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**Study No. NT001 - Doppler Ultrasound for Prediction of Reversibility of Acute
Kidney Injury in Septic ICU Patients**

Study protocol and statistical analysis plan

Thessaloniki, 5 October 2017

Study protocol and statistical analysis plan

This was a prospective observational cohort study with unselected, consecutive septic patients being included. Thirty-two adult septic patients hospitalized in a general intensive care unit were studied during the first 24h of treatment and after the discharge from the ICU setting. The unit has a capacity of 15 beds and is located in a large tertiary hospital in northern Greece. The study was conducted from March to August 2017. The protocol had the approval of the ethics committee of the hospital. Subjects were included if they developed sepsis plus AKI during their hospitalization in the ICU, regardless of the underlying cause. The presence of Acute Kidney Injury was determined according to the KDIGO guidelines, i.e. at least stage 1 AKI. The categorization of AKI as transient or persistent was determined by the normalization of the kidney function after the administration of appropriate therapy and support in the ICU environment and after 3 days of hospitalization. For the purpose of the study all consecutive patients suffering from sepsis were included, with the exception of those surviving less than three days, in order to determine whether they had AKI (persistent or not).

After the determination of sepsis, patients were divided into 3 distinct groups: Group 1 included the patients without progression to AKI, Group 2 included the patients with transient AKI and Group 3 comprised of the patients with persistent kidney injury. For every group a series of functional physiological parameters were recorded or measured: Age, sex, mortality, hospitalization days in ICU, baseline Urea and Creatinine, SOFA and APACHE II scores upon admission to ICU, Lactates, Urine Urea and Creatinine, Fractional Excretion of Sodium (FENa), the Urine Urea to Plasma Urea and the Urine Urea to Plasma Creatinine ratio, the Urine Potassium to Urine Sodium ratio, Plasma urea/creatinine ratio, the Daily Diuresis and the Fluids Balance during the first 3 days of ICU stay and the Hematocrit. Other parameters recorded were related to the cardiac function as assessed by the heart ultrasound [Velocity-Time Integral calculated at the aortic valve (VTI) and the Cardiac Index (CI)], as well as respiratory parameters in mechanically ventilated patients (Respiratory Compliance, Tidal Volume - V_t , Oxygen Saturation of Hemoglobin – SaO_2 , PaO_2 to FiO_2 ratio) or circulatory system indexes, like Mean Arterial Pressure (MAP), heart rate (HR) and dosage of vasoactive agents.

The US examinations were performed by three ICU medical doctors under the supervision of a senior consultant, experienced in abdomen US. A standard convex transducer for abdominal exploration was used for the identification of renal vasculature and the calculation of the resistive index. B-mode US in the posterior-lateral approach allowed localization of the kidneys and evaluation of renal parenchyma. In all patients, simultaneous recordings of color Doppler ultrasound for semi-quantitative evaluation of renal vasculature and pulse Doppler ultrasound for the calculation of RI were performed. The former led to a grading from 0 (no identifiable renal vasculature) to 3 (fully identifiable renal vessels up to the arcuate arteries) (Table 1). The Resistive Index is calculated employing the following formula: $RI =$

[peak systolic velocity - minimum diastolic velocity] / peak systolic velocity. Assessment of both kidneys was performed and at least three values for RI were obtained from each kidney. The average value from all the measurements was considered the final value for every case.

Table 1: Color-Doppler for a semi-quantitative evaluation of intra-renal vascularization

Stage	Quality of renal perfusion by color-Doppler
0	Unidentifiable vessels
1	Few vessels visible near the hilum
2	Hilar and interlobar vessels in most of the renal parenchyma
3	Renal vessels identifiable until the arcuate arteries in the field of view

Statistics

The study parameters were tested for compliance with the normal distribution with the Kolmogorov-Smirnoff test. For the comparison between the 3 groups of patients the one-way Analysis of Variance (ANOVA) was utilized. The Bonferroni post-hoc analysis was employed to reveal statistical differences in means between the three groups of patients. For discrete variables, the χ^2 test was used. Correlations between various parameters were explored using the Pearson's correlation test. The identification of independent variables predicting persistent AKI was based on multivariate logistic regression analysis. Finally, the creation of the ROC curve for the calculated RI values was used to determine the ideal cut-off value of the RI concerning the prediction of persistent AKI in this pool of participants, rendering the respective sensitivity and specificity characteristics.

Statistical significance level was set to 0.05. All statistical analyses were performed using the SPSS v.15 (IBM) statistical software.