# Early Detection of Acute Renal Lesions by Serum Cystatin C in Children at Hospital and University Centre of Brazzaville

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## Summary

**INTRODUCTION**

**Acute Kidney Injury (AKI) is considered one of the main public health problems. The effective management of these alterations is based on the early detection of renal lesions. The objective of this study was to evaluate the contribution of the Cystatin C (CysC) assay in the early diagnosis of acute kidney injury (AKI) in children hospitalized in pediatric intensive care units in Brazzaville.**

**MATERIALS AND METHODS**

**Sixty children at high risk of developing AKI were included. Consent form signed was obtained from parents, socio-demographic data, weight and height of children recorded. Creatinine (Cr), CysC and urea were assayed in serum 24 hours after admission. Glomerular filtration clearance was estimated using serum creatinine and CysC. Glomerular filtration rate (GFR) was calculated from CysC and Cr. The diagnostic accuracy was determined by comparing the results of CysC to those of Cr (considered as a reference biomarker).**

**RESULTS**

**The median age was 5 years (with extremes ranging from 1 month to 17 years). Cr, CysC, urea, and GFR/Cr (mean ± standard deviation [range]) were 0.94±1.17 (0.2–1.4 mg/dl), 0.14 ± 0.062 (0.053-0.095 mg/l), 46.65±47.75 (15.0–45.0 mg/dl), 81.85±31.90 (≥190 ml/min per 1.73 m2 , respectively. The level of CysC in patients with ARL was significantly higher than that of children with normal renal function (p<0.001). CysC detected 71.7% of children with AKI versus 26.7% with Cr. The performance characteristics (area under the curve, sensitivity, specificity) were 0.63, 89.6% and 37.5% for creatinine and 0.76, 92.9% and 54.8% for cystatin respectively. Analysis of the characteristics of the two curves revealed that CysC had a significantly higher diagnostic capacity (p<0.001).**

**CONCLUSION**

**Our results show that the performance of serum CysC in detecting AKI early was superior to that of serum Cr in children hospitalized in pediatric intensive care units in Brazzaville.**

***Keywords:*** *Acute Kidney Injury, Cystatin C, Serum Creatinine, Paediatric Intensive Care Unit.*

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## Introduction

Acute Kidney Injury (AKI), defined as a sudden loss of kidney function with a rapid decline in glomerular filtration rate, is considered one of the major public health problems [1, 2, 3]. Although reversible in most cases, it can be life-threatening. In children, AKI are particularly found in those hospitalized in pediatric intensive care units (PICU). Although its prevalence in children is not well known [1, 4], its global incidence would be increasing ranging from 4.5 to 82%, and the global mortality rate varies between 20 and 47% [5, 4, 6]. In sub-Saharan Africa, 66% of children in the pediatric intensive care unit develop AKI, and the mortality rate associated with these ARS varies between 20.9-46.8% [7; 8]. In Congo, no pediatric data on acute kidney injury is available.

AKI screening is based on determining the Glomerular Filtration Rate (GFR) expressed in ml/min/1.73m2 [9, 10, 11] which is estimated from serum creatinine measurements using various equations [12]. The early detection of AKI in pediatric care units is essential for better management [13, 14, 15]. Serum creatinine, considered the gold standard, remains the most practical and widely accepted method [4, 16]. However, there are limits to their use. Serum Cr levels can be affected by many factors including age, gender, medication, diet, muscle mass, metabolic rate, inflammatory disease, or liver disease can influence serum Cr leading to an inaccurate estimate of renal failure [4, 16, 6, 2]. Indeed, creatinine does not increase significantly until 25 to 50% of renal function has been lost, and there may be a temporal dissociation between the increase in serum creatinine and actual glomerular filtration.

These findings have motivated the search for new, more reliable markers of GFR. The ideal marker would be a freely filtered endogenous substance, with an unmodified plasma concentration [1, 4, 17, 2]. Attention is currently focused on Cystatin C, an inhibitor of lysosomal cysteine protease [1, 4, 18]. Cystatin C (CysC) is an endogenous small molecule non-glycosylated (13 kDa) protein produced in all nucleated human cells at a constant production rate, and its production rate is unchanged in the inflammatory state [4, 19]. CysC is freely filtered through the glomerular membrane and is not secreted by the tubule, reabsorbed in serum, or catabolized in the proximal tubule. Therefore, his serum levels reflect the level of glomerular filtration rate (GFR). Our study aimed to prospectively evaluate the value of serum cystatin C as an early marker of deterioration of renal function in seriously ill children admitted to the PICU. The hypothesis is that CysC, as an

early biomarker of AKI, would be strongly correlated with the clinical state, more than Cr.

## Materials and Methods

This was a cross-sectional analytical study carried out between October and December 2020. The study population was represented by children admitted to pediatric intensive care units (PIC) at the Brazzaville Hospital and University Center and the Talangaï Reference Hospital (TRH). The biological analyzes were carried out at the Faculty of Health Sciences (FHS), in the clinical and molecular biochemistry unit.

The study protocol received approval from the Research Ethics and Health Sciences Committee (CERSSA) under number 270/MRSIT/IRSSA/CERSSA and complied with the Declaration of Helsinki. We obtained the signed consent of all parents or guardians of the children included in the study.

A total of 60 children (aged between 1 month and 17 years) were included in the study, within 48 hours of admission. The selection of the study population was random, drawn at random from children hospitalized in the pediatric intensive care unit, according to the diagram in figure 1 in the appendix. All children were at risk of developing renal failure (hemodynamic instability, sepsis, nephrotoxic therapy, and others). Patients receiving corticosteroid therapy or with thyroid disease or those with pre-existing kidney disease were not included.

Socio-demographic data were collected using a survey form. A blood sample was then taken from each patient in the morning (between 7 a.m. and 9 a.m.) for biochemical assays (creatinine, cystatin C and serum urea). AKI was defined by the pediatric «Risk Injury Failure Loss and End-stage (pRIFLE) and a decrease in estimated creatinine clearance (eCCl) of at least 25 % from baseline within 48 h of admission [20]. Serum Cr and urea were determined by the modified Jaffé colorimetric method [21]. An indirect sandwich ELISA competition assay method was used to determine the level of CysC.

GFR was calculated using serum Cr levels, respectively [22, 23].

The diagnostic value of serum Cr and CysC to identify AKI was assessed using ROC curve characteristic analysis. The performance characteristics of CysC were determined and compared to that of Cr.

Microsoft Excel version 2016 software was used for the design of the database and IBM SPSS software (version 26, CHICAGO-USA) for data processing. The study of normality allowed us to determine the distribution of the Laplace-Gauss law by the Kolmogorov-Smirnov test. The qualitative variables were expressed in numbers with percentages and the quantitative variables in mean, median and standard deviation. The nonparametric t-test was used to compare two means and the KHI-2 and Ficher tests to compare proportions. Sensitivity (Se), specificity (Sp), and positive (PPV) and negative (NPV) predictive values were assessed to determine the relevance of cystatin C in the early diagnosis of AKI. The area under the ROC curve defines the performance of the marker. The difference was considered significant when the p-value was less than 0.05.

Research involving human subjects complied with all relevant national regulations,

institutional policies and follows the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Research attestation) and Research Ethics and Health Sciences Committee (270/MRSIT/IRSSA/CERSSA). Informed consent was obtained from all parents of children included in this study.

## Results

The population recruited during the study period is described in Table I. Sixty children admitted to the pediatric intensive care units were included (29 boys and 31 girls). The boy/girl ratio was 0.94. The median age was 5 years, the mean 6.60±5.31 with extremes ranging from one month to 17 years.

Serum Cr, serum CysC, urea, and glomerular filtration rate (mean ± standard deviation [range]) were 0.94±1.17 mg/dl (0.2–1.4 mg/dl), 0, 14±0.06 mg/l (1.31–2.38), 46.65±47.75 (15.0–45.0 mg/dl ) and 81.85±31.90 (≥190 ml/min by 1.73 m2), respectively. These results are shown in Table II. CysC detected 43 children (71.7%) with AKI versus 34 (56.7%) and 16 (26.7%), respectively by DFG/Cr and serum Cr (Table III).

The area under the curve (AUC) was 76.5% [60.3 - 80.8] (0.76 [0.60 - 0.81]) for CysC and 62.8% [48.5 - 67.0] (0.63 [0.49 - 0.67]) for creatinine. CysC therefore exhibits greater discrimination than that of Cr (figure 2). The comparative performance characteristics of creatinine and cystatin C (sensitivity, specificity and positive likelihood ratio), for detecting AKI are shown in Table IV. The sensitivity of the markers was 92.9 and 89.6 for CysC and Cr, respectively.

**Table I:**

***Socio-Demographic Characteristics of the Study Population***

| Variable | Details | *p*-value |
|---|---|---|
| Age (years) | | |
| Mean ± SD | 6.60 ± 5.31 | |
| Median (Min - max) | 5 (0.08 - 17.0) | |
| Age group n (%) | | |
| [0 - 1[ | 7 (11.7) | |
| [2 - 4[ | 18 (30.0) | |
| [5 - 10[ | 22 (36.6) | |
| [11 - 18[ | 13 (21.7) | |
| Sex ratio (M / F) | 29/31 or 0.94 | <0.0001 |

*n: number of patients ; SD: Standard deviation ; Min : minimum value ; Max : maximum value; %: percentage*

**Table II:**

***Profile of Renal Biomarkers***

| Biomarkers | Mean ± SD (range) | Median (Min-Max) |
|---|---|---|
| Creatinine (mg / dL) | 0.94 ± 1.17 (0.2–1.4) | 0.56 (0.27 - 8.39) |
| Cystatin C (mg / L) | 0.14 ± 0.062 (0.053 - 0.095) | 0.13 (0.07 - 0.28) |
| Urea (mg / dL) | 46.65 ± 47.75 (15 - 45) | 28.53 (5.76 - 233.6) |
| GFR / Cr (ml / min / 1.73 $m^2$ ) | 81.85 ± 31.90 (≥120) | 84 (5.0 - 201.0) |

*GFR : glomerular filtration rate ; Cr : creatinine ; CysC : cystatin C ; SD: standard deviation ; Min : minimum value ; Max : maximum value.*

## Discussion

Determination of glomerular filtration rate (GFR) quantifies renal function. In recent years, Cystatin C has been proposed as an alternative to serum creatinine for the estimation of GFR. However, the serum creatinine concentration remains the most widely used marker to determine the intensity of renal lesions in current practice [5, 24, 25]. Indeed, the estimation of GFR via the serum creatinine level would be biased, because the levels could be higher than the real values [15, 26-29]. Thus, several studies have been carried out to identify new early biomarkers of acute kidney injury in different countries.

This work aimed to evaluate the performance of Cystatin C in the early detection of ARS compared to creatinine in children admitted to pediatric intensive care.

**Table III**:

***Distribution of Clinical Groups (AKI+ and AKI-) According to Biomarkers***

| **Biomarkers** | **AKI+** | **AKI-** | ***P-value*** |
|---|---|---|---|
| Creatinine (mg / dL) | n = 16 | n = 44 | |
| Mean ± SD | 2.031 ± 1.814 | 0.567 ± 0.208 | |
| Percentage (%) | 26.7 | 73.3 | 0.0022[a] |
| Cystatin C (mg / L) | n = 43 | n = 17 | |
| Mean ± SD | 0.169 ± 0.053 | 0.075 ± 0.005 | |
| Percentage (%) | 71.7 | 28.3 | 0.0001[a] |
| TFG (ml / min / 1.73m$^2$) | **n = 34** | **n = 26** | |
| Mean ± SD | 78.47 ± 30.80 | 81.95 ± 32.33 | 0.1421[a] |
| Percentage (%) | 56.7 | 43.3 | |

*GFR: glomerular filtration rate ; SD: standard deviation ; (a): AKI+ vs AKI-*

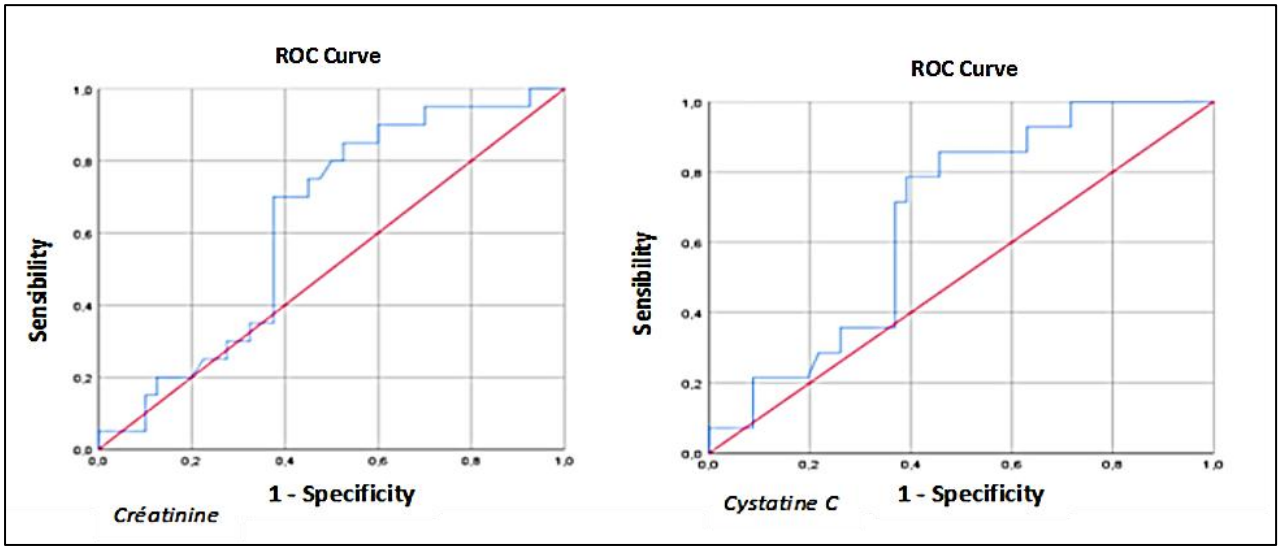


***Figure 2:***

***Receiver Operating Characteristic Curve of Creatinine (Cr) and Cystatin C (CysC) of Patient***

**Table IV:**
***Evaluation of the Performance of Cystatin C***

| Performance index | Characteristics (%) | |
|---|---|---|
| | Creatinin | Cystatin C |
| Sensitivity (Se) | 89.6 | 92.9 |
| Specificity (Sp) | 37.5 | 54.8 |
| Positive predictive value (PPV) | 40.9 | 50.2 |
| Negative predictive value (NPV) | 88.2 | 64.1 |
| Positive likelihood ratio | 1.02 | 1.4 |

This is the first prospective study evaluating the performance of serum CysC in the early diagnosis of AKI in children admitted to the pediatric intensive care departments of two health structures in Brazzaville.

The methodology used was that reported by several authors [4, 17, 30]. In the present study, the mean age of our population was 6.60±5.31 years with extremes ranging from 1 month to 17 years with a median of 5 years. . These results are similar to those of the studies of Chan *et al*. [30] and Tresa *et al*. [31] which found respectively, 4.09±3.98 years and 7.5±4.4 years as mean age, and corroborate the study of Volpon *et al*. in Brazil [17]. All the patients admitted to the different paediatric intensive care units had at least two reasons for admission. The most frequent reasons for admission were infectious diseases (27%) including severe malaria of various forms and respiratory conditions (25%) including respiratory distress. Our results differ from those of Ataei *et al*. [4] and Lagos-Arevalo *et al*. [32] which obtained a predominance of neurological (27% and 29%) and respiratory (11% and 41%) diseases, respectively. However, our results corroborate those of Ademola *et al*., [7] who reported infectious diseases as major reasons for consultation [7, 32].

We observed a statistically significant difference between children with AKI+ and those with AKI- assessed by serum creatinine and cystatin C levels. This lack of diagnostic precision could be explained by the fact that the collapse of the mass bodily is a symptom commonly seen in critically ill children; creatinine is closely dependent on body mass. On the other hand, the difference is not statistically significant with the GFR estimated by the serum Cr. Ataei *et al*., [4] reported statistically significant results with cystatin C, 1.85±0.54 mg/dL ($p<0.001$) but not significant statistically with creatinine and GFR which was 74.1±24.72 ml/min/1.73m2. The age of the study population could explain this difference.

In our study, cystatin C detected more cases (71.7%) than creatinine (26.7%). CysC has been proposed as a new biomarker for the detection of renal failure [4]. Indeed, several studies have shown that high CysC values were associated with a GFR between 88 and 95 ml/min/1.73m2 [4, 25]. CysC could therefore detect kidney damage 24 to 48 hours before Cr [4, 25]. Volpon *et al*. [17] and Lagos-Arevalo *et al*. [32] on the other hand suggest assaying the 2 markers simultaneously to accurately diagnose AKI.

According to the ROC curves of cystatin C and creatinine, the area under the curve is 76.5% [60.3 - 80.8] (0.76 [0.60 - 0.81])

and 62 , 8% [48.5 - 67.0] (0.63 [0.49 - 0.67]) respectively. Our results are close to those of McCaffrey *et al*. [33] who found an area under the Cystatin C curve of 0.79% [0.64 - 0.94], Lagos-Arevalo *et al*. (2015) [32] who also found an area under the curve 0.71 for CysC. However, our results differ from those of Ataei *et al*. [4] who observed areas under the curves of 0.55 and 0.93 for creatinine and cystatin C respectively. Bagheri *et al*. [30] found an area under the curve of 0.93 and 0.67 for cystatin c and creatinine respectively. This proves that cystatin C has a better ability to identify AKI early [1, 4].

We also observed differences in the performance of Cystatin C and creatinine relative to the glomerular filtration rate. The sensitivity and specificity were 89.6, 37.5 and 92.9, 54.8 respectively for Cr and CysC. Our results are different from those found by McCaffrey *et al* who reported a sensitivity of 75% and a specificity of 82% for cystatin C. Lagos-Arevalo *et al*. [32] found a sensitivity of 69% and a specificity of 81 % for cystatin C.

Low molecular weight proteins have been reported to have better diagnostic sensitivity than serum creatinine in children [33] and adult patients in intensive care [34, 35, 28]. On the other hand, the serum level of CysC is independent of muscle mass, it is, therefore, a superior marker of renal function compared to Cr in children and patients with muscle loss [22, 35, 36].

Our study showed that serum CysC is a more precise marker of the evaluation of GFR than serum Cr in affected children admitted to pediatric intensive care. It is therefore reasonable to affirm that the acute change in CysC could more accurately detect an acute reduction in GFR than serum Cr. The diagnostic value of CysC found in this study (area under the curve 0.76) approximates that of previous studies [37].

## Conclusion

The results obtained in this study show that serum Cystatin C detects renal failure earlier than creatinine, in children admitted to pediatric intensive care units in Brazzaville. In addition, Cystatin C has a higher diagnostic value than Creatinine, thus confirming several previous studies. It, therefore, represents an early biomarker in the diagnosis of acute kidney injury.

## Recommendations

Practitioners should include Cystatin C assay in the investigation of acute kidney injury. Further studies of Cystatin C assessment can be performed to determine the occurrence of acute kidney injury by establishing a true baseline for populations in sub-Saharan Africa to improve the performance of Cystatin C as an early biomarker of acute kidney injury.


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### Author contributions

LM Miguel, FT Mabiala Wann, DG N'jilo Tchatchouang, and C Lékana performed complete experimental research. LM Miguel and CR Dobhat-Doukakini performed data

analysis and wrote the manuscript. AA Abena,G Moyen, D Moukassa and E Mokondjimobe revised the manuscript. RB Bolanga, FT Mabiala Wann and AR Okoko contributed to the study design, data analysis, and manuscript writing. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.


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## Data availability

Data will be available upon request.

## Conflict of interests

The authors state no conflict of interest.

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## Appendix

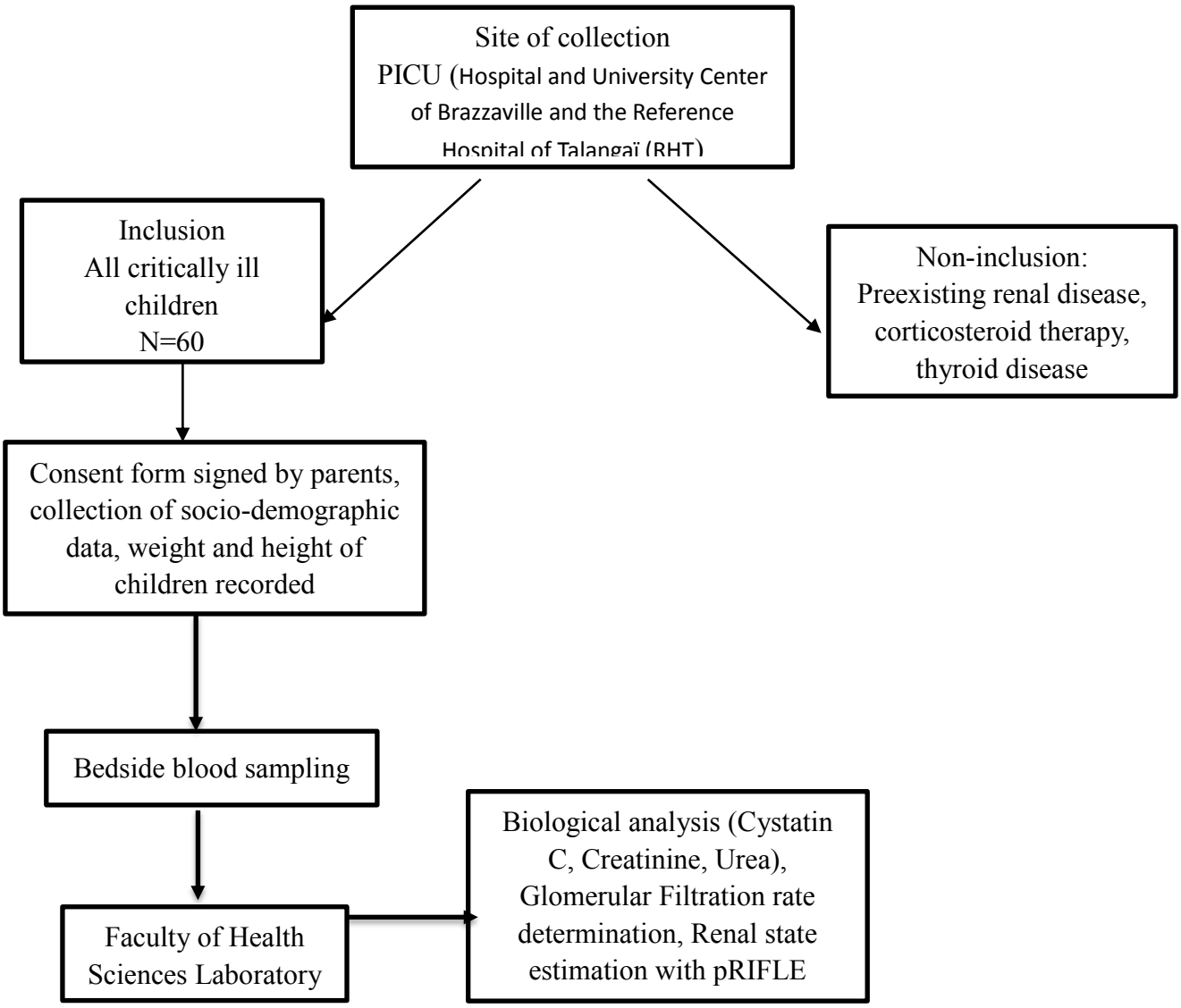


**Figure 1:**
***Study Design***