated Discrimination Improvement (IDI) and Net Reclassification Improvement (NRI) quantify the improvement of the biomarkers in predicting the risk of AKI. Comparing the CLIN-AKI model to the CLIN-EO-AKI, NRI considers an improvement in reclassification as if an AKI patient moves up a risk category or if a non-AKI patient moves to a lower risk category. Similarly a worse reclassification occurs if an AKI patient moves down a risk category or if a non-AKI patient moves up a risk category. Overall, NRI is the difference in the proportion of improvements in reclassification and the proportion of worse reclassifications. The IDI formula quantifies the reclassification continuously instead of categorically.

Values in parentheses are SEM.