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Relation of Pulmonary Artery Pressure and Renal Impairment in ST Segment Elevation Myocardial Infarction Patients

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Background: Recent reports have demonstrated the adverse effects of venous congestion on renal function in patients having heart failure. None of the above trials however included patients with acute ischemia.

Hypothesis: Echocardiographic correlates of increased right ventricular afterload would be associated with an increased risk of acute kidney injury (AKI) in ST elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary intervention (PCI).

Fig. 1. ROC curve for systolic pulmonary artery pressure to predict acute kidney injury (AUC 0.739, 95% CI 0.671–0.806, $p < 0.001$) (for Abstract 1).

Methods: We conducted a retrospective study of consecutive 930 STEMI patients who underwent primary PCI and had a full echocardiography study performed within 72 hours of hospital admission between June 2011 and December 2014.

Results: Echocardiography demonstrated that patients with AKI had significantly lower left ventricular (LV) ejection fraction, higher systolic pulmonary artery pressure (SPAP) and right atrial pressures ($P < 0.001$ for all). Following the performance of logistic multivariate analysis model SPAP (HR 1.07, 95% CI 1.04–1.11; $p < 0.001$) and LV ejection fraction (HR 0.95, 95% CI 0.92–0.99; $p = 0.03$) emerged as independent predictor of AKI. On receiver operating characteristic (ROC) curve analysis, the optimal cut-off value of SPAP to predict AKI was measured as more than 32 mm Hg, with 71% sensitivity and 62% specificity (AUC 0.739, 95% CI 0.671–0.806, $p < 0.001$).

Conclusions: Among STEMI patients undergoing primary PCI SPAP is an independent predictor of AKI.

2

Prognostic Implications of Fluid Balance in ST Elevation Myocardial Infarction Patients Complicated by Cardiogenic Shock

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Background: Positive fluid balance (FB) has been associated with adverse outcomes among patients admitted to general intensive care units, however no information is present to date on its possible prognostic implications in critically ill patients due to myocardial ischemia. We analyzed the relation of a positive FB and its persistence over time on in-hospital outcomes among patients with cardiogenic shock (CS).

Methods: We retrospectively studied fluid intake and output for 96 hours following hospital admission in 48 consecutive adult patients with ST elevation myocardial infarction (STEMI) complicated by CS, all undergoing primary angioplasty. Daily and accumulated fluid balance was registered up to 96 hours following admission. The cohort was stratified into two groups, based on the presence or absence of positive FB on day 4. Patients records were assessed for in-hospital adverse outcomes as well as 30 day all-cause mortality.

Results: A positive FB was present in 19/48 patients (40%). Patients with positive FB were more likely to develop acute kidney injury, need for new intubation and less likely to have renal function recovery as well as successful weaning from mechanical ventilation ($P < 0.05$ for all). Patients with positive FB had significantly higher 30 day mortality (68% vs. 10%; $P < 0.001$). In a multi-

Fig. 1. Comparison of accumulated mean fluid balance in milliliters at 24, 48, 72, and 96 hours following admission, between survivors and non-survivors (for Abstract 2).

variate logistic regression model for every 1 liter increase in positive FB, the adjusted risk for 30 day mortality increased by 48% (OR = 1.48, 95% CI 1.09–2.02; $P = 0.01$).

Conclusions: A positive FB was strongly associated with higher 30-day mortality in STEMI patients complicated by CS.

3

Dynamic Predictive Scores for Cardiac Surgery Associated Acute Kidney Injury

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Introduction: Acute kidney injury (AKI) is a common complication after cardiac surgery, in the last decade, studies were carried out to predict AKI/severe AKI in order to improve patients' outcome. With the new 2012 Kidney Disease: Improving Global Outcomes (KDIGO) AKI definition, the predictive efficiency of the existing risk scores remains uncertain, especially in the other racial population. And the recognition of perioperative risk factors contributing to the AKI is rising. Thus a clinical risk score by which clinicians can estimate the risk of cardiac surgery associated acute kidney injury in different time is needed.

Objective: To develop a risk score model for predicting cardiac surgery associated acute kidney injury (CSA-AKI) in Chinese patients.

Methods: A total of 7233 cardiac surgery patients in Zhongshan hospital, Shanghai Medical College, Fudan University during Jan, 2010–Apr, 2013 were enrolled prospectively. Logistic regression was used to analyze preoperative, at the day of ICU and 24 hours after ICU admittance CSA-AKI risk factors among 6081 patients as derivation group. Receiver operator characteristics (ROC) curves with area under ROC curves (AUROC) were used to create a point scoring of risk factor combinations at each time point. Another 1152 patients served as validation group and Hosmer–Lemeshow goodness-of-fit tests were applied to estimate the predictive values of the scoring system.

Results: The incidence of CSA-AKI and mortality in the derivation group were 23.8% (1446/6081) and 2.8% (170/6081), while in the validation cohort they were 19.3% (222/1152) and 3.4% (39/1152).

According to the logistic regression male, age ≥ 40 yrs, valve combined with coronary artery bypass grafting surgery, preoperative NYHA score > 2 , left ventricular ejection fraction $\leq 35\%$, preoperative kidney (without renal replacement therapy) disease, intraoperative cardiopulmonary bypass application, intraoperative erythrocyte transfusion ≥ 4 U and postoperative low cardiac output syndrome were independent risk factors for CSA-AKI. The point scoring of risk factor combinations led to AUROC values for CSA-AKI prediction of 0.74 (preoperative), 0.75 (at the day of ICU admission) and 0.82 (postoperative) in the validation group and Hosmer–Lemeshow goodness-of-fit tests revealed a good agreement of expected and observed CSA-AKI rates.

Conclusions: A dynamic predictive score system was developed and predictive efficiency for CSA-AKI was validated in Chinese cardiac surgery patients.

4

Prognostic Value of Urine NGAL and Urine IL18 in Critical Patients at the Admission to an Intensive Care Unit

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Introduction: The serum and urine biomarkers (creatinine, urea, and diuresis) currently in use are not sensitive or specific for detecting kidney damage, limiting treatment options and potentially compromising the outcome. New biomarkers are being studied in order to diagnose an earlier and more specific AKI, like NGAL and IL18. However the comparative performance of u NGAL and u IL18 in ICU remains to be validated.

Objective: To estimate the diagnostic accuracy of uNGAL and uIL18 at the admission to an adult general ICU for early detection of AKI, 30 days mortality and need of RRT.

Methods: We conducted a prospective observational study of 302 consecutive adult patients admitted to a general ICU, from November 2013 to April 2014. The study was approved by the institutional review board. Informed consent was obtained from the patient surrogate. AKI was defined by KDIGO criteria. Patients

were follow up 30 days until their discharge from Hospital or death.

Clinical Information: Gender, age, cause for admission, APACHE, SAPS, Charlson index, need for RRT.

Samples: u NGAL, u IL18 at the admission in the ICU, Creatinine daily till 96 h, weekly and discharge creatinine.

NGAL was done by Standardized Clinical Platform ARCHITECT assay, provided by Abbott Diagnostics. IL18 was made by ELISA (*Human IL-18 instant NN267*).

Statistical analysis: SPSS17 was used. Diagnostic characteristic of u NGAL were evaluated with receiver –operating characteristic (ROC) curves for AKI diagnosis.

Results: 73/302 (24%) patients developed AKI and 56/302 (18.5%) died.

AKI patients had significant higher mortality, 26/73 (35, 6%) vs. non-AKI 30/229 (13%) $p < 0.0001$; APACHE (22 ± 9 vs. 15 ± 6) $p < 0.0001$; and SAPS (48 ± 20 vs. 33 ± 15) $p < 0.005$ but no differences were found in age, sex and Charlson index (5 ± 2 vs. 4 ± 2).

There is a moderate correlation between NGAL and IL18 (Pearson 0.54; $p < 0.01$).

The ROC curve for u NGAL at admission and the occurrence of AKI was 0.79 (IC 0.74 a 0.84) $p < 0.001$.

We found for u NGAL values higher than 61 ng/ml, 76% sensitivity and 68% specificity.

The ROC curve for u IL18 at admission and the occurrence of AKI was 0.69 (IC 0.62–0.76) $p < 0.001$.

We found for u IL18 higher than 29 ng/ml, 64.8% sensitivity and 63% specificity.

Conclusions: Urine NGAL and urine IL18 at admission in ICU predicts AKI. There is a moderate correlation between NGAL and IL18, but in our experience ROC curve for NGAL is better.

5

Hepato-Renal Syndrome Post Liver Transplantation, Bridge Therapy with Continuous Renal Replacement Therapy in Type 1A Emergency. Succesful Re-Transplant. A Case Report

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Introduction: Renal dysfunction after liver transplantation (LT) is common, with an incidence of 20 to 40%. Nephrotoxicity is a common cause. The presentation of sudden anuria and persistent liver dysfunction forces us to suspect different pathologies.

Case Report: 61 year old female, with liver cirrhosis (NASH) Child-Pugh C 10 and MELD 14 points had a succesful deceased LT. Explant report was: cirrhosis, steatosis 5% and Mallory bodies.

24 hrs after LT she presented cardiogenic shock, elevated LFTs; acute renal failure (ARF) with oliguria, continuous renal replacement therapy (CRRT) was started. No improvement in LFT'S was seen in 24 hrs, she was reoperated for suspected outflow obstruction. A congestive, discolorating graft was found, which improved with positioning. A biopsy was taken, which reported massive hepatic necrosis 85% predominantly in zones 2 and 3. State 1A emergency was reported, a successful retransplantation was performed on day +5. CRRT was suspended because of spontaneous diuresis. Liver explant confirmed 90% necrosis, intense pericentral sinusoidal congestion regarding venous obstruction. Postop evolution was uneventful and she she was finally discharged on day +23 with normal liver and kidney function.

Discussion and Conclusion: The continuous elevated transaminases suggests significant hepatic necrosis. Etiological reasons of early graft dysfunction after transplantation are multiple and include: ischemia, reperfusion injury, primary dysfunction, technical complications, rejection and infection. It has been suggested that 5% of ARF secondary LT require CRRT. It is recommended the use of CRRT in anuric patients and intensive resuscitation, as in our case.

6

Acute Kidney Injury in Critically Ill Children: A Prospective Analysis of Risk Factors

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Introduction: Children admitted to pediatric intensive care unit (PICU) are at risk of acute kidney injury (AKI). Pediatric AKI syndrome is going to be, nowadays, a well-known disease, with a well-studied physiopathology and a specific epidemiology. However, few pediatric studies have focused on the identification of factors potentially associated with the development of this condition. The aim of our study was to identify risk factors of AKI in a large sample of critically ill children.

Methods: All patients admitted to our PICU (age between 0–16 years) over a 6-months period (January-June 2014) were prospectively enrolled. AKI was defined according to KDIGO criteria. A comparison between patients with and without AKI was carried out, and the risk factors playing a significant role in the manifestation of AKI were analyzed. These factors were first evaluated by univariate analysis; a multivariate analysis by stepwise regression was then performed using odds ratio (OR) with 95% confidence interval (CI).

Results: There were 79 cases included in this study, with the incidence rate of AKI being 31.6%. In 19 out of 25 AKI cases, a stage 1 AKI was diagnosed, whereas in 6 patients CRRT was required (stage 3 AKI). Patients with and without AKI were comparable as far as gender, age, body weight, and non-renal comorbidities. In the AKI group, PIM3 score was significantly higher than for non-AKI group (0.038 vs. 0.019; $p = 0.05$) The most common

PICU admission diagnoses in AKI cases were cardiac disease (28%), respiratory failure (24%) and infections (12%). In univariate analysis, risk factors for AKI resulted to be inotrope exposure (OR 2.57; 95% CI 1.01–6.84; $p = 0.05$), hypotension (OR 3.58; 95% CI 1.29–9.98; $p = 0.012$), MODS (OR 4.08; 95% CI 1.23–13.55; $p = 0.016$), and thrombocytopenia (OR 7.14; 95% CI 1.66–30.8; $p = 0.04$). Overall, hypotension was the only independent risk factor for AKI in a multiple logistic regression model ($p = 0.0047$). The mortality rate was estimated to be higher in AKI patients compared with non-AKI cases (12% vs. 1.8%; $p = 0.05$).

Conclusions: The incidence of AKI in critically ill children is high, and this is associated with high mortality. In the PICU setting, AKI represents a marker of illness severity and it is mainly associated with hypotension.

7

Using eGFR to Estimate the Extent of Renal Recovery after Cardiac Surgery Associated AKI

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Objective: The extent of renal recovery after acute kidney injury (AKI) is often assessed by serum creatinine. Previous studies showed that the long-term risk to develop progressive chronic kidney disease (CKD) in cardiac surgery associated AKI (CSA-AKI) patients is higher than non-AKI patients even when the renal has been completely recovered after AKI. Our study tried to find out if the estimated glomerular filtration rate (eGFR) can do better and the relationship between different extent of eGFR decrease and the outcome of the AKI patients.

Methods: We collected data of patients who underwent cardiac surgery from April 2009 to December 2012 for prospective analyses. Complete renal recovery was defined as an SCr concentrations at discharge ≤ 44 mmol/l above baseline values. The eGFR was determined according to the simplified MDRD formula and EPI formula. During the follow-up period of 2 years, the primary endpoint was all-cause of mortality and the secondary endpoint was progressive CKD. progressive chronic kidney disease was defined as CKD stages 4–5 (GFR ≤ 30 ml/min/1.73 m²) including ESRD (receive renal replacement therapy or renal transplantation).

Results: Among the total of 3245 patients enrolled, the AKI incidence was 39.9% ($n = 1295$). According to SCr criteria, there are 72.9% ($n = 944$) patients had ‘completely renal recovery’. Using the simplified MDRD formula to calculate the eGFR of AKI patients who was assessed as ‘complete renal recovery’ by SCr criteria, the eGFR at discharge was significantly lower than that at hospital admission (79.1 ± 37.8 vs. 94.4 ± 34.2 ml/min/1.73 m², $p < 0.001$). According to simplified MDRD formula, there are 32.1% ($n = 416$) patients whose eGFR ≥ 90 ml/min/1.73 m² and 67.9% ($n = 879$) patients whose eGFR < 90 ml/min/1.73 m² at discharge.

Using the EPI formula to calculate the eGFR of AKI patients who was assessed as ‘complete renal recovery’ by SCr criteria, the eGFR at discharge was significantly lower than that at hospital admission (75.4 ± 30.1 vs. 91.1 ± 19.8 ml/min/1.73 m², $p < 0.001$). According to EPI formula, there are 33.3% ($n = 431$) patients whose eGFR ≥ 90 ml/min/1.73 m² and 66.7% ($n = 864$) patients whose eGFR < 90 ml/min/1.73 m² at discharge.

After 2 year period followed up, we first analyze the outcomes of AKI patients with ‘complete renal recovery’ assessed by SCr criteria. Cox proportional hazards regression model showed that after adjusted for age, diabetes, hypertension, type of surgery, cardiopulmonary bypass time et al, AKI with completely renal recovery is still the risk factor for accumulated overall survival (RR 1.79, 95% CI 1.28 to 2.52) and progressive CKD (RR 1.92, 95% CI 1.37 to 2.69).

Then we analyze the outcomes of AKI patients whose extent of renal recovery assessed by eGFR criteria (both simplified MDRD & EPI formula). Cox proportional hazards regression model showed that AKI patients with eGFR < 90 ml/min/1.73 m² at discharge is the risk factor for accumulated overall survival and progressive CKD. But AKI patients with eGFR ≥ 90 ml/min/1.73 m² at discharge is not the risk factor anymore for accumulated overall survival and progressive CKD.

Conclusions: The change of SCr criteria may over-estimate the extent of renal recovery of CSA-AKI patients. AKI patients with eGFR (both simplified MDRD or EPI formula) < 90 ml/min/1.73 m² at discharge significantly increase the risk for accumulated overall survival and progressive CKD. CSA-AKI patients with eGFR (both simplified MDRD or EPI formula) ≥ 90 ml/min/1.73 m² at discharge can be better for estimate completely renal recovery.

8

Central Venous Pressure Is Useful to Predict Acute Kidney Injury after Cardiac Surgery

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Objective: Previous studies showed that acute kidney injury (AKI) incidence and mortality increased along with the increase of central venous pressure (CVP) of infective shock patients. Considered that CVP may be much more important to evaluate the volume and cardiac function of patients after cardiac surgery, we try to analyze the relationship between CVP and AKI & AKI-RRT incidence and mortality of patients received cardiac surgery.

Methods: Data of patients underwent valve and CABG surgery from Jan 2013 to December 2013 in our hospital was collected for retrospective analyses, including demography, preoperative comorbidity, postoperative input and urine output, first CVP at ICU admission, short-term outcome et al. Percent fluid overload (PFO) = [Total input (L) – Total output (L)] / basic body weight (kg) $\times 100\%$. The patients were grouped as AKI and non-AKI group.

Results: A total of 1192 patients were enrolled. The AKI incidence was 25% ($n = 298$) and AKI-RRT incidence was 1.2% ($n =$

14). The mortality in AKI group was much higher than in non-AKI group (7.4% vs. 0.7%, $P < 0.001$). The CVP at ICU admission and PFO within first 24 h in AKI group is significantly higher than in non-AKI group (12.2 ± 2.4 vs. 8.5 ± 2.7 mm H₂O, $P < 0.001$; $1.6 [-0.7, 3.1]$ vs. $1.0 [-0.9, 2.5]\%$, $P = 0.005$). The CVP at ICU admission and PFO within first 24 h in death group is significantly higher than in survival group (11.4 ± 3.0 vs. 9.4 ± 3.0 mm H₂O, $P < 0.001$; $2.8 [0.9, 6.1]$ vs. $1.1 [-0.8, 2.6]\%$, $P = 0.002$). Logistic multivariate regress analyze showed that gender (male), age (every 10 years added), BMI, preoperative serum creatinine >106 $\mu\text{mol/l}$, cardiopulmonary bypass time (every 1 hour added), CVP at ICU admission (every 1 mm H₂O added) are independent risk factors of AKI, and only preoperative serum creatinine >106 $\mu\text{mol/l}$, CVP at ICU admission, PFO within first 24 h were independent risk factors of AKI-RRT after cardiac surgery. The areas under the ROC curves to predict AKI and AKI-RRT by CVP were 0.837 and 0.789.

Conclusion: The CVP at ICU admission is an independent risk factor for AKI and AKI-RRT after cardiac surgery. Its predictive value is even better than PFO within first 24 h which make sense for the hemodynamics management after cardiac surgery.

Fig. 1. (for Abstract 9).

9

Importance of the Increased Serum Creatinine and Its Relationship with the Consult to the Nephrologist

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Objective: The Objective of this paper is to investigate the number of patients with elevated serum creatinine admitted to hospital in a period of time, and the percentage of them that were consulted to the nephrologist.

Patients and Methods: We included all the patients admitted to hospital from March to October 2015. It was defined elevated serum creatinine when it was higher than 1.2 mg/dl with the Jaffe method. The patients were grouped by specialty. We calculated the percentage of patients with elevated serum creatinine, in the whole group and by speciality. It was evaluated the percentage of consults to the nephrologist, by elevated serum creatinines.

Results: See table 1 for result.

Conclusion: In light of the results obtained, we realized that the percentage of elevated serum creatinines consulted to the nephrol-

Fig. 2. (for Abstract 9).

Table 1. (for Abstract 9)

Specialty	Number of patients	Number of increased creatinines	Number of consults by increased creatinines
Traumatology	943	128 (13.57%)	5 (3.91%)
Surgery department	1,318	219 (16.61%)	40 (18.26%)
Internal medicine	1,187	392 (33.02%)	65 (16.58%)
Cardiology	641	452 (70.51%)	9 (1.99%)
Proctology	316	95 (30.06%)	12 (12.63%)
Gynecology	491	18 (3.66%)	6 (33.33%)
Urology	565	358 (63.36%)	38 (10.61%)
Total	5,461	1,662 (30.43%)	175 (10.53%)

Fig. 3. (for Abstract 9).

ogist is low in all the hospital. When we compared the percentage of elevated serum creatinines consulted to the nephrologist, between the different specialties, we observed that the percentage of consults made by the surgical specialties, was greater than that one made by the clinical specialties. In spite of being well known that the impaired renal function, evaluated by the elevation of the serum creatinine, even though it is transient, it is a risk factor for the development of chronic renal disease, the importance of this elevation is still nowadays undervalued. Therefore, we should implement medical conducts, directed to stimulate the referrals of the patients to the specialist.

10

Validation of a Simple and Economic HPLC-UV Method for the TDM of Antibiotics Most Used in Critically Ill Patients

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Introduction: Critically ill patients are often affected by several pathophysiological conditions, as septic shock, multiple organ dysfunction syndrome and acute kidney injury, that significantly alter the normal pharmacokinetics of drugs. The treatment of these patients require antibiotic administration and, frequently, extracorporeal circulation or continuous renal replacement therapy in

order to support organ failure. The right antibiotic dosing is particularly difficult in this patient population because the dosage schedules are conventionally defined from pharmacokinetic studies performed in healthy volunteers, whereas in critically ill patients the pharmacokinetics parameters are modified by both the clinical condition and the extracorporeal therapy. Therapeutic drug monitoring (TDM) may assist to overcome this problem, but a TDM service is usually available only for some aminoglycosides and glycopeptides.

Objective: The aim of this study is the validation of an HPLC-UV method for the simultaneous quantification of meropenem, vancomycin, piperacillin and tazobactam in human plasma samples.

Methods: Aliquots of 250 µl of human plasma were spiked with analytes and cefotaxime as internal standard, then 100 µL of saline and 500 µL of acetonitrile were added and vortex-mixed. Precipitated proteins were separated by centrifugation for 10 min at 10,000×g and the supernatant was evaporated to dryness under a stream of nitrogen. Samples were reconstituted with 250 µL of mobile phase and transferred into HPLC microvials. Chromatographic separation was performed by an Agilent 1100 series HPLC system equipped with a quaternary pump, a degassing line, an autosampler, a Kinetex C18 (5 mm, 150 mmx4.6 mm) column and an UV/Vis detector set at 220 and 298 nm. The volume of injection was 100 µL, and the mobile phase was a mixture of phosphate buffer 0.1 M pH 3.15 and methanol in gradient, delivered at a flow rate of 1 ml/min.

Results: The HPLC-UV method assure an adequate separation from interferences for each analyte and was validated over clinical concentration ranges. For all the analytes, lower limit of quantification (LOQ) and lower limit of detection (LOD) were 1 and 0.1 µg/mL respectively, and the standard curves were linear between 1 and 100 µg/ml with coefficients ≥ 0.998 . The intra-day and inter-day precisions were all within acceptance criteria ($\pm 10\%$). The recovery was $>70\%$ for all the analytes.

Conclusions: The method presented here is simple, economic, accurate, precise and reliable for the determination of the antibiotics most used in critically ill patients. In comparison to the classical methods used in the TDM, that are often based on immunological test requiring patented instrumentation and kit, the principal advantage of HPLC analysis consist in its versatility, since it is possible to dose, with a same equipment and instrumentation, all the antibiotic and antifungal drugs used in the clinical practice, also those for which a commercial kit for TDM is not available. In addition, when the patient is treated with combination therapy, HPLC method is able to separate and quantify at the same time several different drugs from the same sample, thus optimizing the costs, the amount of sample needed and the time required for the analysis.

11

Molecular Dysregulation in Plasma from Non Dialytic Patients with Chronic Kidney Disease

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Introduction and Aims: Cell-free plasma DNA (cfDNA) is short double-stranded DNA originates from necrotic and apoptotic cells reflecting inflammation. It has been documented that cfDNA is present in blood and urine in healthy and pathological conditions. cfDNA has been found in many clinical conditions in which apoptosis and necrosis were involved suggesting that such events are the main source for its presence. High levels of cfDNA have been also reported in pathological conditions involving kidneys (HD, PD, severe sepsis) and there are few data about the role of cfDNA in CKD patients (pts). The aim of this study was to eval-

uate plasma cfDNA and Caspase-3 (Casp3) in pts with different stages of CKD. Furthermore, we evaluated indices of inflammation and ox stress in this population.

Methods: We enrolled 40 nondialytic CKD pts who came to our center for routine visit and we divided them depending on CKD stage (CKD-EPI creatinine equation). 29 healthy volunteers were enrolled as control. cfDNA was extracted from plasma and quantified by Real Time PCR for the β -actin gene. Quantitative plasma level of Casp3, IL-6/18/1 β , EPA (Eicosapentaenoic acid), MPO (Myeloperoxidase) and NO (Nitric Oxide) were also performed by ELISA assay.

Results: Quantitative analysis showed significantly higher levels of cfDNA in CKD pts compared with control ($p = 0.001$) (fig. 1a). Furthermore, cfDNA distinguished CKD pts from healthy controls with a sensitivity of 81.4% ($p < 0.0001$) (fig. 1b). The increase of apoptotic events in CKD group was confirmed by Casp3 activation ($p = 0.003$) (fig. 1c) and a significant positive correlation was observed between cfDNA and Casp3 levels ($\rho = 0.779$, $p < 0.0001$). Positive correlations were observed between cfDNA and urea ($\rho = 0.340$, $p = 0.043$) and between Casp3 and urea ($\rho = 0.564$, $p = 0.015$). cfDNA levels were significantly different depending on CKD stages ($p = 0.025$); furthermore, we observed an increasing levels of cfDNA from CKD stage 1 to CKD stage 5. Inflammatory status of CKD pts (plasma concentrations of IL-6/1 β /18), and ox stress status (plasma concentrations of MPO, EPA and NO), were statistically different from control population. Furthermore there was a positive correlation between cfDNA and IL-6 ($\rho = 0.363$, $p = 0.0070$).

Conclusions: Our data suggest that cfDNA is increased in the plasma of CKD pts and cell apoptosis could be one of its potential source. CKD pts enrolled in the study were non-dialytic pts, and we hypothesize that cfDNA could be influence by urine elimination and could be find and detect in urine. Further experiments are necessary to our confirm this hypothesis. We concluded that inflammation, ox stress, apoptosis and higher levels of cfDNA could be potential causes of cytotoxicity could be implicated in the pathological mechanism involved in CKD.

Fig. 1. (for Abstract 11).

Cardiac Surgery-Associated AKI: A Distinctive Variant with Differential Roles for Chitinase 3-Like Protein 1, Urinary Neutrophil Gelatinase-Associated Lipocalin and Serum Creatinine

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Introduction: Acute kidney injury (AKI) is frequent after cardiac surgery and associated with worse outcomes. This syndrome encompasses kidney damage without dysfunction (subclinical), kidney dysfunction without damage (functional), and kidney damage & dysfunction (established) [1].

Aims: To evaluate the novel damage biomarker urinary chitinase 3-like protein 1 (UCHI3L1; ref. 2) as diagnostic tool for AKI in cardiac surgery patients.

To validate UCHI3L1 against the damage biomarker urinary neutrophil gelatinase-associated lipocalin (UNGAL), and a frequent assessment of the functional biomarker serum creatinine (SCr).

Methods: Prospective cohort study at the cardiac surgery ICU of Ghent University Hospital from May 2012 till February 2014. Patients were included if: age ≥ 18 y; elective surgery (planned ≥ 4 h in advance); and written informed consent. Participation was excluded if: AKI KDIGO stage ≥ 1 at time of enrollment; CKD KDO-QI stage 5; recent kidney transplant (≤ 3 mo before); or surgery during weekend.

Blood and urine were collected pre-(t0), and post-operatively starting at ICU admission (t1) and then at 2 h (t2), 4 h (t3), 6 h, 12 h, 24 h and 48 h after ICU admission.

At each time-point we determined the concentration of SCr, serum CHI3L1 (SCHI3L1) and UCHI3L1; UNGAL was measured at t1 and t3 only; urine output was monitored hourly.

The primary endpoint was AKI stage ≥ 1 within 48 h after ICU admission. A secondary endpoint was AKI stage ≥ 2 within 12 h after ICU admission. Reference SCr was defined as the lowest SCr value within the last 3 mo prior to enrollment. Tubular damage was defined as UNGAL ≥ 150 ng/ml or as Δ UNGAL > 100 ng/ml with second value ≥ 125 ng/ml [3].

Results: Of the 211 enrolled patients, 8 already had AKI stage ≥ 1 at enrollment or at ICU admission, and were therefore excluded in the primary analysis. In this cohort (n = 203), 95 patients (46.8%) developed AKI stage ≥ 1 within 48 h after ICU admission: 67 (70.5%) had stage 1, 19 (20.0%) stage 2, and 9 (9.5%) stage 3. These patients were older (74.0 vs. 67.0 y; $P < 0.001$), had a lower

Table 1. AUC-ROC with 95% CI for predicting AKI stage ≥ 1 within 48 h after ICU admission (for Abstract 12)

		Time		
		t1 (ICU admission)	t2 (= t1 + 2 h)	t3 (= t1 + 4 h)
Biomarker	SCr	0.735 (0.669–0.794)	0.761 (0.694–0.819)	0.792 (0.728–0.847)
	SCHI3L1	0.671 (0.601–0.735)	0.649 (0.577–0.716)	0.664 (0.593–0.730)
	UCHI3L1	0.556 (0.484–0.627)	0.575 (0.503–0.645)	0.649 (0.578–0.715)
	UNGAL	0.583 (0.511–0.653)	Not applicable	0.612 (0.541–0.679)
	[SCHI3L1]-[UCHI3L1]	0.621 (0.549–0.690)	0.643 (0.571–0.711)	0.684 (0.613–0.749)
	[SCHI3L1]-[UNGAL]	0.646 (0.575–0.713)	Not applicable	0.665 (0.594–0.731)

Table 2. AUC-ROC with 95% CI for predicting AKI stage ≥ 2 within 12 h after ICU admission (for Abstract 12)

		Time		
		t1 (ICU admission)	t2 (= t1 + 2 h)	t3 (= t1 + 4 h)
Biomarker	SCr	0.780 (0.718–0.834)	0.821 (0.760–0.871)	0.857 (0.801–0.902)
	SCHI3L1	Not significant	0.628 (0.557–0.695)	0.720 (0.653–0.781)
	UCHI3L1	0.621 (0.550–0.688)	0.686 (0.617–0.748)	0.678 (0.610–0.741)
	UNGAL	0.650 (0.581–0.716)	Not applicable	0.656 (0.587–0.720)
	[SCHI3L1]-[UCHI3L1]	0.633 (0.562–0.699)	0.773 (0.708–0.829)	0.758 (0.692–0.816)
	[SCHI3L1]-[UNGAL]	0.659 (0.590–0.724)	Not applicable	0.774 (0.710–0.830)

eGFR_{CKD-EPI} (73 vs. 87; $P < 0.001$), a higher EuroSCORE (6 vs. 5; $P = 0.004$), and less isolated CABG surgery (35.8 vs. 54.6%; $P = 0.005$). Median time to diagnosis was 12.2 h (IQR: 7.2–18.4 h) and 84.6% of the AKI was functional. Subclinical AKI, which was missed by KDIGO, occurred in 5.1% of the patients.

In the secondary analysis, 1 patient that already had AKI stage ≥ 2 at enrollment was excluded. In this cohort ($n = 210$), 5 patients (2.4%) developed AKI stage 2 within 12 h after ICU admission.

In the early post-operative period, only the functional biomarker SCr was a good predictor of AKI stage ≥ 1 within 48 h after ICU admission (table 1). However, the combination of SCHI3L1 with a urinary renal stress or damage biomarker could predict AKI stage ≥ 2 within 12 h after ICU admission (table 2).

Conclusions: In this single-center cohort of patients who underwent elective cardiac surgery, AKI was frequent, predominantly mild and functional (without damage). Post-operative 2-h measurements of SCr had good predictive value for AKI, while the combinations [SCHI3L1]·[UCHI3L1] and [SCHI3L1]·[UNGAL] had good predictive value for AKI stage ≥ 2 only.

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Molecular and Genetic Mechanisms Involved in the Pathogenesis of Cardio-Renal Crosstalk

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Heart performance and kidney function are closely interconnected. The physiological crosstalk between these two organs is necessary to maintain the regular homeostasis and the normal functioning of the human body. However, during disease states, the damaged organ can induce structural and functional dysfunction in the other organ. The term ‘cardiorenal syndrome’ (CRS) encloses a scenario of clinical interactions in which cardiac and renal dysfunction coexist. Observational and clinical data showed that acute/chronic worsening of kidney function directly contributes to acute/chronic cardiac disease and viceversa, constituting the CRS. The CRS classification includes a huge array of acute and chronic conditions of these two systems, where the primary failing organ can be either the heart or the kidneys.

The crosstalk between the heart and the kidney is clearly evidenced but not fully understood. Indeed, different factors have been shown to be involved in the pathogenesis of CRS, such as systemic inflammation, oxidative stress, apoptosis and immune dysregulation. Recently, considerable attention has been paid to the role of new alternative mechanisms which may be implicated in the pathogenesis of cardiorenal crosstalk. In this abstract, we will focus on epigenetics, prenatal programming, small non-coding RNA and extracellular vesicles, underlying their possible role in heart and kidney disease (table 1).

Deeping insight the cardio-renal puzzle demands for expanded knowledge of many still missing pieces. Heart-kidney crosstalk has

Table 1. Possible new mechanisms implicated in CRS crosstalk (for Abstract 13)

Epigenetic mechanisms	Changes in gene function that are mitotically and/or meiotically heritable and that do not entail a dx in DNA sequence	Covalent modifications of DNA bases (e.g. DNA Methylation) Histone modification RNA interference Chromatin remodeling
Prenatal programming	Environmental cues during fetal development can permanently affect the composition, homeostatic systems and functions of multiple organs and systems	Nutritional and other
Small non-coding RNA	RNA that does not encode a protein and regulates gene expression	MicroRNAs (miRNAs), Small interfering RNA (siRNAs), Piwi-interacting RNAs (piRNAs)
Extracellular vesicles (EVs)	Cell-derived vesicles that are enclosed by a lipid bilayer, ranging from 30 nm to 2,000 nm	Apoptotic bodies Microvesicles (MVs) Exosomes

significant clinical relevance and the management of CRS is challenging, due to the multitude and complexity of the pathophysiological interactions between these two organs. Alternative factors, such as epigenetics, prenatal programming, small non-coding RNA and extracellular vesicles, might represent a new way of communication between cells and organs in the setting of cardio-renal crosstalk. Nevertheless, this field of research still remains largely unexplored. Further studies are needed, because genetic mechanisms may represent very attractive therapeutic targets, thus improving clinical outcomes of patients with heart and kidney disease.

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Aetiology, Prognostic Factors and Treatment Outcomes of Acute Kidney Injury in Children in a Tertiary Hospital in South Africa

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Background: Few reports on aetiology and outcome of acute kidney injury (AKI) originate from Sub-Saharan Africa. This study aimed to determine the aetiology, prognostic factors and treatment outcomes of AKI in children.

Methods: A retrospective file review was done of 63 children with AKI (median age 38.9 months [IQR 1.8–105.9]) referred to the Paediatric Nephrology unit, Steve Biko Academic Hospital, a tertiary hospital in Pretoria, South Africa, from 2003 to 2016. Features analysed included age, sex, aetiology of AKI, renal function, morbidity, mortality, dialysis requirement and outcome. Patients were categorized as Group 1 (complete/partial renal function recovery without need for continued dialysis), and Group 2 (died/required continuation of dialysis).

Results: Male: female ratio was 2.9:1. The causes of AKI were acute glomerulonephritis 18/63 (29%), obstructive uropathies 18/63 (29%), acute tubular necrosis (ATN) associated with hypovolaemia, 8/63 (13%), sepsis 7/63 (11%), toxins 6/63 (9%), post-surgery 4/63 (6%), and haemolytic uraemic syndrome 2/63 (3%). Thirty-one per cent developed AKI in hospital. Of those with ATN due to hypovolaemia, 50% were neonates: 25% breastfed, 50% mixed fed. In Group 1, 34/59 (58%) completely recovered (8 received peritoneal dialysis (PD)), and 25/59 (42%) had on-going renal impairment. In Group 2, 4/63 (6%) demised (2 received PD). Sixty per cent of patients with multi-organ failure died.

Conclusion: Acute glomerulonephritis and obstructive uropathies are common causes of AKI in this on-going study. Exclusive breastfeeding becomes a risk factor for the development of AKI in the face of insufficient breastmilk.

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Assessment of Competitive Adsorption Kinetics of Teicoplanin and β 2-Microglobulin with Modified Cellulosic Polymer (Lixelle S-35): An *in vitro* Screening Test

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Background: Lixelle S-35 cartridge is an adsorption column developed for β 2-microglobulin (β 2-MG) removal in dialysis-related-amyloidosis (DRA). Lixelle S-35 contains modified cellulosic polymer which could lead not only the adsorption of β 2-MG but also other compounds. In this setting, the adsorption phenomenon could involve antimicrobial agents resulting in changes of their pharmacokinetic profiles. The aim of the present *in vitro* study was to investigate the competitive behavior between Teicoplanin (TEC) and β 2-MG by using Lixelle's polymer.

Methods: We performed 9 experiments using plasma obtained from human healthy volunteer donors. Each test was carried out using 210 ml of plasma separately spiked with TEC (21.06 ± 2.58 mg/l; $N = 3$) and β 2-MG (14.38 ± 0.30 mg/l; $N = 3$) in order to evaluate the adsorption kinetic profiles of each compound. Thereafter, we assessed the competitive adsorption kinetics between TEC and β 2-MG ($N = 3$) spiking plasma with both compounds (TEC 26.25 ± 2.66 mg/l; β 2-MG 14.09 ± 0.65 mg/l). Plasma solution contained into a Erlenmeyer flask was incubated with 1.5 ml of Lixelle's polymer for 120 min. During the experiment plasma solution was stirred and maintained at temperature of 37° C. Sample were collected at 1, 2, 3, 4, 5, 8, 10, 20, 30, 60 and 120 min. TEC levels were analyzed by validated High Performance Liquid Chromatography (HPLC) whereas β 2-MG levels were measured by immunochemiluminometric assay (Liason® System, β 2-Microglobulin reagent, DiaSorin S.p.A, Saluggia, IT). Results were used to calculate mass balance analysis.

Results: Free mass baseline levels slowly decreased during experiments for both compounds (TEC and β 2-MG) when their kinetic profiles were separately evaluated (figure 1a and 1b). Those trends suggested that Lixelle's polymer is able to absorb not only β 2-MG but also TEC. Data were confirmed by the comparable mass adsorbed at the end of the experiment: 1.20 ± 0.54 and 1.42 ± 0.18 mg for TEC and β 2-MG, respectively. On the other hand, no difference in kinetic profiles were observed when TEC and β 2-MG were evaluated at the same time in the same plasma solution (figure 1c and 1d). Results suggest that TEC and β 2-MG did not compete for Lixelle's binding sites and therefore the adsorption phenomenon was led by non-specific force like Van Der Waals forces and/or hydrogen bonds. On the contrary, β 2-MG seems to enhance the adsorption of TEC considering its maximum adsorption value achieved when the experiment was concluded (1.90 ± 0.22 mg).

Fig 1. Mass balance analysis of Teicoplanin and β 2-Microglobulin during the experiment. Change in adsorbed mass and free mass of TEC and β 2-MG at experiment time points. In **a** adsorption kinetic profile of TEC; in **b** adsorption kinetic profile of β 2-MG; in **c** adsorption kinetic profile of TEC with β 2-MG; in **d** adsorption kinetic profile of β 2-MG with TEC (for Abstract 15).

Conclusion: The present study focused on the competitive adsorption kinetics of TEC and β 2-MG with modified cellulosic polymer. Our data highlight that Lixelle's polymer has comparable adsorption capacity for both compounds without competitive behavior. Consequently, the TEC administration does not alter the goal of the extracorporeal treatment with Lixelle medical device in β 2-MG removing. Although *in vivo* investigation is needed, the *in vitro* results suggest that patients treated with TEC undergoing to extracorporeal therapy with Lixelle S-35 may be exposure to underdosing of the antibiotic.

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Cardiorenal Syndrome Type 5 in Sepsis: Role of Endotoxin on Inflammation and Cell Death Pathways

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Background: Cardiorenal Syndrome Type 5 (CRS Type 5) is characterized by concomitant cardiac and renal dysfunction in the setting of different systemic disorders, such as sepsis. In this study, we investigated the possible relationship between endotoxin levels, renal cell death and inflammation in patients with CRS Type 5.

Methods: We enrolled 11 patients with CRS type 5 and 16 healthy controls (CTR). Serum endotoxin activity was measured

by the EAATM. Plasma from patients and CTR was incubated with renal tubular cells (RTCs) and cell death levels were evaluated. Plasma cytokines levels were measured as well.

Results: Accordingly to EAA levels, patients were divided into two groups: 36.3% of patients had low endotoxin activity level (negative EAA), while 63.6% of patients showed high endotoxin activity level (positive EAA). Renal cell death, pro- and anti-inflammatory cytokines were all significantly elevated in CRS Type 5 patients. In particular, RTCs incubated with plasma from EAA positive patients showed significantly higher apoptosis levels and higher caspase-3 activation compared to cells incubated with plasma from EAA negative patients. Moreover, a significant positive correlation was observed between EAA levels and RTC apoptosis levels. Furthermore, IL-6 and IFN- γ levels were significantly higher in CRS Type 5 patients with positive EAA.

Conclusion: In conclusion, this study confirms the high immune deregulation and the strong inflammation in CRS type 5 patients, especially in those with high endotoxin activity. Our data demonstrate, indeed, a strong relationship between endotoxin levels, renal apoptosis and inflammatory markers levels in patients with CRS Type 5.

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The Role of Cell-Free Plasma DNA in Critically Ill Patients with Sepsis

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Background: The identification of highly reliable outcome predictors in severe sepsis is important to define disease severity, monitor response to treatment and predict patient outcome. Cell-free plasma DNA (cfDNA) has been recently proposed as possible prognostic marker of clinical outcome in patients with sepsis. In this study, we investigated the prognostic value of cfDNA in patients with sepsis and its possible correlation with caspase-3, IL-6 and IL-18 levels.

Methods: We enrolled 34 patients admitted to intensive care unit (ICU). 27 patients were septic and 7 were non-septic. cfDNA was extracted from plasma and quantified by real time PCR. Plasma levels of caspase-3, IL-6 and IL-18 were measured by ELISA.

Results: We observed significantly higher levels of cfDNA in septic patients. No significant differences were found in terms of cfDNA levels in patients with Gram+, Gram- and fungal infections. 12/27 septic patients developed acute kidney injury (AKI) requiring CRRT (continuous renal replacement therapy) and showed higher cfDNA levels. 11/27 septic patients had a negative outcome during ICU stay; their cfDNA concentrations at admission were higher compared to survivors. Caspase-3, IL-6 and IL-18 levels were significantly higher in septic patients compared to non-septic patients and correlated with cfDNA levels.

Conclusion: cfDNA can be considered a good prognostic marker of clinical outcome in septic patients. Its levels increase in case of AKI complicating sepsis, in particular if CRRT is needed, and are associated with poor outcome. Caspase-3, IL-6 and IL-18 levels are higher in septic patients and correlate to cfDNA concentrations.

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Severe Intrahepatic Cholestatic Hyperbilirubinemia Treated with CytoSorb Hemoadsorption

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Introduction: Recently introduced extracorporeal cytokine hemoadsorption devices acquired attention as a strategy to help the control of severe SIRS, by removing a wide range of inflammatory mediators [1], such as TNF-alpha, IL-1 beta, IL-6, and IL-8 [2]. Animal studies [3] and clinical trials in septic patients [4, 5] demonstrated a decrease in cytokine levels during sepsis and possibly an improved outcome.

The so called 'cytokine-adsorbing column' CytoSorb is based on size selectivity, that target molecules up to 50 kDa, such as pro- and anti-inflammatory cytokines, through hydrophobic interactions with its polymer neutral lipophilic surface [6]. Manufacturer indicates a high removal capacity for other hydrophobic mid-molecular substances, like free haemoglobin, myoglobin, bilirubin and various bacterial enterotoxins.

We report the case of a patient treated with CytoSorb for extremely high levels of bilirubin.

Case: A 53-years old man, with no past medical history, was admitted to our ICU after ischemic cardiopulmonary arrest treated with venous-arterial extracorporeal membrane oxygenation and intra aortic balloon pumping. After 5 days we could suspend extracorporeal support because of progressive cardiac recovery. The patient recovered consciousness, but maintained need for mechanical ventilation and CRRT, and developed progressive severe hyperbilirubinemia. Percutaneous liver biopsy revealed severe cholestasis with biliar thrombosis and diffuse biliar infarctions, with limited attenuation and collapse of the reticular structure and no signs of ischemic necrosis, nor inflammation. Intrahepatic cholestasis being the main problem, with total bilirubin (Bil,tot) exceeding 50 mg/dl, we tried to treat liver dysfunction by sequential use of an artificial liver support system (MARS – Molecular Adsorbent Recirculating System) and CytoSorb.

MARS treatment was conducted continuously for 2 days and 9 hours, with 2 cartridges. Bil,tot decreased from 54.9 to 26.4 mg/dl (reduction by 52%), and direct bilirubin (Bil,dir) from 36 to 19.1 mg/dl.

After one month, with Bil,tot at 56.0 mg/dl, we applied one CytoSorb cartridge for 24 hours, that resulted in Bil,tot reduction to 44.4 mg/dl (reduction by 21%). After 3 days, with Bil,tot again at 55.0 mg/dl, we applied 3 CytoSorb cartridges continuously for

Fig. 1. Bil,tot and Bil,dir trends. It is possible to note reductions during MARS and CytoSorb treatments. Progressive numbers indicate different cartridges applied for each cycle (for Abstract 18).

3 days, obtaining a decrease of Bil,tot to 24.9 mg/dl (reduction by 55%).

The patient died for septic shock 5 days after the end of the second treatment, with Bil,tot at 32.2 mg/dl.

Conclusions: This case confirms the capability of CytoSorb to remove both indirect and direct bilirubin. In order to achieve an effect similar to 57 hours of MARS with 2 cartridges, we used continuously 3 CytoSorb cartridges for 69 hours. Further studies are needed to evaluate differences between MARS and CytoSorb for molecules other than bilirubin.

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Trajectories of Acute Kidney Injury and Long-Term Survival after Severe Sepsis

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Background: The 16th ADQI Consensus Conference has recently introduced concepts of persistent acute kidney injury (AKI) and acute kidney disease (AKD) to incorporate duration and renal

recovery in the current diagnosis. In the prospective observational study of 54 patients with surgical severe sepsis we have determined epidemiology of persistent AKI and AKD and their effect on major adverse kidney events (MAKE), development of chronic critical illness (CCI) and long term survival.

Methods: Two independent nephrologists clinically adjudicated AKI types using KDIGO criteria and ADQI recommendations. In contrast to rapidly reversed AKI, persistent AKI is characterized by the persistence of KDIGO creatinine and urine output criteria beyond 48 hours of the onset. Patients who did not completely recover kidney function at the discharge or the last follow up were considered to have AKD. MAKE was defined as the composite hospital outcome of death, dialysis, or AKD and CCI was defined as at least 14 days of ICU stay. Development of chronic critical illness, readmission and survival after discharge were assessed at six months after patient enrollment.

Results: Prior to enrollment, one patient had preexisting end-stage renal disease (ESRD). During hospitalization, 67% (n = 36) developed AKI. Among 36 patients with AKI, 53% (n = 19) had persistent AKI and 47% (n = 17) had rapidly reversed AKI. Composite hospital outcome (death, dialysis, or AKD) occurred only in persistent AKI group with a prevalence of 89% (17/19) (figure 1a). Among hospital survivors 19% was discharged with AKD stage 1, 6% with AKD stage 2, and 7% with AKD stage 3 while 35% had complete recovery of their AKI episode (AKD stage 0) (figure 1b). Median kinetic estimated glomerular filtration rate decreased with increasing AKD stages with medians of 57, 45, and 34 ml/min/1.73 m² for AKD stages 1, 2 and 3, respectively. Cumulative survival probabilities at 6 months for patients who had AKD stages 1 to 3 (50%) were significantly lower than those who completely recovered at hospital discharge (94%, p = 0.001) and those who did not develop AKI (93%, p = 0.03) (figure 1c).

The prevalence of chronic critical illness was significantly higher for those that had persistent AKI (74%, 14/19) compared to those that had rapidly reversed AKI (41%, 7/17) (p = 0.04). Although patients with CCI had significantly worse cumulative sur-

Fig. 1. Trajectories of AKI and Survival in Sepsis. **(a)** MAKE and CCI after persistent AKI; **(b)** AKD after persistent AKI; **(c)** Kaplan-Meier curves by AKD stages; **(d)** Kaplan-Meier curves by chronic critical illness and AKD stages (for Abstract 19).

vival (64% CCI vs. 96% no CCI at 6 months $p = 0.001$), when stratified by the occurrence of AKD, only patients with AKD stage 1–3 and CCI had significantly worse survival compared to all other groups. Cumulative survival probabilities at 6 months for CCI patients who had AKD stage 1–3 (42%) were significantly lower than patients who had CCI but had no AKD (90%, $p = 0.006$) and patients with no CCI (96%, $p < 0.0001$) (figure 1d).

Conclusions: Persistent AKI and AKD are significant risk factors for development of chronic critical illness and contribute to long-term mortality among these patients.

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Metformine Induced Lactic Acidosis in a Diabetic Patient

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We report a case of a 72 year-old lady, who was admitted due to altered consciousness level and with difficulty to arouse, vomiting and muscle weakness. The relatives refer that she 'had not gone to the toilet at all' the day of admission. They reported episodes of high fever and loss of appetite one week before.

Her comorbidities include: diabetes mellitus six years ago, arterial hypertension since twenty years. She had no documented history of chronic kidney disease. Her medication was metformine, vildagliptin, telmisartan, hydrochlorthiazide, atenolol and atorvastatine.

Laboratory findings revealed: BUN 77.3 mg/dl, Creatinine 9.4 mg/dl, pH = 7.07, K^+ 6.19 mmol/l, BE = -22, HCO_3^- = 7.3 mmol/l,

lactates = 10 mmol/l, AnGap = 37.7 mmol/l. The rest of laboratory data was unremarkable.

The constellation of clinical features and biochemical data was highly suggestive of metformine induced metabolic acidosis. Metformine was stopped and urgent dialysis was performed six hours after admission. Marked clinical improvement was observed. She had another dialysis session the next day to remove metformine from the circulation. Lactate level normalized and last creatinine was 2.5 mg/dl. She was discharged on day four.

Metformine induced metabolic acidosis is a medical condition which is associated with high mortality. A high level of suspicion is required to diagnose it. Early diagnosis and prompt dialysis can reduce complications and mortality of this condition.

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Does Dialysis Influence Regulatory T Cells?

A Systematic Review and Meta-Analysis

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Background: Conflicting results have been reported about the influence of dialysis on Regulatory T cells (Treg). Tregs comprise a sub-population of the total lymphocyte yet regulate key immune responses across a variety of disease settings. Patients on dialysis have iper-activated but impaired immune system. One possible explanation is altered Treg cells. Increase in Tregs may cause immunosuppression and a decrease in Tregs may increase inflammation.

Our aim is to determine whether the available literature support a positive or negative influence of dialysis on Tregs.

Methods: We performed a systematic search of relevant studies published through May 2015 in the MEDLINE database and integrated the results in a meta-analysis.

Mean differences in Tregs number of haemodialyzed patients (HD) and healthy controls (HC) were compared. In accordance with the p value of heterogeneity, random-effects model was applied. All statistical analyses were performed using the general package for meta-analysis (Version 4.3-0, depends R ($\geq 2.9.1$)).

Results: After screening 84 published studies, 5 were included in the meta-analysis. A total of 99 HD patients and 88 age-matched HC were compared. The difference in percentage of Tregs in total CD4⁺ T cells between HDs and HCs was not statistically different.

We performed two additional analyses: patients with chronic kidney disease not on dialysis (CKD-V) vs. HC ($p = 0.002$; mean difference in Treg percentage was -2.47% in CKD-V vs. controls) and CKD-V vs. HD (not significant).

Conclusions: This meta-analysis and systematic review demonstrate an association between uremic state in lowering Treg number and support the hypothesis that HD is associated with alterations of Treg number. Our findings highlight the need for new clinical studies focused on the effect of first dialysis treatment on

Tregs. These will be critical to understanding: 1) the influence of uremia and dialysis on Tregs and 2) the role of Tregs in disease in dialysis patients.

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SNP Profiling of Kidney Transplant Patients: Highlighting the Differences in the Genetical Background to Maximalize Immunosuppressive Therapies

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Introduction: Acute renal allograft rejection is define as a sudden decline in the function of the transplanted kidney, that gives rise to the creatinine levels. Whenever acute rejection is confirmed, the possibility of inadequate immunosuppression, whether due to inadequate dosing or noncompliance, must be addressed. The failure to recognize this contributing factor increases the risk of continued acute rejection episodes and, due to inadequate long-term immunosuppression, may eventually lead to graft failure. However, there is not a pre-defined protocol available to predict the probability of an adverse event in patients receiving the same medications. Moreover a more personalized immunosuppressive therapy may prevent complications and help to avoid graft rejection. Several Single Nucleotide Polymorphisms (SNPs) have been already associated with acute renal allograft rejection (AR). Therefore we aim to identify a pre-operative SNP profile assay that might underline the differences in the genetic background of the patients. SNPs are analyzed for allele and genotype frequencies in genes involved in immune responses and pharmacokinetics/pharmacodynamics of immunosuppressive drugs.

Methods: This is a cohort study. Genomic DNA isolated from 200 μ l of whole blood of 153 kidney transplant patients (42 with AR (wAR) and 111 without AR (w/oAR)) are genotyped for 20 SNPs in TNF-alpha, IL-10, ABCB1, UGT1A9, IMPDH2 genes.

Results: The average age of our kidney transplanted patients is 52.8 years and their group consists of 48 females and 105 males. Based on our preliminary results all SNPs are in Hardy-Weinberg equilibrium (HWE) in our study population. No transplant patients wAR were C/C homozygote for ABCB1 G-2677T/A, T/T and C/C homozygotes for UGT1A9 C-440T and T-331C, respectively. In our ongoing study so far 9 SNPs have been analyzed by Trend Test between the two transplanted groups; the p values are above 0.05 for each SNP, with the exception for UGT1A9 C-440T and T-331C ($p = 0.043$). The allele frequencies of C for UGT1A9 C-440T and T for UGT1A9 T-331C are both 70% in the w/oAR group; while, the allele frequencies of those polymorphisms are increased to 83% in the wAR group.

Conclusions: The homozygotes T/T and C/C for UGT1A9 C-440T and T-331C are missing in the wAR group and may define a possible protective haplotype for patients w/oAR. Thus, to confirm our results a case-control, multicenter research study is needed. Data from the newly enrolled patients will allow us to perform an age- and sex-matched analysis of the patients' cohort. Understanding the exact link between these SNPs and acute kidney rejection is crucial to more successfully maximalize antirejection therapies.

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Maintenance of Residual Urine Output and Type of Dialysis Can Influence FGF23 Levels

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Background: Bone mineral disorders are common in patients with chronic kidney disease (CKD). Hyperphosphatemia is frequent in dialysis patients. Recently, several studies investigated the role of fibroblast growth factor 23 (FGF23) in regulation of renal phosphate excretion in CKD. However, patients with residual urine output (UO) seem to better control serum phosphorus (P) levels than those without UO. Our aim was to determine if the dialysis modality and the maintenance of UO influence levels of FGF23.

Methods: We performed a cross-sectional study in two groups: hemodialysis (HD) and peritoneal dialysis (PD) patients. The variables analyzed were creatinine, urea, calcium, P, parathyroid hormone, 25 hydroxy vitamin D and C-terminal FGF23 (cFGF23). The UO collection refers to one week. All p value were two sides and statistical significance was set at $p < 0.05$.

Results: We enrolled 58 HD and 64 PD patients. Residual UO was present in 78 patients and the median volume was 5600 (3412–9450) ml/week. The median cFGF23 were higher in HD versus PD (2634 [1192–6880] vs. 1395 [609–2660] RU/ml; $p = 0.012$) and higher in patients without residual UO (2634 [970–8348] vs. 1428 [1042–3047] RU/ml; $p = 0.030$). We found a significant correlation between FGF23 and age, P, dialysis modality and residual UO. Univariable and multivariable adjusted analysis showed that P, dialysis modality and residual UO are independent predictors of cFGF23 respectively with OR = 0.495 [$p < 0.001$], OR = -0.184 [$p = 0.040$] and OR = -0.189 [$p = 0.034$].

Conclusions: In our study, cFGF23 levels were lower in patients with residual UO and in PD patients. P levels and dialysis modality are independent predictors of cFGF23 levels.

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In vitro and Preliminary in vivo Evaluation of a New CRRT Machine: KIBOU™

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Introduction: Kibou™ (Asahi Kasei Medical Co., Tokyo, Japan) is a new multifunctional automatic machine for Continuous Renal Replacement Therapy (CRRT) (figure 1). Kibou can perform different CRRT therapies with the possibility to use only a single platform; in particular, modalities for both adults and pediatrics, counter-current and co-current configurations, the use of heparin or citrate-calcium anticoagulation therapies and plasma exchange can be delivered (figure 2).

Methods: Based on the traditional experience of International Renal Research Institute of Vicenza (IRRIV), *in vitro* and a test evaluation of Kibou and related disposables has been conducted. In particular, during *in vitro* phase all the possible modalities and treatments have been performed, even for 24 hours. The 12 *in vivo* treatments were performed: 3 SCUF, 3 CVVH, 3 CVVHD, 3 CVVHDF, all for adult patients. The treatment parameters have been set based on the clinical needing of the patients. Machine's usability and accuracy has been evaluated by the staff (clinicians, nurses and engineers) through scores table. The measurement of the accuracy of fluid balance system (gravimetric) has even been performed comparing the displayed values in the monitor of the machine and in the bed scales. Cureflo™ ACF-130W filter has been used for the *in vivo* evaluation.

Results: Based on score given by the staff, the machine hardware results compact and well organized (figure 1), with 3 well separated compartments (dialysate/replacement, blood and effluent) that facilitate the preparation phase. Auto-priming function allows short priming time. The interface is user friendly in all the modalities (SCUF, CVVH, CVVHD, CVVHDF, TPE, for adults and pediatrics). During the α test *in vivo*, the measured fluid balance error was always lower than 0.3%, in all the modalities. On the contrary CVVHDF pre-infusion and pre + post infusion are not performable. For pediatric treatment the continuity of the flow of peristaltic blood pump need to be maintained and improved.

Conclusion: Kibou is a promising CRRT machine that can perform multiple continuous therapies with just one platform. In particular, we evaluated a highly accurate gravimetric fluid balance control system and a user friendly interface. Kibou is one of the first machines of the new frontier of CRRT devices: the fifth generation of CRRT machines.

Fig. 1. The new CRRT multifunctional machine: Kibou™ (for Abstract 24).

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Any Advantage from Polymethylmethacrylate Hemofilters for Patients on Extracorporeal Life Support?

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Introduction and Aim: ExtraCorporeal Life Support (ECLS) is a rescue therapy for the intensive care management of acute cardiac and/or respiratory failure in patients who fail conventional treatments. Despite continuous developments of ECLS-systems, there are many related complications to face up.

The non-biologic surface of an extracorporeal circuit leads to a systemic inflammatory response, triggering activation and consumption of pro- and anti-coagulant blood components [1]. Increased or high levels of plasma D-dimer, in the absence of other explaining pathologies, reveal circuit coagulation activity [2], as a sign of membrane oxygenator (MO) aging process, thus necessitating ExtraCorporeal Membrane Oxygenation (ECMO) circuit exchange [3].

Technical-induced hemolysis during ECMO is another relevant issue, with a reported incidence up to 18% [4], and could be associated to a number of factors, including pump-head and MO thrombosis [5].

Fluid overload and Acute Kidney Injury (AKI) are commonly associated to severe respiratory failure on ECMO. The use of Continuous Renal Replacement Therapy (CRRT) is therefore common for ECMO patients; it seems to be a good method for treating patients who develop AKI, improving fluid balance and treating electrolyte disturbances [6], and it may contribute to reduce mortality rates [7].

Toray CRRT filters (BK 2.1 and BG 1.60) have a dialysis membrane made with PolyMethylMethAcrylate (PMMA). This membrane has, at the same time, high porosity and unprecedented hemoadsorption capacity, which permit removal of low molecular weight proteins [8] (like beta 2-microglobulin) and cytokines [9].

The aim of our study was to evaluate, in patients on ECMO, the effect on coagulation and hemolysis tests of PMMA filters compared to the traditional AcryloNitrile (AN) hemofilters (AN69 – ST150 Gambro).

CRRT treatment	Replacement			Dialysate		Adult		Pediatric	
	Pre	Post	Pre and post	Co-current	Counter-current	Heparin	Calcium-citrate	Heparin	Calcium-citrate
SCUF	—	—	—	—	—	x	x	x	x
CVVH	x	x	x	—	—	x	x	x	x
CVVHD	—	—	—	x	x	x	x	x	x
CVVHDF	—	x	—	x	x	x	x	x	x
TPE	—	—	—	—	—	x	x	x	x

Fig. 2. The therapies performable with Kibou™ (for Abstract 24).

Material and Methods: In this retrospective, observational study we analyzed ECMO patients undergoing CRRT in our ICU, from January 2013 to March 2016.

We analyzed data from 17 patients who made a total of 27 CRRT cycles. A CRRT cycle was defined by lasting at least 24 hours and being preceded by a period free from CRRT of at least 48 hours. We divided these cycles into two groups: Group AN using ST150 Gambro filters and Group PMMA using PMMA Toray filters. 9 cycles were eliminated due to insufficient data, thus excluding 7 patients. On the other 10 patients, we compared 13 cycles for Group AN with 5 cycles for Group PMMA.

We compared percent variations of D-dimer (dDD), haptoglobin (dHPT), platelets (dPLT) and urea (dUREA) during the first 24 hours of each CRRT cycle, by using data before and after 24 hours from the beginning of hemodialytic treatment.

Results: Data are expressed as means $\pm$ SD. The reduction of urea was similar in the two groups: it decreased of $37.9 \pm 26.8\%$ in Group AN and of $34.1 \pm 30.2\%$ in Group PMMA (NS).

About coagulation parameters: D-dimer increased of $2.9 \pm 31.1\%$ in Group AN while decreased of $23.2 \pm 52.9\%$ in Group PMMA (NS); PLT decreased of $5 \pm 16.8\%$ in Group AN and increased of $2.2 \pm 36.9\%$ in Group PMMA (NS).

About the hemolysis index HPT, we found a reduction of $16.9 \pm 31.1\%$ in Group AN and of $5.8 \pm 21.4\%$ in Group PMMA (NS).

Conclusions: Both filters have valid depurative capability, with similar urea clearance efficiency. Although this study could not find a statistical difference for platelets, D-Dimer and Haptoglobin between the two kinds of hemofilters, PMMA filters tended to be associated with a decrease of D-Dimer and less effect on platelets and Haptoglobin. A much larger study, including more cases and more parameters, should be warranted to confirm the possible advantages of PMMA filters in patients on ECMO.

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Pressure and Hemolysis Related to Pediatric CVCs with Different Peristaltic Pumps

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Background: Pediatric patients with Acute Kidney Injury (AKI), fluid overload or hemodynamic instability are often treated with Continuous Renal Replacement Therapies (CRRT), which offer accurate management and control of ultrafiltration flows, uremia and solute levels, fluid balance and electrolyte imbalance. On the other hand, the administration of CRRT to pediatrics with adult's machine leads to several problems: vascular access site and catheter dimension have to be adequate both for the needful blood flow and the size of the patient; catheterization with larger catheters may lead to venous damage and thrombosis; a large percentage of patient's blood volume (10–15%) can circulate in the extracorporeal circuit (consequences are thermodynamic instability and hypotension). Information concerning pediatric CRRT are lacking: data about children are limited, retrospective and referred to particular clinical situations; moreover, knowledge acquired from adult patients cannot be easily shifted in pediatric area. Our aim is the estimation of hemolysis, flow and pressures related to central venous catheters of 4, 5 and 7 French; analysis are conducted with pediatric dedicated pumps (CARPEDIEM) and adult's pumps in order to study the influence of pump type on pressure in the catheter.

Methods: With a simplified version of a real extracorporeal dialysis system we collect pressures and flows for different test conditions (4, 5 or 7 French catheters; two roller or pediatric three roller peristaltic pumps). Pressures are measured at access and return points of the catheter and downstream of the pump, for blood flux of 7, 10, 15, 20, 30 ml/min. Secondly, we perform an estimation of hemolysis induced by the extracorporeal circuit; we use two separated lines, one with adult's pump and the other with CARPEDIEM pump, both with 4 French catheter at the end of the lines.

Results: Amplitude of pressures near the catheter is low with the pediatric pump instead of adult's one (figure 1). Pressure at the access and return points of the catheter increases with flux and is always low if pediatric pump is used (figures 2 and 3). Hemolysis is greater in the circuit with pediatric pump (table 1).

Conclusions: Lower pressures in the catheter translate into reduced stresses into patient's vascular access. Results about pressure insisting in the catheter in function of flux and pump type can be useful to evaluate the best solution for the pediatric treatment. Hemolysis is an important factor in dialysis treatment, but it has never been investigated for pediatric area.

Fig. 1. (for Abstract 26).

Fig. 2. (for Abstract 26).

Fig. 3. (for Abstract 26).

Table 1. (for Abstract 26)

Pump type	Free hemoglobin (induced by the circuit in 2 hours) (mg/dl)
Adult	11.06
Pediatric	14.92

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Predictive Models of Flow-Induced Hemolysis in Pediatric CVC

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Background: Blood is damaged when it is subjected to non-physiological stress inside medical devices; in a dialysis extracorporeal circuit red blood cells are particularly damaged by catheter, peristaltic pump and filter. The index of hemolysis (HI) gives an experimental quantification of the hemolytic event, but for the optimization and prediction of the hemolytic potential of medical devices, mathematical models are required. We identify from literature four classes of predictive models of hemolysis: power-law, threshold, strain-based and numerical models. The aim of our study is to evaluate these models with our experimental data; in

particular, we are interested in the estimation of hemolysis induced by a 4 French catheter when it is inserted in an extracorporeal circulation system, with a blood flow equal to 10 ml/min and two different pump types (adult's and pediatric). This configuration may represent the optimal setting for CRR in newborns with dedicated machines.

Methods: With a simplified version of a real extracorporeal dialysis system we collect pressures and flows for different test conditions (4, 5 or 7 French catheters; two roller or pediatric three roller peristaltic pumps). Pressures are measured at access and return points of the catheter and downstream of the pump, for blood flux of 7, 10, 15, 20, 30 ml/min. With a second testing circuit (where blood circulates without the use of a pump) we obtain an estimation of the release of hemoglobin induced by the 4 French catheter only. With data from the first circuit we evaluate power-law and threshold predictive models; analytical results from the specific configuration of 4 French catheter and 10 ml/min blood flux are then compared with the variation of free hemoglobin estimated with the second circuit.

Results: Figure 1 shows our analytical data of shear stress and exposure time compared with a threshold curve of hemolysis: all the points are below the threshold but they are interested by sub-lethal trauma. Results confirm that the increase of blood viscosity due to ultrafiltration is related to more hemolysis in the circuit. The variation of plasma free hemoglobin induced by the catheter is greater with the adult's pump in the circuit (table 1).

Conclusions: There aren't yet some shared 'guide lines' for the estimation of hemolysis induced by medical devices. For our specific working conditions and theoretical assumptions, power-law model is suitable for the description of hemolytic phenomenon inside the catheter.

Fig. 1. (for Abstract 27).

Table 1. (for Abstract 27)

Pump type	Free hemoglobin – power law (induced by the catheter in 1 hour) (mg/dl)	Free hemoglobin – experimental results (induced by the catheter in 1 hour) (mg/dl)
Adult	2.58	3.35
Pediatric	1.32	2.03

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CYTOSORB® Membrane May Improve Hemodynamic and Pro Inflammatory Response in Cardiac Patients with Post Operatory MOF

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Introduction: CYTOSORB® may adsorbe inflammatory mediators in septic patients, but data on post cardiac surgical patients are inconclusive. Aim of this study is to evaluate in post cardiac surgery patients with cytokine storm and multiorgan failure (MOF): 1 The change of inflammatory mediators and endotoxin. 2 The haemodynamic response. 3 The changes in organ dysfunction after CYTOSORB® hemoperfusion during the post operatory period.

Methods: 10 post operative cardiac surgery with post operative MOF were enrolled in the study. All patients were submitted to RRT with a Prismaflex system (Baxter USA) in which was assembled the adsorbing membrane CYTOSORB®.

All patients had monitoring of the cardiorespiratory function and evaluation of of endotoxin, IL6, procalcitonin at basal time (T0), at 24 hours (T1) and 36 hours (T2). SOFA score was evaluated at T0 and T2.

All data are expressed as Mean. ANOVA test was used to compare the changes during time course.

Results: 10 cardiac surgery patients with post operative MOF (APACHE II 28 ± 2 , SOFA 13 ± 2) were enrolled in the study. Every patients had a mean 18 ± 6 hours of CYTOSORB® perfusion. with blood flow rates = 180 ± 20 ml/min. Anticoagulation was achieved using systemic heparine (2 cases) and regional citrate (8 cases). There was any complication related to the procedure. IL6 ($p < 0.05$) and Procalcitonin ($p < 0.05$) decreased during the procedure. SOFA score decreased from 13 ± 2 to 10 ± 6 ($p < 0.01$) and MAP increased from 62 to 75 ($p < 0.01$) with decreasing of inotropic support.

Conclusions: In post cardiac surgery patients with MOF and cytokines storm CYTOSORB® membrane may be useful to improve organ failures and cardiovascular function. Larger studies are warranted in cardiac surgery patients with depressed function.

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pH and Free Calcium Plasmatic Level Variations during Continuous Veno-Venous Hemofiltration with Citrate Anticoagulation in Critically Ill Patients

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Introduction: Critically ill patients with acute kidney injury (AKI) have a mortality rate up to 50%. Continuous veno-venous hemofiltration (CVVH) is commonly used as renal replacement

Table 1. Values of general population are reported as mean $\pm$ SD (for Abstract 29)

	Pre-CVVH	1-H CVVH	12-H CVVH	24-H CVVH
pH	7.38 $\pm$ 0.07	7.33 $\pm$ 0.08	7.39 $\pm$ 0.08	7.41 $\pm$ 0.08
paO ₂ mm Hg	95 $\pm$ 32	93 $\pm$ 21	86 $\pm$ 26	97 $\pm$ 14
paCO ₂ mm Hg	35 $\pm$ 10	39 $\pm$ 12	40 $\pm$ 8	41 $\pm$ 10
HCO ₃ ⁻ mmol/l	22 $\pm$ 3.8	20 $\pm$ 4.4	24 $\pm$ 5.1	26 $\pm$ 7.0
Base excess	-3.8 $\pm$ 4.8	-5.3 $\pm$ 5.7	-0.1 $\pm$ 6.58	2.4 $\pm$ 9.3
Ca ⁺⁺ mmEq/l	1.15 $\pm$ 0.07	1.04 $\pm$ 0.15	1.21 $\pm$ 0.13	1.21 $\pm$ 0.09
Ca ⁺⁺ post-filter mmEq/l	/////	0.26 $\pm$ 0.05	0.33 $\pm$ 0.02	0.29 $\pm$ 0.06
Na ⁺ mmEq/l	142 $\pm$ 4.0	141 $\pm$ 3.6	140 $\pm$ 3.0	140 $\pm$ 1.7
Ca-gluconate flow ml/h	/////	131 $\pm$ 58	140 $\pm$ 58	133 $\pm$ 41
Citrate flow ml/h	/////	350 $\pm$ 31	343 $\pm$ 28	325 $\pm$ 41
K ⁺ mmEq/l	3.9 $\pm$ 0.7	4.0 $\pm$ 0.74	3.6 $\pm$ 0.36	3.6 $\pm$ 0.29
Creatinine mg/dl	3.9 $\pm$ 2	4.0 $\pm$ 2.1	2.3 $\pm$ 1.3	1.7 $\pm$ 0.9
Lactates mmol/l	3.8 $\pm$ 3	3.1 $\pm$ 2.9	3.8 $\pm$ 3.4	4.2 $\pm$ 4.6
Cl ⁻ mmol/l	109 $\pm$ 2.6	109 $\pm$ 3.5	105 $\pm$ 2.3	102 $\pm$ 1.7

Table 2. Values of patients with liver failure and septic shock are reported as mean $\pm$ SD (for Abstract 29)

	Pre-CVVH	1-H CVVH	12-H CVVH	24-H CVVH
pH	7.31 $\pm$ 0.04	7.32 $\pm$ 0.08	7.32 $\pm$ 0.07	7.34 $\pm$ 0.05
paO ₂ mm Hg	90 $\pm$ 13	84 $\pm$ 13	109 $\pm$ 30	120 $\pm$ 26
paCO ₂ mm Hg	34 $\pm$ 14	31 $\pm$ 11	39 $\pm$ 14	44 $\pm$ 8
HCO ₃ ⁺ mmol/l	18 $\pm$ 2.8	17 $\pm$ 1.0	20 $\pm$ 1.6	23 $\pm$ 1.5
base excess.	$\pm$ 8.5 $\pm$ 4.5	$\pm$ 9.7 $\pm$ 2.05	$\pm$ 5.6 $\pm$ 2.4	$\pm$ 1.1 $\pm$ 2.1
Ca ⁺⁺ mmEq/l	0.89 $\pm$ 0.13	0.84 $\pm$ 0.05	1.05 $\pm$ 0.16	1.11 $\pm$ 0.08
Ca ⁺⁺ post-filter mmEq/l	/////	0.23 $\pm$ 0.007	0.27 $\pm$ 0.06	0.32 $\pm$ 0.04
Na ⁺ mmEq/l	135 $\pm$ 0.6	135 $\pm$ 1.2	138 $\pm$ 2.9	137 $\pm$ 4.2
Ca-gluconate flow ml/h	/////	100	183 $\pm$ 58	133 $\pm$ 29
Citrate flow ml/h	/////	350	300 $\pm$ 50	316 $\pm$ 58
K ⁺ mmEq/l	4.6 $\pm$ 0.57	4.3 $\pm$ 0.6	3.6 $\pm$ 0.2	3.6 $\pm$ 0.4
Creatinine mg/dl	5.1 $\pm$ 1.0	4.5 $\pm$ 0.9	3.3 $\pm$ 0.4	2.6 $\pm$ 0.2
Lactates mmol/l	9.3 $\pm$ 6.4	9.3 $\pm$ 4.7	7.1 $\pm$ 4.5	3.8 $\pm$ 0.6
Cl ⁻ mmol/l	101 $\pm$ 3.0	101 $\pm$ 4.0	101 $\pm$ 1.0	100 $\pm$ 0.5

therapy (RRT) in intensive care unit (ICU) [1]. The risk of clotting with the resultant filter down-time needs continuous anticoagulation, but excessive anticoagulation may result in bleeding complications. No evidence about the best anticoagulation method exists, although citrate anticoagulation seems to have less bleeding risk than heparin and it could be associated with less mortality. However it is reported that citrate carries disturbance of free plasmatic calcium level, and acidosis or alkalosis if insufficiently metabolized or too much administered respectively [2, 3].

Aim of this study was to evaluate variations in pH and free plasmatic calcium levels in ICU patients during CVVH with citrate anticoagulation.

Material and Methods: We collected data from patients admitted to ICU from January 2015 to January 2016 needing RRT with CVVH (Prismaflex, HF 1400 PAES membrane, Gambro Industries, Meyzieu Cedex, France) under regional anticoagulation with citrate (ACD-A, Fresenius Kabi AG, Germany). According to our local protocol citrate was infused via inflow line and calcium

gluconate 1% was infused in reinfusing line. Replacement solution, commercially prepared used, was PrismaSol 2 mmol/l Potassium (GAMBRO). We performed acid-base balance analysis just before starting with CVVH and then at 1–12–24 hours during CVVH treatment. Calcium gluconate and citrate infusion rate was registered. Duration of plasmafilter was also recorded. Important complications related to anticoagulation were listed.

Results: There were enrolled 11 patients (8 Males and 3 Females, 67 $\pm$ 10 years old) admitted to ICU due to respiratory failure (5 pts), septic shock (4 pts; 3 out of them had also liver failure) and cardiac failure (2 pts). Mean prescribed dose of CVVH was 27 $\pm$ 5 ml/kg/h. Mean plasmafilter duration was 90 $\pm$ 10 hours. Acid-base balance analysis values are reported in the table 1 and table 2.

Conclusion: We did not record significative variations in pH and Ca⁺⁺ values during CVVH in ICU. Only one filter clotting was recorded but no episode of major bleeding was recorded although all patients were at high risk for bleeding due to their main disease, including patients with septic shock and liver failure.

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The Treatment of AKI in Nephrology Hospitalization: The SLE-HDF 15 litres in 10 Hours

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The AKI in intensive care has been widely treated by international and national guidelines.

The treatment of AKI in patients not requiring admission in Intensive Care Unit, but often hospitalized in Nephrology Unit, it is showed of less relevance.

For over 5 years we have used for the treatment of AKI of patients admitted in Nephrology Unit an intermittent slow technique, implemented in approximately 600 patients with AKI for a total of about 3000 treatments.

In this study we report the clinical results obtained in 100 consecutive patients referred to our Nephrology Unit from 1/1/2014.

We excluded the patients with AKI and lactic acidosis by metformin, which were treated with CVVHDF.

The Dialysis Protocol provides a slow low efficiency intermittent treatment called SLE-HDF (Sustained Low Efficiency Hemodia-Filtration), with 10-hour duration, 1.5 L/h dialysate for a patient up to 75 kg, 2 L/h up to 85 kg, 2.5 L/h over 85 kg.

Half of the dialysate was used in convention in post and half in diffusion.

End points were the renal survival and the survival of the patient.

On each patient was calculated on at least one seat, the Kt/V urea (UKt/V) according to the formula $UreaD/P \times \text{volume} \times 0.58 \times BW$, where D/P is the ratio of Dialysate/Plasma and BW is body weight in Kg.

Were studied 100 patients, 45 females and 55 males, with mean age 79.4 ± 11 years. The weight was $74 \text{ kg} \pm 18 \text{ kg}$ at the start of treatment. The 65% of patients had diuresis $< 500 \text{ ml}/24 \text{ hours}$, the 16% $< 1000 \text{ ml}$. The causes of AKI were: 41% heart failure, 31% AKI on MRC, 7% post-renal, 6% Hepatorenal Syndrome, 4% sepsis, 11% other causes. Major comorbidities were heart disease (63%), diabetes (50%), COPD (38%), age over 85 years, cancers 23, liver disease 16, hypotension requiring amine 15, sepsis 10. The mean UKt/V is showing equal to 0.4 ± 0.05 per session.

The deaths were 43. Patients discharged were 57. Of these, 43 had a renal functional recovery. Fourteen patients have not recovered renal function and were admitted for chronic dialysis treat-

ment. In total in the 100 patients, 512 treatments were performed, average 5.12 ± 3.7 . In 5 patients with oligoanuria we evaluated daily values of urea, creatinine, P, K and bicarbonates that showed the effectiveness of SLE-HDF in controlling the electrolyte and uremic poisoning framework.

In conclusion, our protocol of SLE-HDF, which uses volumes of dialysate sharply lower than used in literature, has been shown to be effective in correcting the biochemical profile of the patient with AKI. The results are considered satisfactory, having obtained the clinical improvement in 57% of patients, considering that the 43 deaths, 10 were suffering from Hepato-cirrhosis and 13 from malignant neoplasm.

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Acute Kidney Injury in Patients with Multiple Myeloma: Clinical Presentation and Outcomes

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Background: Renal involvement in multiple myeloma (MM) is common and may influence clinical outcomes. In this study we evaluated clinical presentation and outcomes of patients with MM and acute kidney injury (AKI).

Methods: We performed a retrospective single center study including patients with a diagnosis of MM and AKI (defined according to KDIGO criteria) between January 2009 and December 2015. We collected at multiple time points clinical and laboratorial data, including histological picture, need for renal replacement therapy (RRT) and clinical outcomes.

Results: Sixteen patients were evaluated with a mean age of 62.5 ± 13.5 years (11/16 men). Thirteen (81%) patients were at their first presentation of MM. The main paraprotein types were light-chain (46%) followed by IgG (38%) and IgA (16%). At the presentation all the patients presented a stage 3 AKI with a mean eGFR of $8.4 \pm 3.9 \text{ ml/min}$. Eleven patients (68%) required RRT that was performed by standard bicarbonate-dialysis with the use of polymethylmethacrylate membrane in 3/11 patients. 13 patients underwent kidney biopsy that showed: cast nephropathy in 8 (61%), light-chain deposition disease in 3 and AL-amyloidosis in 2 subjects. Thirteen patients were treated with a bortezomib-based chemotherapy and three with other regimens. After a median follow-up of 38.8 months, 6 (37.5%) patients had died, of whom 4/6 within the first 3 months after AKI diagnosis. The median survival time was 22.7 months. Three patients remained dialysis-independent until death, one is still undergoing RRT, while 7/11 patients recovered the renal function after a median of 105 (17.2–239) days. Neither the dialysis membrane nor the histological diagnosis was related with time for renal recovery. However, time on RRT was significantly and directly correlated with creatinine level at the end of follow-up ($r = 0.88$, $p = 0.03$).

Conclusions: In patients with MM AKI is often the first manifestation of the disease and may be severe, requiring RRT in the majority of subjects. These patients are at high risk of early mortal-

ity and median survival time is short, even if renal recovery is possible. However, patients experiencing MM-related AKI may be also at risk for development of chronic kidney disease and should be strictly monitored.

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Fluconazole Administration in Critically Ill Patients Undergoing Continuous Veno-Venous Hemofiltration

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Introduction: Invasive fungal infection (IFI) in critically ill patients is mostly represented by *C. albicans* even if in last years non-*albicans* species became more and more frequent [1]. In EPIC II study *Candida* were responsible for 20% of positive microbiological culture [2]. IFI increases critically ill patients mortality so an appropriate antifungal treatment is necessary and also should be promptly prescribed before any positivization of sample culture that normally requires many time [3]. Fluconazole is an anitmicotic agent administered normally once a day and eliminated via the kidney. Little data exist about fluconazole dosage and timing of administration in critically ill patients undergoing continuous veno-venous hemofiltration (CVVH) [4].

Fig. 1. C_{min} and C_{max} and dosage of fluconazole are shown and expressed as mean ± SD (for Abstract 32).

Aim of the study was to evaluate plasma concentration of fluconazole in patients undergoing CVVH.

Methods: ICU adult patients undergoing CVVH requiring fluconazole treatment were enrolled. Different dosages and timing of fluconazole (Diflucan, Pfizer Italiana, Latina, Italy) were prescribed and adjusted on Therapeutic Drug Monitoring (TDM). Lower (C_{min}) and upper (C_{max}) fluconazole concentration were recorded; also mean dosage and timing of administration of antifungal therapy were reported.

Results: 17 patients admitted to ICU were enrolled. All patients required CVVH because of renal impairment and CLcr <20 mg/ml. Fluconazole 200 or 300 mg q12 h, 100 or 200 mg once daily was used based on CLcr. TDM was performed after 48 h and every 2 days trying to achieved a desired concentration between 8–12 mg/l. C_{min} and C_{max} and dosage of fluconazole are shown in figure 1.

Conclusion: In critically ill patients undergoing CVVH only the administration of 300 mg twice daily allowed to achieve a mean C_{min} above the lower limit of efficacy for *Candida*. Single administration of 150 mg should not be prescribed in critically ill patients undergoing CVVH because of probably inefficacy of the antifungal therapy.

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Meropenem Continuous Infusion in Critically Ill Patients Undergoing CVVH

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Introduction: Acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT) affects approximately 25% out of critically ill patients in intensive care unit (ICU) [1]. Continuous veno-venous hemofiltration (CVVH) may significantly alter antibiotics clearance especially for those eliminated mainly through renal emunctory [2]. In critically ill patients, particularly the ones with multi drug resistant bacteria (MDR) infection, inadequate ex-

posure to antimicrobial agents could represent a life-threatening condition. To avoid it, meropenem continuous parenteral infusion (CI) has been suggested [3].

Aim of this study was to analyze CI meropenem steady state concentration (C_{ss}) in critically ill patients undergoing CVVH.

Methods: ICU adult patients undergoing CVVH requiring meropenem treatment were enrolled from January 2015 to July 2015. Different dosages of meropenem were prescribed according to our local protocol based on creatinine clearance (CL_{Cr}), microbiological data and adjusted on Therapeutic Drug Monitoring (TDM). Reconstituted solution of meropenem (Merrem, Astra-Zeneca, Milano) with 50 ml of saline 0.9% in 60 ml syringe (Bene-fis, Genova, Italy) was administered via central venous line with an Alarys GH pump (BD Carefusion) at 8.3 ml/h every 6 hours. Steady state concentration (C_{ss}), dosage and total clearance (CL_{Cr} TOT) of meropenem were recorded.

Results: 7 patients admitted to ICU due to severe sepsis were enrolled (1 Female, 6 male). All patients required CVVH because of renal impairment and CL_{Cr} <20 mg/ml. Meropenem 125 mg q6 h, 250 mg q6 h was used based on CL_{Cr}, meropenem 500 mg q6 h was used in patients with MDR infections to reach C_{ss} target above 20 mg/l. TDM was performed after 48 h and every 2 days. C_{ss}, CL_{Cr} TOT and dosage of meropenem are shown in figure 1.

Conclusions: Continuous infusion of meropenem in critically ill patients undergoing CVVH allowed to reach a effective C_{ss} (8–16 mg/l for meropenem) with at least dosage of 250 q6 h. Lower dosage increased the risk for inefficacy of antimicrobial therapy. To ensure an adequate C_{ss} against MDR pathogen it larger doses (500 q6h) should be administered.

Fig. 1. C_{ss} (mg/dl reported as mean plus SD), CL_{Cr} TOT (ml/min reported as mean plus SD) and dosage of meropenem are shown (for Abstract 33).

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Safety and Efficacy of Citrate Anticoagulation for Continuous Renal Replacement Therapy for Acute Kidney Injury Following Liver Transplantation: A Single Centre Experience

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Background: Acute kidney injury (AKI) following liver transplantation (LTx) is a frequent and serious complication with an incidence from 12% to 95%, associated to an unfavorable effect on the prognosis of patients. Among them, the incidence of AKI requiring continuous renal replacement therapy (CRRT) ranges from 10 to 30%. Usually CRRT requires anticoagulation in order to prevent clotting of the extracorporeal circuit. KDIGO guidelines indicate the use of citrate as loco-regional anticoagulant drug for CRRT regardless patient's hemorrhagic risk. Despite this indication, however, the use of citrate is still under debate in patients with liver failure and/or LTx because of the potential risk of plasmatic citrate accumulation due to reduced liver clearance.

Aim of the Study: To evaluate the use of citrate as anticoagulation drug in continuous renal replacement therapy (CRRT) for acute kidney injury (AKI) following LTx.

Materials and Methods: Between January and December 2015, 5 liver transplant patients (pts) were treated with citrate-CRRT for post-operative AKI. A retrospective analysis of data has been performed to evaluate safety and efficacy of the treatment.

Results: Indications for liver transplantation were HCV cirrhosis (1 pts), re-transplantation due to HCV recurrence (1 pt), hepatocellular carcinoma on post-alcoholic cirrhosis (1 pt), urgent re-transplantation due to primary non function (2 pts). AKI's etiology was secondary to hypovolemia (2 pts), hypovolemia associated to drug nephrotoxicity (1 pt) and impaired liver function (2 pts). All the patients developed AKI during the early post-operative course (median 1 day; range 1–26 days). The main indication to CRRT was oligo-anuria.

All the patients were treated with CRRT via continuous veno-venous hemodiafiltration using citrate anticoagulation (median 3 mmol/l; range 2.5–3 mmol/l) due to the high hemorrhagic risk related to the liver function impairment. Median CRRT duration

was 7 days (range 3–50) with a median of 3 treatments (range 2–23) before renal function recovery.

Conclusions: At our center 5 patients have been successfully treated with CRRT with citrate for acute kidney injury during the post-LTx course. All the patients reached full renal function recovery and no one experienced complications related to the citrate anticoagulation use. Our results, even if on a small series of patients, confirm that continuous renal replacement therapy with citrate is a safe and promising treatment for post liver transplantation acute kidney injury.

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Pathogenesis of Kidney Injury in Course of Cirrhosis Induction, in a Contest of CCl₄-Inhalation Rat Model

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Background: Acute kidney injury (AKI) is common in advanced liver dysfunction with cirrhosis. The most influential hypothesis in the field of it, is peripheral arterial vasodilation hypothesis with subsequent hemodynamic impairment resulting in kidney disfunction. On the basis of recent knowledges, was born the need to revise the classical hypothesis, focusing the attention on cholemic nephropathy (CN), a comprehensive term describing renal disfunction in course of obstructive jaundice, and on inflammatory syndrome as responsible of advanced cirrhosis and systemic complications. We investigated an animal model of liver cirrhosis induced in rats by CCl₄ inhalation.

Methods: Decompensated liver cirrhosis was induced by carbon tetrachloride (CCl₄) inhalation for 13 weeks in 21 Male Wistar Han rats. Urine and plasma samples were collected during induction and Luminex analysis was carried out for analysis of multiple biomarkers involved in inflammation (EPO, G-CSF, GM-CSF, GRO/KC, IFN- γ , IL1 α -1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17A, IL-18, M-CSF, MCP-1, MIP-1 α , MIP-3 α , RANTES, TNF- α , VEGF) and kidney injury (NGAL, Clusterin, Osteopontin, MCP1, KIM1, IL-18, B2microglobulin, cistatine-C, calbindine). Kidney histology was evaluated at the sacrifice. Immunohistochemistry was used to study the main bile acids tubular transporters SLC10A1, P-Glycoprotein, Asbt, Mrp4 and Ost- β . Also Caspase-3 and TLR-4 were analyzed.

Results: At sacrifice, the liver was consistent with cirrhosis. Creatinine shows incremental mean values. Major histopathological features were not present, some cases presented mild pathological features of tubulo-interstitial damage. By quantifying cytokine two behaviors are evident a Gaussian pattern with peak at week 4 for all cytokines. While biomarkers of kidney injury had a peak at week 7. Immunohistochemistry demonstrate differences between treated rats and control group.

Discussion: The main our goal was to analyze the pathogenesis of kidney injury in course of cirrhosis induction, in a rat model. The peripheral arterial vasodilation hypothesis has been most influential in the field of cirrhosis and its complications, but the expansion of modern knowledge would require a different approach to advanced cirrhosis.

New hypothesis have already placed the immune system as central role in the pathogenesis of cirrhosis and its complications. In the present study we detect, for the first time, a chronological relationship between systemic inflammation response and kidney injury: we demonstrated a kidney involvement during a phase of compensated cirrhosis. Also Cholemic nephropathy represents a widely underestimated, but important cause of kidney dysfunction in patients with cholestasis and advanced liver disease: we demonstrate. Through Immunohistochemistry results we underline a possible key role of bile acids in kidney disease in course of cirrhosis, which seems to be involved in a damage to the proximal tubular cells through simultaneous adaptive and apoptosis signals. Therefore AKI in patients with liver disease represents a wide group of different entities and also Cholemic Nephropathy seems to be one part of it.

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Functional Abnormalities and Thyroid Nodules in a Cohort of Patients with Chronic Renal Failure Awaiting Kidney Transplant

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Introduction: Alterations of thyroid function, especially hypothyroidism, both in clinical and subclinical forms, are more common in people with terminal chronic disease (ESKD) than the general population. Patients with ESKD hypothyroid have more frequent a cardiovascular disease (CVD) and an increased risk of mortality compared to euthyroid ones. In the patient with ESKD also an increased incidence of benign thyroid nodules has been described with respect to the general population and an increase in thyroid cancer incidence.

Material and Methods: We conducted a retrospective study on a population of 2,147 patients on the waiting list for kidney transplants in the last 15 years in order to identify functional anomalies and/or instrumental ones and possible risk factors.

Results: The most frequent thyroid functional abnormality is hypothyroidism, in 437 patients (20.3% of the population considered), and in particular subclinical hypothyroidism, present in 289 patients (13.5% of the population). Factors associated with hypothyroidism are the presence of CVD ($p < 0.001$) and age compared with euthyroid patients (mean age 53.9 years $\pm$ 12.3 versus 57.6 $\pm$ 12.0 years, $p < 0.001$). We found hyperthyroidism in 24 patients (1.1%) and it is statistically related to the female sex ($p < 0.003$) and CVD ($p < 0.001$). About the presence of instrumental anomalies, 804 (37.4%) of patients have thyroid nodules of which 770 (35.9%) benign and 34 (1.6%) malignant. The benign and malignant thyroid nodules are associated with a statistically significant CVD and presence of extra thyroid cancer. The autosomal dominant polycystic kidney disease (ADPK) is a nephropathy predisposing the presence of goiter ($p < 0.001$).

Discussion: Our study highlights in a population of patients suffering from ESKD an high percentage of dysfunction of thyroid

function, in particular hypothyroidism that is correlated with CVD. The presence of benign or malignant nodules is associated with an increased risk of CVD and extra-thyroid cancer. 45.1% of patients with ADPK have a thyroid goiter. The presence of functional abnormalities and benign and malignant nodules should always be investigated in patients on the waiting list for kidney transplantation, in order to reduce cardiovascular complications and the risk of cancer that are both already increased by the necessary immunosuppressive anti-rejection therapy.

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Is Uremia Associated with the Presence of Endothelial Progenitor Cells with a Calcifying Phenotype?

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Background: Disorders of mineral and bone metabolism, common in patients with chronic kidney disease, are collectively known as chronic kidney disease-mineral and bone disorder (CKD-MBD), and they have been implicated in the pathogenesis of vascular calcification. CKD-MBD patients show alterations in the number and function of circulating endothelial progenitor cells (EPCs), physiological regulators of angiogenesis and vessel repair, that might acquire a calcifying phenotype, expressing mineralization biomarkers. This study investigated the expression of vitamin D receptor (VDR) and osteocalcin on EPCs of healthy individuals and hemodialysis patients, and their possible associations with circulating biomarkers of inflammation and vascular calcification.

Methods: We compared CD133±EPC counts, expressing VDR or osteocalcin, in 23 healthy subjects vs. 53 hemodialysis patients, 17 of them without vitamin D receptor agonist (VDRAs) therapy and 35 treated with calcitriol (n = 17) or paricalcitol (n = 18). The correlations of each CD133±EPC subset with serum levels of inflammatory and calcification indexes were also analyzed.

Results: The percentage of all EPC phenotypes examined here, either CD133 positive or CD133 negative, expressing VDR or osteocalcin, were found to be significantly increased in hemodialysis patients as compared to healthy controls (CD133+VDR+ p = 0.011, CD133-VDR+ p = 0.014, CD133+OC+ p = 0.018, CD133-OC+ p = 0.016). Moreover, in dialysis patients not under VDRA therapy CD133+EPCs expressing osteocalcin correlated positively with calcium levels (p = 0.047), while in VDRAs-treated patients CD133+EPCs expressing VDR correlated positively with interleukin 6 levels (p = 0.024) and CD133-EPCs expressing osteocalcin correlated positively 25-hydroxyvitamin D levels (p = 0.05).

Conclusions: Our data suggest that in CKD-MBD patients, EPCs undergo an endothelial-to-procalcific shift, thus representing an influential risk factor, within the bone-vascular axis, for vascular calcification. Moreover, a possible link between mineral disorders and vitamin D replacement therapy emerged, that might result in adverse effects in CKD patients.

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Simkania Negevensis, Seroprevalence and Clinical Impact of a 'New' Bacterium in Renal Transplant Recipients and in Hemodialysis Patients

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Background: *Simkania negevensis* is an obligate intracellular bacterium belonging to the family Simkaniaceae in the Chlamydiales order. It is considered an ubiquitous microorganism and aquatic environments may be involved as a source of infection for humans. It was just isolated in samples from domestic water supplies and from mains water supplies, like spa water or swimming pool water, confirming its ability to resist to the common chlorination treatments.

Evidence indicates a possible role of the microorganism in respiratory tract infections, in gastroenteric disorders and in the pathogenesis of cardiovascular disease, furthermore it has hypothesized that it could play a role in lung transplant rejection. However prevalence and possible effects in nephrology are yet not known.

Methods: To evaluate this we examined the occurrence of *Simkania negevensis* in two different populations, both characterized by a high susceptibility to infectious complications: 105 hemodialysis patients, 105 renal transplant recipients and 105 healthy subjects through the IgG and IgA response to *Simkania negevensis* in their sera.

Serum antibodies to *Simkania negevensis* were detected by a homemade ELISA performed according to the Kahane's protocol. Furthermore water samples from hemodialytic circuit were collected, to evaluate *Simkania negevensis* resistance to usual treatment of disinfection.

Results and Conclusion: Our results were unexpected, showing a higher seroprevalence of antibodies against *Simkania negevensis* in the hemodialysis patients, compared to those with renal transplant patients, suggesting the treated water used in hemodialysis as a possible source of infection. Findings on samples confirm that the common fluid treatments in hemodialysis are not able to purify the water from the *Simkania negevensis* contamination. However our findings do not define a clinical risk for positive patients to the bacterium.

Dialyzability of Oxycodone and Its Metabolites in Chronic Non-Cancer Pain Patients with End Stage Renal Disease

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Severe chronic pain is common among dialysis patients. While opioids are often used in this population as preferred analgesic drugs, knowledge about their safety in this context is limited. Oxycodone is increasingly used for the treatment of chronic moderate-severe pain conditions as oral controlled release (CR) tablets however it has mostly a renal excretion, leading to accumulation in case of kidney failure. Evidence about this drug and its metabolites' dialyzability is lacking.

We assessed the effect of standard hemodialysis (HD) and on-line hemodiafiltration (HDF) methods on the plasma concentration of oxycodone and its metabolites during a 4-hour dialysis session in $n = 20$ End-Stage-Renal-Disease chronic-pain patients treated with oral CR oxycodone. Chromatographic techniques were used to evaluate the studied compounds' plasma concentration at 3 different time points along dialysis. Mean plasmatic concentration of oxycodone and noroxycodone in the sample showed an overall reduction trend over time but it was less enhanced for noroxycodone. Mean reduction of oxycodone and noroxycodone arterial concentration was significantly higher with HDF (54 and 27%, respectively) than with HD (22 and 17%, respectively). Analysis of the regression of these compounds' clearance on their increasing arterial concentration showed a more stable and linear clearance prediction with HDF (roughly, 85 ml/min); with HD, for increasing arterial concentration, clearance of oxycodone decreased while noroxycodone clearance increased. Dialyzability of oxycodone and noroxycodone, though limited, was documented. The dialyzability with HDF is better than HD and it persists along the four hours session. This evidence will contribute towards considerations as to the safety of the use of oxycodone in dialysis patients in the future.

Coupled Plasma Filtration and Adsorption and Liver Depuration: Preliminary Results of HERCOLE Study

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Introduction: Coupled plasma filtration and adsorption (CPFA) is currently used in the treatment of severe sepsis with the intention of removing the proinflammatory mediators from the systemic circulation. Some evidence exist about the bilirubin adsorbing ability of the neutral styrenic resin which is part of the extracorporeal circuit of CPFA. The aim of this study is to assess efficacy and safety of CPFA in extracorporeal detoxification of liver toxins in patients affected by acute or acute-on-chronic liver failure.

Methods: 7 patients (age 23–61 years) with acute ($n = 2$) or acute-on-chronic ($n = 5$) liver failure were enrolled. A total of 16 CPFA treatments were carried out. Each CPFA treatment lasted 6 hours. Unfractionated heparin was used as anticoagulation of the extracorporeal circuit. The number of treatment for each patient was established on his/her clinical status. The reduction ratios per session of bilirubin and bile acids were considered. Hemoglobin, platelets, white blood cells, coagulation tests, urea, creatinine, and electrolytes were also checked on starting CPFA and CPFA end as biocompatibility measures.

Results: All the sessions were well tolerated by the patients. Alcohol was the most common etiology of the liver injury ($n = 5$), 1 patient had a jaundice with Cholangitis and 1 patient had normal liver function. Median reduction rate per session for total bilirubin was 27.99% (range 2.15–40.01), for direct bilirubin was 31.37% (range 8.52–48.61), for indirect bilirubin was 29.12% (range 6.62–65.02) and for bile acids was 30.56% (16.67–59.62). In six out of seven patients we observed a recovery of liver function.

Conclusions: Although CPFA is a non-standardized technique for the liver depuration, its use in patients with acute or acute-on-chronic liver failure has shown favorable effects on safety and efficacy in terms of detoxification.

Different Kidney Ageing, Scintigraphic Comparison of Left and Right Renal Function Along Time

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Introduction and Aims: The renal vascular pathology in all its forms (hypertensive, nephroangiosclerotic and ischemic) has a continuously increasing incidence and clinical relevance. The aim of this study was researching a functional asymmetry between the

left and the right kidney that could reflect the organ aging both in physiological and pathological conditions.

Methods: 600 patients (pts) (313 M, 287 F) mean age 61.2 ± 18.3 (years) were studied with ^{99m}Tc -MAG3 dynamic renography. In 132 cases the examination was carried out with the Captopril test in order to increase the diagnostic sensitivity to ischemia. A group of patients (pts) (53), whose renography suggested the presence of renal artery stenosis, has been studied with doppler ultrasound too.

Results: In the enrolled patients: mean serum creatinine levels was $(1.7 \pm 0.8 \text{ mg/dl})$, eGFR $(45.9 \pm 24.9 \text{ ml/min})$, right Longitudinal Diameter (LD) $(101.4 \pm 12.3 \text{ mm} - \text{Normal Value (NV): } 109 \pm 12 \text{ to } 13 \text{ mm})$, left LD $(98.9 \pm 12.6 \text{ mm} - \text{NV: } 112 \pm 12 \text{ to } 13 \text{ mm})$. • 465 pts (77.5%) had bilateral functional kidneys, of these 248 (53.3%) had a symmetric right/left functionality ($50 \pm 5\%$), versus (vs) 217 with asymmetric functionality (of these: right Functional Contribute (FC) $53.4 \pm 15.4\%$ vs. left FC $46.6 \pm 15.4\%$, $p < 0.0001$). • In pts with asymmetric functionality, 131 (60.4%) showed a prevalent right FC vs 86 with prevalent left FC ($p < 0.001$). • 135 pts, were Solitary Functional Kidney (SFK) (79 right SFK, 56 left SFK, $p < 0.001$). In these subgroups, eGFR of the right solitary kidney was of $50.4 \pm 22.3 \text{ ml/min}$ compared to eGFR of $33.8 \pm 7.2 \text{ ml/min}$ of the left SFK ($p < 0.04$). The functional scintigraphic contribution of the right kidney resulted superior to the left kidney ($53.1 \pm 21.4\%$ vs. $46.9 \pm 21.4\%$, $p < 0.0001$), in both males and females. An even greater evidence of functional asymmetry with right renal prevalence was detected in the male population. Further more the functional asymmetry is particularly incident at an age higher than 50 years: in these pts the right vs. left FC was $53.6 \pm 21.9\%$ vs. $46.4 \pm 21.9\%$, $p < 0.0001$. Between the SFK, 61.8% was solitary right kidney vs 39.2% of left SFK, $p < 0.001$). Concerning the pts with functional asymmetry, the doppler ultrasound, performed in 53 cases, 14 stenosis, 57.1% interested the left renal artery, 28.6% the right renal artery, 14.3% both renal arteries.

Conclusions: Left kidney resulted significantly more susceptible to vascular ageing. Both FC decrease and kidney lost were in fact more frequent in the left kidney. A renal asymmetry has indeed indication to further investigation s regardless the presence of renin-angiotensin-system activation.

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Reversible AKI from Repeated Exposure to Ethylene Glycol. A Renal Biopsy Diagnosis

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Background: Ethylene glycol is nephrotoxic after enzymatic transformation into calcium oxalate and undergoes intratubular precipitation, provoking tubular necrosis and AKI.

Case Presentation: A 41 years-old Caucasian male, smoker with history of alcoholic abuse, was admitted to a nearby hospital for severe metabolic acidosis, neurologic signs and worsening of renal functional parameters. EGA showed pH 7.2, HCO_3^- 9.2

mEq/l, anion gap 30 mmol/l, lactate 10 mmol/l. The biochemical profile showed serum creatinine 8.38 mg/dl and BUN 73 mg/dl. Because of accompanying seizures, the patient was admitted to the Intensive Care Unit and treated with continuous renal replacement therapy for a week. The patient recovered completely and was discharged.

After three months, the patients was admitted to our Nephrology Unit for novel occurrence of AKI, elevation of pancreatic enzymes and refractory arterial hypertension. Serum creatinine was 35 mg/dl, BUN 475 mg/dl and EGA showed a high anion gap metabolic acidosis (pH 7.1, HCO_3^- 9.7 mEq/l, anion gap 28.5 mmol/l, lactate 15 mmol/l), oliguria and peripheral edema.

The patient had psychomotor agitation and mydriatic pupils. Magnetic resonance imaging (MRI) of central nervous system (CNS) showed edema in occipital regions, while hypodense lesions were identified on computed tomographic (CT) scans. The toxicological serum and urine examination did not show evidence of toxic substances, because the assay was carried out a few days after the clinical onset; therefore, at this time the cause of this severe acidosis and renal failure was not apparent. A dialysis treatment was initiated. Two days after admission the patient manifested generalized seizures that required oro-tracheal intubation and admission to the Intensive Care Unit. During hospitalization a status epilepticus occurred in association with a paralysis of the seventh cranial nerve. MRI of CNS at this time showed occipital lesions suggestive of PRES. Two days later clinical conditions improved and the patient was readmitted to our Unit. The patient strongly denied any drug-of-abuse or suicidal toxic assumption. Hemodialysis was carried out for a total of four days, then stopped for spontaneous amelioration of renal function parameters and improvement of diuresis. Two weeks after admission, a renal biopsy was performed. The gross microscopic examination of core biopsy showed several translucent crystals of calcium oxalate. The histologic examination confirmed the presence of several crystals mainly within tubular lumens that resembled calcium oxalate monohydrate crystals because of their shape and polarized light optical characteristic. Eventually, the patient's relatives confirmed that the patient had assumed on both occasions a small quantity of car antifreeze solution.

Discussion and Conclusion: In this case the exact diagnosis of ethylene glycol poisoning that caused AKI was evident only after performing renal biopsy. The patient was unwilling to admit assumption of this toxic substance and toxicological assays were not promptly available for a correct diagnosis. Renal biopsy is a relevant procedure for diagnostic purposes in AKI.

A New Adsorptive System for Simple Apheretic Treatments Directly on Blood

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Introduction: A new sorbent (*Cytosorb*, *Cytosorbents*), able to adsorb hydrophobic molecules, could be a valid artificial support in many conditions of organ failure through the reduction of cytokines and other toxic molecules directly from blood. The extracorporeal systems presently available are based instead on plasma-adsorption, that implies a more complex circuit and management.

Aims: We designed an in vitro and in vivo study. The in vitro study evaluated the system's adsorption capacity on bilirubin (a protein-bound solute), while the in vivo study verified the congruence between in vitro and in vivo results and clinical outcomes.

Methods: The in vitro study included 2 tests, carried out with equimolar solution of Albumin (Alb) and Bilirubin (Bil), containing unconjugated (strongly Alb-bound) Bilirubin [1] in order to verify removal of protein-bound solutes. The third test, 24 h long, with higher concentration of Bil and lower of Alb was carried out to study the kinetics of adsorption in this particular condition. The in vivo study is currently enrolling patients with severe hepatic dysfunction with hyperbilirubinemia. Primary outcome is to eval-

uate in vivo Bil removal, whereas secondary outcomes are hemodynamic stabilization, reduction of inotropes, improvement of hepatic function and inflammatory status.

Results: The in vitro experiments showed the adsorption capacity of the system concerning Bil (table 1), with a minimal Alb loss. We could not demonstrate any release of the adsorbed Bil. For the in vivo study, we enrolled until today, 3 patients (table 2). Bil Mass Balance (MB) in vivo was higher than in vitro and showed the system's capacity of Bil removal, whereas removal rate (RR) is not significant for that purpose, being affected by many factors, first at all Bil production.

Conclusions: The in vitro study shows the sorbent efficacy in removing Bil along 24 h at least, without significant Alb loss. By the same adsorption mechanism, inflammatory cytokines are also expected to be removed. Cytosorb might represent a simple aid in organ dysfunctions and other clinical situations. In fact it may work directly on blood, alone, or in combination with CRRT. Other experiences are ongoing to confirm these data.

Reference

- 1 Weber, et al: Biomacromolecules 2008;9:1322–1328.

The Spectrum of the Modern Extracorporeal Blood Purification Therapy in the Intensive Care Units

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Background: A modern view of the extracorporeal blood purification (EBP) therapy in the intensive care Units (ICU) goes well beyond the support to renal failure. This is particularly true in a tertiary care hospital, where most seriously ill patients are treated, frequently coming from the emergency room or a secondary care hospital, or the transplant area. A high level of expertise and special organisation are necessary. In our Unit there is a restricted group of skilled nurses, chosen among the largest group of nurses for RRT, and one doctor with the responsibility for the activity in the ICU. The decision-making process to start with a 'special' (non conventional) technique is shared between Nephrologists and Intensivists. The selection of patients is especially critical, mainly due to the high cost of these treatments. The operational parameters and managing the treatment is only up to the Nephrologist and dedicated nurses.

Materials and Methods: Post-surgical, cardiological, post-heart surgery or transplant, post-liver transplant, general ICU... are the situations where a specific EBP could provide a therapeutic help are different. The removal of bilirubin, ammonia and liver-related toxins mainly affecting the neurological status, may be a valid help both in the patient with a liver disease on waiting list for liver transplant or with a primary-non function. Potassium, myoglobin, free Hb and cytotoxic products in a patient undergoing heart-lung machine may be removed as well with sorbents. The

Table 1. (for Abstract 43)

	1st test	2nd test	3rd test
Bil, mM	0.4	0.8	0.8
Alb, mM	0.4	0.8	0.4
MB Bil, g	0.94	1.02	2.49

Table 2. (for Abstract 43)

	Tot. h treatment	Bil. Conc t ₀ , mg/dl	Bil. MB, g	RR %	Other
1 post-transplant Cirrhosis	28	45.1	2.25	36	Gram – Sepsis
2 post-transplantat Cirrhosis	24	36.7	6.00	0	Gram – Sepsis
3 post-transplant Hyperbilirubinemia	96	53.06	20.88	64.1	Gram – Sepsis

Table 1. (for Abstract 44)

	PEX	Plasmasorption	Combined PlasmasHD	Charcoal hemoperfusion	Endotoxin removal	CPFA	LDL-apheresis
Patients	7	6	3	4	10	10	4
Treatments	28	13	5	6	18	47	8

PEX = Plasmaexchange; CPFA = coupled plasmafiltration-adsorption.

same applies to patients with severe post-traumatic rhabdomyolysis in a general ICU. Sepsis is another condition where different treatments, with convection and adsorption may be used, alone or in association. The LDL-apheresis, a selective apheretic treatment able to remove apoB-lipoproteins, has been used by us in an ICU to treat hypertriglyceridemia-related pancreatitis. Patients with life-threatening complications of an immunological disease such as a vasculitis or LES, should be treated on site with plasmaexchange, frequently associated to dialysis.

Results: In table 1 the list of the non renal EBP treatments we may perform on site in the ICUs, and the number of patients and procedures applied in the recent 3 years. As to the clinical outcomes, it is difficult to state how much do these therapies may actually positively affect survival, due to the extreme complexity of the clinical picture of patients. The efficiency parameters of the different techniques (removal of bilirubin, ammonia, myoglobin, endotoxin, tryglycerides, immune-complexes, autoantibodies...) are always really satisfactorily. The number of treatments is decided both on the measured reduction ratios and the post-treatment rebound.

Conclusion: EBP therapy in these conditions may represent a *plus value* able to induce a more rapid and favourable illness trend. Due to the high complexity and costs, an appropriate selection of patients potentially candidate to these treatments is mandatory. A special organisation model in the Nephrology Unit is advisable.

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Contrast-Induced (CI)-AKI: Much ADO About Nothing?

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Background: Contrast-induced acute kidney injury (CI-AKI) is commonly defined as a decline in kidney function, shown by absolute (at least 0.5 mg/dl) or relative (at least 25%) increase of the serum creatinine levels, occurring within 48 hours after administration of iodinated contrast media (CM). *Data from the published literature* are heterogeneous about incidence and prognosis. However, different clinical conditions are included under the supposed diagnosis of CI-AKI and the only point in common is the

raise of serum creatinine levels after administration of iodinated CM, even though it is likely not the pathogenic cause in the majority of post-CM AKI cases.

Aim of the Study: The aim of this study was to estimate the real occurrence, the risk factors and the clinical impact of CI-AKI, considering other differential diagnosis of nephrotoxicity.

All patients (excluding those who were on dialysis before CM administration) who receive intravenous or arterial administration of CM were included: 429 patients, who performed 550 CM examinations were enrolled in 10 consecutive months. We analysed the following parameters: technical features, clinical context, basal level of serum creatinine and its variation in 48 and 72 hours after the infusion of CM according to KDIGO criteria, standard therapy and prophylactic protocols eventually applied.

Results: 23 events that can be defined as CI-AKI were observed in 21 patients, with an incidence (4%) and need for dialysis (5%) and death (5%) with results similar to those observed in the literature (1–33%, 0.4–5%, 6.3–34%, respectively). The main risk factor identified (OR 61, IC 17–215) was represented by hemodynamic instability (shock, sepsis, arrhythmias), otherwise the basal level of serum creatinine and CM volume did not have statistic significance in the multivariate analysis.

Basing on the collected data, we created an unexpected category of events that was characterized by an improvement of renal function after CM; we called them CI-AKI-REVERSE when they satisfied symmetrical criteria of CIN such as an absolute (at least 0.5 mg/dl) or relative (at least 25%) decrease of serum creatinine levels occurring within 48 hours after administration of iodinated CM.

Of interest, 53 events defined as CI-AKI-REVERSE were observed in 45 patients. In the multivariate analysis, the only factor responsible for CI-AKI-REVERSE occurrence was intravenous profilactic hydration (OR 62, IC 3.788–1027).

Comparing CI-AKI and CI-AKI-REVERSE events, renal function was significantly worse (mean eGFR 53 ml/min vs. 62 ml/min, $p < 0.0001$) and CM quantity was higher (volume/renal function, mean 4.3 vs. 3.3, $p < 0.0001$) in CI-AKI-REVERSE.

Conclusions: We showed that a lot of different clinical conditions are included in the supposed diagnosis of CI-AKI. Paradoxically, if we follow 'post hoc ergo propter hoc', we could hypothesize that CM may improve renal function, since CI-AKI-REVERSE were more frequent than CI-AKI events. In addition, intravenous hydration represents the real element that improves renal function, and it is much more clear in CI-AKI-REVERSE, where negative risk factors (shock, sepsis, hemodynamic instability) were absent. The role of basal renal function and of CM volume as potential risk factors for CI-AKI were minimized since they were worse in CI-AKI-REVERSE than in CI-AKI group. The worsening of renal function in the days after CM needs a differential diagnosis,

evaluating not only the theoretic profile of the 'patient at risk' but also clinical conditions occurring near CM infusion. 'Real CI-AKI' was a rare event and characterized by a low clinical impact and easy to prevent with adequate intravenous hydration.

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Radioisotopic Evaluation of Renal Function in Kidney Transplant Donors after Nephrectomy for Detection of Early AKI and Long Term Compensatory Renal Hypertrophy

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Background: After nephrectomy, as a result of the decrease of the nephron mass, kidney transplant donors (KTD) develop a partial loss of renal function, defined as AKI (Acute Kidney Injury) according to KDIGO criteria (Clinical Practice Guideline, 2012). The recovery of renal function following AKI is mainly ascribed to the concept of renal reserve function (RFR), defined as the capacity of the kidney to increase glomerular filtration rate. However, there are only few studies on RFR in KTD and a correlation with long term functional outcomes.

Aim of the Study: The aim of the present study is to analyze 20 KTD renal function before nephrectomy, in the immediate post-operative period and 1 year after the surgical procedure.

Results: KTD mean age at the time of donation was 54.7 years (min-max, 30–78), mean serum creatinine 0.73 mg/dl (0.5–0.96), eGFR (CKD-EPI) 97.7 ml/min/1.73 m² (69–119) and a radioisotope (⁵¹Cr-EDTA) GFR 100.91 ml/min (78–129). The split function was evaluated by a concomitant scintigraphy using ^{99m}Tc-MAG: the mean percentage of renal function of right kidney was 48.12% (43–56) and left 51.88% (44–57), respectively. As expected, immediately after nephrectomy all KTD worsened renal function: the mean percentage increase of serum creatinine was 81% (50–105%) within 72 hours post-surgery (we observed the peak of creatinine after 24–48 hours in the most of cases). Seven days after surgery, renal recovery was observed in all cases: the mean percentage increase of serum creatinine was 53.2%, significantly lower than the zenith of serum creatinine. These results suggest a potential gain of about 30% in comparison to the starting value of the right kidney. One year after nephrectomy (all left nephrectomies), we studied KTD renal function (GFR) using a radioisotopic evaluation of renal function using ⁵¹Cr-EDTA and we then compared it with the split radioisotope (⁵¹Cr-EDTA) GFR of right kidney at the first scintigraphic evaluation. Mean GFR was 67.9 ml/min (50–87) vs. 48.5 ml/min (39.7–60.4) before donation with an average GFR increase of 19.2 ml/min (0.5–45.6) and a percentage increase of the right renal function up to 110% (mean 41%, min 1.4%).

Conclusions: The results of this study that radioisotopic evaluation is feasible and allows a precise determination of renal func-

tion at different time points after nephrectomy in KTD. Moreover, we observed a compensatory hypertrophy in all the 20 KTD included in the study independently from age and from the presence of co-morbidities such as elevated BMI and hypertension.

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High Cut-Off Hemodialyzers Efficiently Remove Immunoglobulin Free Light Chains and Reduce Tubular Injury Induced by Plasma of Patients with Multiple Myeloma

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Introduction and Aims: Patients with multiple myeloma (MM) develop acute kidney injury (AKI) due to free light chains (FLC) deposition in tubular epithelial cells (TEC) with formation of tubular casts and triggering of apoptosis. High cut-off (HCO) hemodialyzers have been shown to remove FLC allowing renal recovery. The aim of this study was to correlate FLC removal by HCO filters with established markers of tubular injury such as urine NGAL, retinol binding protein (RBP), α 1-microglobulin (α 1-M) and with the pro-apoptotic effect of plasma of patients with MM on cultured TEC.

Methods: We selected 5 MM patients (IgA λ or IgG κ type) with AKI (RIFLE criteria) requiring dialysis (HCO Gambro Theralite, 18 sessions of 6 hr, blood flow 300 ml/min, dialysate flow 500 ml/min). Plasma FLC and urine NGAL were analyzed by nephelometry. Urine immunoelectrophoresis was also performed. *In vitro* on isolated human TEC, we evaluated FLC binding by FACS/immunofluorescence, MM plasma-induced apoptosis (TUNEL, caspase-3-8-9 activities), mitochondrial function (MitoTracker) and NGAL mRNA/protein expression.

Results: At study admission, MM patients showed mean serum creatinine 6.74 ± 1.12 mg/dl, plasma FLC 10246.53 ± 2249.38 mg/l, urine NGAL 226.73 ± 41.85 ng/ml, urine RBP 62.82 ± 12.64 mg/g creatinine, urine α 1-M 287.28 ± 39.75 mg/g creatinine. At day 28 from the inclusion in the study, we found that 4/5 (80%) of MM patients recovered renal function after HCO treatment. After 5 days, we observed the removal of more than 50% FLC and a significant decrease of urine NGAL, RBP and α 1-M. Serum albumin levels remained stable during the study period. *In vitro*, MM plasma induced dose-dependent TEC apoptosis via mitochondrial dysfunction, caspase-3-8-9 activation and an increase of NGAL mRNA/protein expression. HCO treatment significantly reduced FLC binding to TEC, MM plasma-induced apoptosis and NGAL up-regulation.

Conclusions: The results of the present study suggest that HCO hemodialyzers efficiently remove FLC and reduced MM plasma-induced TEC apoptosis. Urine NGAL, RBP or α 1-M and *in vitro* TEC apoptosis may be new potential biomarkers to evaluate FLC removal by HCO filters, tubular injury and renal recovery.

Acute Kidney Injury Requiring Dialysis in Non Renal Solid Organ Transplantation: A 13-Year Retrospective Monocentric Analysis

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Introduction and Aims: Acute kidney injury (AKI) is a frequent complication of critically ill patients in Intensive Care Units (ICUs). AKI is characterized by an enhanced incidence of multiple organ failures, mortality and progression toward chronic kidney disease (CKD). Several studies reported the increased incidence of CKD in non renal solid organ transplant (NRSOT) recipients due to the nephrotoxicity of immunosuppressive drugs, particularly calcineurin inhibitors. However, only a few studies analyzed the relevance of AKI in NRSOT population. The aim of this study was a 13-year retrospective analysis of AKI incidence in NRSOT recipients, trying to identify its clinical relevance on outcome and progression toward CKD.

Methods: We retrospectively analyzed all critically ill patients treated by renal replacement therapy (RRT) in ICUs in the period 2001–2013. For all patients RIFLE and SOFA scores and the severity index ATN_ISS were evaluated at the start of RRT. For each NRSOT recipient, we calculated RIFLE, SOFA and the severity index ATN_ISS at RRT start. The percentage of AKI requiring RRT in the NRSOT population and for single transplanted organ (liver, heart or lung graft) was studied. Renal function (eGFR) was evaluated at the end of observation (30 days). Hemmer-Lemeshow statistical test was performed.

Results: We treated by RRT 2648 critically ill patients with AKI for a total of 20932 sessions performed: 291/2648 (10.99%) were NRSOT recipients. In the study period, 1673 liver, 317 heart and 34 lung transplantations were performed. We treated by RRT 174/1673 (10.4%) liver, 83/317 (26.2%) heart and 34/110 (30.9%) lung transplanted patients. NRSOT patients' characteristics were: age 59.8 ± 7.6 yrs, male 67.7%, serum creatinine 3.88 ± 1.27 mg/dl, SOFA 12.8 ± 3.1 , RIFLE (Risk 12%, Injury 17.2%, Failure 70.8%) and ATN_ISS score 0.8 ± 0.11 . The prevalent cause of AKI was sepsis (44%). Mortality rate of NRSOT patients treated by RRT was 44.3% (129/291), 42.5% (74/174) for liver, 49.4% (41/83) for heart and 41.2% (14/34) for lung recipients, respectively. AKI was a complication of the early post-transplantation period since the need for RRT occurred in the first 30 days after surgery in 69.1% of NRSOT recipients (64.9% for liver, 60.2% for heart and 82.3% for lung, respectively). In survivors, mean serum creatinine at the end of the study period was 2.37 ± 0.82 mg/dl (1.88 ± 0.94 mg/dl in liver, 2.42 ± 0.75 mg/dl in heart and 2.81 ± 0.92 mg/dl in lung graft recipients, respectively).

Conclusions: Our retrospective 13-year analysis of critically ill patients treated by RRT in ICUs revealed an increased incidence of AKI. Sepsis was the main cause of AKI in NRSOT population. AKI requiring RRT in the first 30 days after transplantation was associated with a worse outcome and with progression toward CKD.

Coupled Plasma-Filtration Adsorption Reduces Tubular Injury in Bile-Associated Cast Nephropathy through Direct Adsorption of Bilirubin and Liver-Type Fatty Acid Binding Protein

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Introduction and Aims: Bile-associated deposition of intraluminal casts and proximal tubular epithelial cell (TEC) apoptosis are possible causes of acute kidney injury (AKI) in the course of severe liver dysfunction. TEC injury is mediated by bilirubin adsorption through a mechanism dependent on the activity of the endocytic receptor megalin. Liver-type Fatty Acid Binding Protein (L-FABP) is a 15 kDa peptide belonging to the free fatty acid family able to bind hydrophobic molecules including bilirubin. During liver failure, the increase of L-FABP plasma levels is known to enhance bilirubin uptake with consequent damage of tubular cells. Indeed, L-FABP is able to bind to megalin mediating bilirubin uptake in TEC. The aim of this study was to investigate the protective role of Coupled Plasma Filtration Adsorption (CPFA) on bile cast nephropathy through direct L-FABP and bilirubin resin adsorption.

Methods: We reported the case of a kidney transplant patient who developed sepsis, AKI and liver dysfunction treated by CPFA. We evaluated plasma levels of bilirubin and L-FABP. Renal biopsies, urine sediment and NGAL were performed at different time points. In vitro, we tested: 1) static and dynamic adsorption of L-FABP to polystyrene resin; 2) pro-apoptotic effect (TUNEL) of patient's plasma drawn before and after CPFA on human tubular epithelial cells (TEC): the role of L-FABP was further confirmed in tubular cells engineered to knock-down megalin, the L-FABP receptor, by small interfering RNA (siRNA).

Results: A 50-year-old man was subjected to kidney transplantation with slow recovery of graft function. Kidney biopsy revealed acute tubulo-interstitial and vascular rejection treated with thymoglobulin. He then developed septic shock for Legionella infection with multiple organ failures (serum creatinine 5.2 mg/dl and oliguria requiring dialysis; bilirubin 42 mg/dl with liver biopsy showing marked cholestasis; plasma L-FABP 52 ng/ml). Urine analysis showed the presence of tubular cells, casts and intense positivity for bilirubin: urine NGAL level was 356 ng/ml. A new kidney biopsy showing bile cast nephropathy was performed. After CPFA was started, we observed an increase of urine output, a decrease of bilirubin (<15 mg/dl), L-FABP (9 ng/ml) and urine NGAL (82 ng/ml).

In vitro, we observed that the polystyrene resin efficiently adsorbed L-FABP in static and dynamic condition. When we incubated TEC with patient's plasma, we observed a cytotoxic and pro-apoptotic effect with activation of caspases. After CPFA treatment, the pro-apoptotic activity of patient's plasma on TEC

was significantly reduced. In addition, plasma-induced TEC apoptosis was dependent on the presence of megalin, the L-FABP receptor: indeed, the pro-apoptotic effect of plasma was significantly reduced in TEC engineered to knock-down megalin by siRNA.

Conclusions: CPFA may have a protective role in bile-associated cast nephropathy and TEC apoptosis through the direct adsorption of bilirubin and L-FABP to the synthetic polystyrene resin. CPFA may be exploited as therapeutic option to limit AKI during sepsis and in bridge to transplantation of patients with end stage liver failure.

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Regenerative Potential of Extracellular Vesicles Derived from Endothelial Progenitor Cells in Sepsis-Associated Acute Kidney Injury

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Introduction and Aims: Sepsis is the main cause of acute kidney injury (AKI) in hospitalized patients. Sepsis-associated AKI is not merely related to tissue hypoperfusion, but also to a direct detrimental effect of pro-apoptotic circulating plasma factors able to induce endothelial (EC) and tubular epithelial (TEC) cell injury. Endothelial Progenitor Cells (EPC) are bone marrow-derived precursors able to accelerate tissue regeneration through the release of paracrine mediators such as extracellular vesicles (EV). EV are nanoparticles involved in cell-to-cell communication through transfer of proteins, lipids, mRNA and microRNA. The aim of this study was to evaluate the protective role of EPC-derived EV on EC and TEC injury induced by plasma of septic patients.

Methods: We enrolled in the study 20 critically ill patients with septic shock and AKI (Failure group RIFLE criteria) requiring renal replacement therapy. Peripheral blood EPC were evaluated by FACS (CD34+/CD133+/flk-1+ cells) and isolated on fibronectin-coated plates. EV were isolated from EPC supernatants by ultracentrifugation and characterized for protein, mRNA and microRNA content. EV isolated from EPC of healthy subjects were isolated and added in culture to human kidney-derived EC and TEC in presence of septic plasma, evaluating apoptosis and functional alterations.

Results: In comparison to healthy subjects, patients with sepsis-associated AKI presented an increased number of circulating CD34+/CD133+/flk-1+ EPC. However, EPC of septic patients iso-

lated on fibronectin-coated plates showed a decrease of proliferation and of EV release. EV isolated from EPC of healthy donors were internalized in both EC and TEC through a L-selectin-mediated mechanism, protecting from septic plasma-induced injury. Indeed, on EC, septic plasma induced leukocyte adhesion, decreased angiogenesis through eNOS reduction and induced apoptosis through activation of the complement cascade (increase of C5b-9). EPC EV reduced all these detrimental effects through the transfer of specific pro-angiogenic microRNA (miR-126, miR-296) and mRNA coding for inhibitors of apoptosis (Bcl-xL) and of complement activation such as CD55 (DAF), CD59 and Factor H. On TEC, septic plasma induced death receptor (Fas, TNF-R) apoptosis and mitochondrial dysfunction through decrease of PGC-1 α . In addition, septic plasma induced loss of cell polarity and function as assessed by trans-epithelial electrical resistance (TEER) and decreased expression of the tight junction protein ZO-1, the endocytic receptor megalin and other TEC-specific solute carriers. All these septic plasma-induced injurious effects on TEC were reduced by EPC EV stimulation. However, the EV-associated protection on both EC and TEC was significantly abrogated by their pre-treatment with RNase, the enzyme able to destroy mRNA and microRNA within EV.

Conclusions: EPC isolated from peripheral blood of patients with sepsis-associated AKI showed functional alterations and decreased proliferation and EV release. EPC-derived EV may be exploited as innovative therapeutic approach in sepsis-associated AKI for their ability to inhibit apoptosis and functional alterations of EC and TEC.

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Quorum Sensing Molecules Released by Gram Negative Bacteria Induce Tubular Injury Interacting with Lipopolysaccharide: A New Potential Mechanism of Sepsis-Associated AKI

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Introduction and Aims: Quorum sensing (QS) molecules are small diffusible signal molecules adopted by Gram negative and Gram positive bacteria for intercellular communication. QS are a system of transcriptional regulation dependent on cell density and formed by 2 elements: the signal molecule (usually an acyl-homo-

serine lactone) able to be internalized in the cytoplasm of neighbouring cells and the transcriptional activator.

QS play a crucial role in biofilm formation and are known to bind the lipid A fraction of LPS. Recent studies suggested that QS can also interact with eukaryotic cells exerting immunomodulatory effects and playing a key role in urinary tract infection. The aim of this study was to evaluate the potential role of QS in sepsis-associated AKI by studying their biological effects on human kidney tubular epithelial cells (TEC).

Methods: Human TEC were isolated from kidneys of patients subjected to nephrectomy. QS from *P. aeruginosa* were purchased by Sigma Aldrich. In selected experiments supernatants of QS-negative mutants of *P. aeruginosa* were used on TEC.

We evaluated QS-induced: cytotoxicity (XTT), apoptosis (TUNEL, caspase-3, -8, 9 activities), alteration of cell polarity (trans-epithelial electrical resistance, TEER), ROS production, PGC-1 α expression, NGAL mRNA/protein expression, *in vitro* leukocyte adhesion and FACS or immunofluorescence analysis of molecules typical of fully differentiated TEC (ZO-1, megalin, AQP-2, E-cadherin) or involved in inflammation (ICAM-1, CD40).

Results: QS exerted a dose-dependent cytotoxic and pro-apoptotic effect on TEC inducing Fas and caspase activation. The percentage of tubular cell apoptosis was similar to that observed in presence of LPS. The pro-apoptotic effect of QS on tubular cells seems to be mediated by an alteration of mitochondrial membrane potential (MitoTracker), increase of ROS production and NGAL release. QS induced mitochondrial dysfunction through decrease of PGC-1 α expression. In addition, QS induced de-differentiation in TEC as assessed by loss of cell polarity with a significant decrease of TEER and of expression of ZO-1, megalin and AQP-2. QS induced a pro-inflammatory effect on TEC increasing leukocyte adhesion and surface expression of ICAM-1 and CD40. A similar effect on TEC was observed using supernatants produced from wild type *P. aeruginosa*, but not from QS negative mutants. The cytotoxic and pro-apoptotic effect of QS on TEC was enhanced by co-incubation with LPS. Preliminary results obtained using HPLC-MS revealed that QS are detectable in the bloodstream of patients with Gram-negative sepsis.

Conclusions: QS derived from Gram negative bacteria may induce different detrimental effects on TEC such as cytotoxicity, apoptosis, mitochondrial dysfunction, ROS generation, NGAL release, leukocyte adhesion and de-differentiation with loss of polarity and of expression of the tight junction protein ZO-1. QS may enhance the pro-apoptotic activity of LPS.

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Association between Regional Citrate Anticoagulation and Enhanced Permeability Hemodialyzers Limits Sepsis-Associated Acute Kidney Injury through the Increased Clearance of Inflammatory Cytokines (IL-6) and Microvesicles

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Introduction and Aim of the Study: During extracorporeal blood purification for sepsis, regional citrate anticoagulation (RCA) inhibits inflammation and decreases mortality. Enhanced permeability (EP) hemodialyzers reduce plasma levels of inflammatory mediators including IL-6. Microvesicles (MVs) are small particles released from activated leukocytes and platelets involved in tissue injury. MVs can transfer proteins and genetic information to target cells. The aims of this study were: 1) to quantify IL-6 and to characterize MV from plasma of septic patients correlating their concentration with outcome; 2) to define a potential role of MVs in the mechanisms of sepsis-associated AKI; 3) to evaluate the synergic role of RCA and EP hemodialyzers in the limitation of sepsis-associated AKI.

Methods: Plasma samples were collected from 10 septic patients to analyze IL-6 (ELISA) and MVs (FACS, Nanosight and RNA profiling). RIFLE/SOFA scores were calculated. CVVH or CVVHD with heparin or citrate (CiCa Multifiltrate, Fresenius Medical Care) and with EP hemodialyzers (Emic2, Fresenius Medical Care) were performed. *In vitro*, whole blood or separated leukocytes and platelets were activated by LPS and cytokines in presence or absence of citrate or heparin to evaluate MV release. The biological effects of septic plasma MVs were studied on cultured human kidney-derived endothelial and tubular epithelial cells.

Results: Plasma IL-6 and MV concentrations were higher in septic than non septic or healthy subjects and correlated with severity of illness and mortality. MVs from septic patients expressed HLA antigens Fas-L, CD40-L, integrins and carried mRNAs and microRNAs involved in inflammation and apoptosis. Plasma MVs induced functional alterations and apoptosis of kidney-derived endothelial and tubular epithelial cells. Association between RCA and EP hemodialyzers significantly decreased plasma IL-6 and MV levels. This effect was less marked using heparin as anticoagulant. Similar findings were observed *in vitro* during CVVH or CVVHD with LPS-activated blood. Citrate significantly reduced the release of MVs from leukocytes and platelets activated by LPS and their ability to induce apoptosis of cultured kidney cells.

Conclusions: During sepsis, MVs are released by activated leukocytes and platelets and their concentrations associated with severity of illness and mortality similarly to IL-6. MVs play a potential role in the pathogenetic mechanisms of AKI. Association between RCA and EP filters may inhibit sepsis-associated AKI through the removal of pro-apoptotic MVs and inflammatory mediators.