Salivary Urea Nitrogen as a diagnostic tool in Acute Kidney Injury

Clinical Research & Prevention Program

Research Proposal

Curitiba – PR - Brazil
Summary

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**Section A: General Project Information**

**Country/region where the project takes place:**

Curitiba, Brazil, Latin America.

Angola, Sudan and Malawi, Africa.

**Project title:**

Salivary urea nitrogen as a diagnostic tool in Acute Kidney Injury

**Name and address of the coordinating Institution (Applicant):**

Pontifícia Universidade Católica do Paraná - Division of Renal Medicine

Av. São José, 300 – Cristo Rei - 80050-350 - Curitiba – PR – Brazil

**Head of the Institute:**

Dr. Roberto Pecoits – Filho, MD, PhD, FASN, FACP

Position: Director, Graduate Program in Health Science – Pontifícia Universidade Católica do Paraná

**Local coordinator of the project:**

Viviane Calice da Silva, MD, ISN Scholar

Position: Nephrologist

Contact Address: Av. São José, 300 – Cristo Rei - Curitiba – PR – 80050-350

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Duration of the project (in months): 12 months
**Section B: Project description**

### a. Rationale of the project in the context of the need of the Applicant’s country

Acute kidney injury (AKI) is defined as an acute worsening or loss of kidney function and can be caused by different mechanisms, shows high incidence with a high morbidity and mortality to patients \(^{(1)}\).

AKI can be classified as per the Acute Kidney Injury Network (AKIN) criteria which bases essentially on the worsening in the baseline serum creatinine over time and urine output to classify patients according to the severity of their underlying disease and helps to identify those at high risk and higher need of additional health care support like dialysis \(^{(2,3)}\).

Despite technological progress in the field of diagnostic methods, the incidence of acute kidney injury (AKI) remains high, particularly in developing countries where medical infrastructure is often lacking. The etiology of AKI differs between developed countries and developing countries. In developed countries, elderly patients predominate, most of cases are treated in large and specialized medical centers and more than 20% of AKI cases develop in the setting of an intensive care unit \(^{(4)}\).

In developing countries this AKI is often a disease of the young. Etiology is dependent on geographical location and may be secondary to infections such as malaria, leptospirosis and dengue fever, but also by envenomation (snakes or arthropods), and can also be subject to seasonal variation \(^{(4,5,6)}\).

Common causes in developing countries are also dehydration, shock, nephrotoxicity from medications and obstructive nephropathy. Particularly in these settings the underlying disease and the resulting AKI often results in high mortality rates of affected patients. Furthermore there is reportedly a larger fraction of volume responsive AKI which could be successfully treated given preceding immediate diagnosis. This makes the need of diagnostic tool which are simple to use and do not require time-delaying laboratory methods obvious \(^{(2)}\).

Kidney function can be evaluated by many methods such as the quantification of inulin clearance (considered as the reference method of kidney clearance capabilities), but also by estimations based on serum and urine creatinine and urea measurements and the calculating of the actual clearance over a defined period.

Furthermore it can be estimated by regression models which allow the estimation of glomerular filtration rate (eGFR as per inulin clearance) as a function of serum creatinine and anthropometric and demographic parameters (such as age, gender, race, weight,
height and muscle mass). Well established examples are the Crockcroft-Gault and the MDRD (Modification of Diet in Renal Disease) estimation equations \(^{(9-15)}\). However, all those methods are laboratory dependent and it implies in a big structure and huge costs.

As for the diagnostic markers, which could serve this purpose, urea has been proposed in the past. It is present in total body water and in exocrine secretions such as saliva, tears, and sweat. Saliva urea nitrogen levels (SUN) and blood urea nitrogen levels (BUN) were shown to correlate in both healthy subjects and chronic kidney disease patients (CKD) in stages 1 to 5D with correlation coefficients (R) between 0.86 and 0.98. Receiver operating characteristic (ROC) curve analyses revealed good diagnostic performance as reflected by an area under the ROC curve between 0.85 and 0.90\(^{(16,17-20)}\).

Recently, the use of a SUN dipstick was studied in AKI settings in cross-sectional pilot study and the higher sensitivity (0.91) and negative predictive value (0.97) were found to diagnose AKIN 3 which is considered the worse degree of disease and when patients need to be referred to treatment immediately \(^{(21)}\). In addition the dipstick offers advantages by its noninvasive nature and the fact that neither a clinical laboratory nor trained personnel are required \(^{(16,21)}\).

Disadvantages of this tool are mainly caused by the bacterial flora from mouth which may interfere with the results because some of these microorganisms produce urease, which cleaves urea and falsely lowers the SUN producing false negative results and delaying the treatment. The result of dipstick is obtained by changes of pH after application of saliva on the test pad, which makes changes in pH during the day and dialysis correlated possible confounders to the results too \(^{(22,23)}\).

However, it is possible that this tool, used in concert with medical history, clinical signs and symptoms, could be useful for an early diagnosis of AKI particularly in the triage during major disasters.

The current study is designed to analyze the diagnostic performance of the dipstick in a longitudinal setting in the screening and response to treatment initiation (such as hemodialysis or peritoneal dialysis amongst others) or during substantial travel times to specialized medical attention in a first phase in Brazil and in a second phase in some places in Africa where this tool would be most helpful and valuable.
b. Objectives of the program

**Primary aims:** Investigate the diagnostic performance of the SUN dipstick in patients diagnosed with acute kidney injury (AKI) according to the AKIN criteria.

![Figure 1. AKIN Criteria](image)

**Secondary aims:** Analyze the capability of the SUN dipstick to detect changes (improvement or worsening) of kidney function (reflected by blood urea nitrogen; and creatinine) or initiated treatment during the observation period.
c. Plan of the project and methodology

Research design: The study will be conducted as a longitudinal, prospective and observational study. Patients with AKI diagnosis (as per the AKIN criteria – creatinine criteria) will be studied and monitored with dipstick measurements (simultaneous to blood measurements) for a maximal period of 7 days.

Sample size calculation: To estimate the sample size a pilot study was conducted at Hans Dieter Schmidt Regional Hospital, Joinville, Santa Catarina last January 2014, where 16 patients with AKI where enrolled. A mean difference between BUN and SUN was found during the follow-up days for each day to all patients. To be able to evaluate the improvement or worsening of kidney function in AKI patients along the days the dipstick result needs to remain approximate of BUN's result, therefore the mean difference between them must maintain the parallelism.

To attend the condition of 5% chance (α=0.05) of rejecting the hypothesis of parallelism when parallelism actually exists (Type I error), and the condition of 80% chance of rejecting the hypothesis of parallelism when the difference between BUN and SUN is higher than 10 mg/dL from the first measurement we found a minimum sample size of 23 patients (80% is the power of the test).

For the reason of this study will be conducted in more centers we extended this sample to 60 patients in phase 01 and 120 patients in phase 02 described below.
**Study population**: Patients admitted to the hospital with the diagnosis of acute kidney injury, regardless of age, gender, race, social status are eligible participate.

**Measurements**: Simultaneous with blood urea nitrogen and creatinine measurements, SUN will be assessed using the SUN dipstick in a maximum of 4 hours between saliva and blood collection.

**Figure 2: SUN dipstick bottled**
d. Expected outcomes

**Primary outcome:** Diagnostic performance of the SUN dipstick applied in the diagnosis in patients with suspicion of acute kidney injury quantified as: sensitivity and specificity, area under the receiver operating curve, positive and negative predictive value.

**Secondary outcome:** Identify the capability of SUN dipstick in detect the improvement or variability (caused by worsening of disease, dialysis influence and recovery) in kidney function during the period of observation.

**Patient selection:** Patients with suspected AKI hospitalized will be enrolled.

**Locations:**
- Hospital Universitário Cajuru, Curitiba, PR, Brazil
- Hospital Regional Hans Dieter Schmidt, Joinville, SC, Brazil
- Santa Casa de Misericórdia de Curitiba, PR, Brazil

**Inclusion/Exclusion criteria:**

**Inclusion criteria:**
- All patients who have some kidney dysfunction shown by an alteration in serum creatinine and blood urea nitrogen will be analyzed.
- After a first medical history evaluation they will be enrolled or not to participate in this study.
- Patients with criteria for AKI detected by AKIN classification will be invited to participate in the study and applied to these consent forms.
- Patients with some degree of Chronic Kidney Disease (CKD) that have a worsening in kidney function will be enrolled.

**Exclusion criteria:**
- Patients with CKD – stage 5d.
- Patients with AKI but unable to give their consent or unable to produce sufficient saliva will be excluded.
Measurements:

Saliva collection: For application of SUN dipstick patients will be asked to provide 1 to 2 ml of unstimulated saliva after refraining from eating and drinking for at least 15 minutes. Saliva will be collected in a plastic cup and allowed to separate in a liquid and foamy phase over a period of 1 – 3 minutes. 50 µl of liquid saliva will be transferred to the two test pads of the SUN dipstick, to the background pad first, then to the reagent pad (Integrated Biomedical Technology) (Figure 2). After 1 minute the color of the test pad will be visually compared to 6 standardized color blocks indicating semi-quantitatively SUN concentration ranges of 5–14, 15–24, 25–34, 35–54, 55 – 74, and >75 mg/dl. If there is an abnormal saliva pH or buffer capacity, the background pad will show color which is not caused by urease. The equivalent saliva urea nitrogen level from this background color will be used to subtract from the reading of reagent pad color level.

This process will be performed twice a day, at the same time, for a maximum of 7 days. Trained nephrologists will collect the samples.

Blood samples collection: At the same time of saliva collection will be collected blood sample for analyzes of blood urea nitrogen and creatinine by the method standardized by internal laboratory of the hospital which will be used to determine their correlation with salivary urea nitrogen.
Statistical analysis and data flow:

**Database:** The database will be created in the first stage in Microsoft Excel 2011 until the end of recruitment period by the principal investigator and updated for him weekly. All data will be stored as a limited dataset in compliance with current local and federal regulation. All the data will be encoded with only a unique anonymous identifier for each enrolled patient to maintenance of confidentiality and just the investigators of the study will have access to these data. After the recruitment period the data will be imported to SPSS 20 for statistical analysis.

**Statistical analysis:** For tests comparing blood urea nitrogen and salivary urea nitrogen will be performed by Spearman’s rank correlation test. Sensitivity and specificity, areas under receiver operating characteristic analysis and calculation of the Youden index, will be calculated respectively, to assess diagnostic performance and the optimal thresholds. For analysis of estimated creatinine clearance will be used formula: Cockroft-Gault that uses age, weight, gender and serum creatinine C&G=\[{(140 - \text{age}) \times \text{weight}}/[72 \times \text{Scr(mg/dl)}}\] × (0.85 if female) and MDRD that uses age, gender, race and serum creatinine (MDRD=\[186.3 \times \text{Scr}^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ female}) \times (1.212 \text{ black})\])\(^\text{10,11}\)

SPSS 20 will be used as the stat software to analyze the data. Inter-observer variability will be assessed using Kappa statistics. Additional parameter (such as stratified and multivariate analysis) will be used to control and identify possible confounding factors.
Study timeline:

Phase 1: Recruitment period

Patients will be enrolled during a 7 months recruitment period in Brazil. During the first two months we will enroll patients only in adult patients in the three hospitals cited above.

Phase 2: Study replication

The same project will be conducted in pediatrics hospitals in Brazil and Sudan as well as in adult patients in general hospitals in Sudan, Angola and Malawi to replicate the experience in these sites and settings where the test would be most helpful and valuable. At month 4 and 5 we will be training the fellows from Sudan, Angola and Malawi; after that period they will enroll patients in their own country.

Figure 3. Study timeline
Figure 4. Participant Sites

Phase 1 - Brazil

- Cajuru Hospital
- Hans Dieter Regional Hospital
- Santa Casa Hospital

Phase 2 – Brazil + Africa

Children

- Pequeno Principe Hospital
- Sudan Sobu University Hospital

Adults

- Angola Clínica Multiperfil
- Sudan Sobu University Hospital
- QECH Malawi Hospital
1. Form for clinical and laboratory evaluation of patients with suspected AKI and data results.

<table>
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<th>Inclusion date</th>
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<td>Patient ID number:</td>
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<tr>
<td>Birth date:</td>
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<tr>
<td>Gender:</td>
<td>Female ( ) Male ( )</td>
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<td>Race:</td>
<td>Black ( ) Non-Black ( )</td>
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<td>Reason for admission:</td>
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<td>Time onset symptoms:</td>
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<td>Previous diseases:</td>
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<td>Baseline serum creatinine (mg/dL)</td>
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<td>Baseline serum Urea or BUN (mg/dL)</td>
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<td>Admission serum creatinine (mg/dL)</td>
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<td>Admission Urea or BUN (mg/dL)</td>
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<td>CKD worsening</td>
<td>( ) Yes ( ) No</td>
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<td>AKI etiology (first one):</td>
<td>Pre-renal ( ) Post-renal ( ) Renal ( )</td>
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<td>AKIN classification at screening date:</td>
<td>I ( ) II ( ) III ( )</td>
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<td>Other tests to the diagnosis of AKI:</td>
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<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>AKIN</th>
<th>Investigator</th>
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e. Description of the Applicant’s Institution and the research group.

The School of Medicine, Pontificia Universidade Católica do Paraná, is a 55 year old school based in Curitiba, Paraná, Brazil. The Renal Medicine Division is part of the Department of Internal Medicine based at the Cajuru and Santa Casa University Hospitals, where the residency program in Nephrology is based. The Renal Medicine Division has strong activities in translational and clinical research, particularly in the area of chronic kidney disease and dialysis, and offers opportunities for Master and PhD students at the Post-Graduation Program in Health Sciences. The Renal Medicine Division is an ISN Sister Renal Center of the University of Nottingham, UK, and has been an academic partner of Renal Research Institute since 2011. Our Institution hosts at the moment 2 ISN Research Fellows from Sudan, who will be actively involved in both phases of the study.

**Figure 5. Working Team**
Viviane Calice da Silva – coordinator of the project and of the Hans Dieter site.

Rafael Weissheimer – coordinator of the Cajuru site.

Fellype Barreto – coordinator of the Santa Casa site.

Eduardo Gubert – coordinator of the Pequeno Príncipe site.

Dalia Yousif and Rasha Hussien – ISN Fellows and coordinator of the Sudan site (Soba University Hospital).

Euclides Scomboio – coordinator of the Angola site (Clínica Multiperfil).

Rhys Evans and Gavin Dreyer – coordinators of the Malawi site (Queen Elizabeth Central Hospital).

Ludimila Campos – study coordinator.
Section C: Relevant references to the project


Section E: Short summary of the project

There are many methods of evaluation and measurement of renal function like inulin dosage, serum creatinine and urea among others. These methods have many limitations like costs and the need of a lab to analysis.

Because of some difficulties about this process a method applied on bedside will improve diagnosis from some kidney pathologies making more easy and cheap for the hospitals and medical centers.

Recently, a SUN dipstick was developed and researchers demonstrated the capability of a semi-quantitative SUN dipstick test to diagnose BUN levels > 25 mg/dl in CKD patients and BUN levels > 47 mg/dL in AKIN 3 patients. ROC analyses revealed excellent sensitivity, specificity and diagnostic performance as reflected by the large area under the ROC curve from 0.85 to 0.90 (CKD) and 0.91 (AKI) respectively\(^{(16,21)}\).

After this discovery there is an interesting in study this method of diagnose to follow patients with acute kidney injury to identify if the correlation between blood urea nitrogen and saliva urea nitrogen is maintained according the treatment applied.

This study aims apply the SUN dipstick in a group of 60 patients with diagnose of AKI for AKIN classification screened daily through the internal laboratory of each service cited above and investigate the capability of this tool to show the changes in BUN at the same time according the treatment applied.

This study will be replicated as a proof principle in another 120 patients, 60 pediatrics and 60 adults in different centers and primary care facilities in Brazil and Africa. We believe that SUN strips could be an important tool to help in the screening, diagnose and follow-up of AKI patients in very poor scenarios where nowadays people are dying without any diagnose or treatment.
Section F: Informed consent document

You are invited to participate in the study: Salivary urea nitrogen as a marker for diagnosis and follow-up for acute kidney injury.

You have been selected through an evaluation form and your participation is not mandatory. At any time you can withdraw your consent to participate. Your refusal will have no impairment in your relationship with the researcher or the institution, or may remain following the treatment with the assistant team according previously planned.

The aim of this study is to identify the capability of salivary urea nitrogen dipstick to diagnose acute changes in renal function and detect improvement or worsening in kidney function after the proposed treatment or according the disease evolution.

Your participation in this research will require a sample of saliva and blood for analysis of daily tests to analyze the kidney function. Blood urea nitrogen, serum creatinine and saliva urea nitrogen will be taken until day seven of follow up.

The risks related to your participation are the possibility of pain at the time of blood collection and a bruise in the same area that will disappear in some days.

The benefits related the participation is to assist an identification of diagnosis method of acute kidney injury, faster and cheaper to the health care system.

The information obtained through this study is confidential and ensure the confidentiality of your participation. Data will not be disclosed in order to enable your identification. You will receive a copy of this term, which states phone number and address of the investigator and may take your questions about the project and your participation at any time.

I declare that I understand the objectives, risks and benefits of my participation in this research and agree to participate.

Name Patient: ___________________________ Name Investigator: ___________________________
Signature Patient: ______________________ Signature Investigator: _______________________
Local and Date: ________________________ Date: _______________________

Information Phone number: (55) 41-3271-3150