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ORIGINAL ARTICLE

Procalcitonin and Pancreatic Stone Protein as markers of infection in children with Chronic Renal Failure in Zagazig University Hospitals.

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ABSTRACT

Background: Infectious complications in children with chronic renal disease (CKD) are a major cause of increased morbidity and mortality rates among these children. This study aimed to determine the diagnostic value of Procalcitonin (PCT) and Pancreatic Stone Protein (PSP) **as markers** of infection in children with chronic renal failure and to decrease morbidity and mortality rates in these children. **Methods**: This case control study was conducted on 36 patients diagnosed with Chronic Renal Failure and admitted at Nephrology Pediatric Unit, Children's Hospital, Zagazig University during the period from **October 2018 to February 2019**. They were divided into 2 groups**, the first group (case group)** included 18 children with CKD diagnosed with infectious complications, **the second group included** (control group) 18 children with CKD **who** didn't develop any infectious complications. The serum expression of PCT and PSP was measured. Pearson correlation analysis was conducted to correlate PCT & PSP with each other and with CRP, temperature, HR, pus cells and blood culture. ROC analysis was used to test the value of PCT, PSP and CRP in early diagnosis of infection in Children with chronic renal failure.

Results: The serum levels of PCT and PSP in case group were higher than in control group (P<0.01). Serum PCT concentrations showed a significantly positive correlation with PSP levels (P<0.001). ROC analysis showed that the **area under curve (AUC)** values of PCT, PSP and CRP were 0.944 (95% CI, 866–1.000), 0.870 (95% CI, 0.757–0.984) and 0.931 (95% CI, 0.729 –0.859) respectively and suggested cutoff levels were >0.62 ng/ml for PCT, >22.8 ng/ml for PSP and >6.5mg/L for CRP with sensitivity 88.9%, 81.8% and 83.3% respectively and specificity 95.6%, 72.2% and 94.4% respectively. **Conclusion:** Serum levels of PCT and PSP are promising biomarkers of early detection of infectious complications in Children with Chronic renal failure.

Keywords: Chronic kidney disease (CKD), Procalcitonin (PCT), Pancreatic stone protein (PSP), Chronic renal failure (CRF) and Heart rate (HR).

INTRODUCTION

Chronic kidney disease (CKD) is growing rapidly as a major public health issue in children in the 21st century. Infectious complications in persons with chronic kidney disease (CKD) are a major cause of increased morbidity and mortality rates in these patients. Accurate and prompt diagnosis of infection remains a problem for both clinical and laboratory doctors **[1].**

Procalcitonin (PCT) is increased 3 to 6 hours after systemic bacterial infection, but not due to local infection or viral infection, non-infectious inflammatory reactions or autoimmune disease. Therefore, it would appear that PCT testing may improve the specificity of the diagnosis of bacterial infection more than checking for other

inflammatory markers, such as C-reactive protein (CRP) or leukocyte counts **[2].**

In addition, pancreatic stone protein (PSP) **is** associated with inflammation, infection, and other disease-related stimuli. Nevertheless, the prognosis of PSP in critically ill pediatric patients is uncertain **[3].** Historically, PSP functions have been documented mainly with regard to the pancreas, and pancreatic acinar cells are known to be the main source of this protein **[4].** Recently, PSP research has bypassed pancreatic diseases and focused on infection and inflammation **[5].**

The present study was conducted to determine the diagnostic value of PCT and PSP as markers of infection in Children with Chronic renal failure and to decrease morbidity and mortality rates in these children.

METHODS

The current case control study was conducted on 36 patients diagnosed with Chronic Renal Failure (CRF) and admitted at Nephrology Pediatric Unit, Children's Hospital, Zagazig University during the period from October 2018 to February **2019. They were divided into 2 groups, the first group included** 18 children with CKD diagnosed with infectious complications (clinically and investigated by chest x-ray, complete blood count (CBC), C-reactive protein (CRP), blood culture, urine analysis, urine culture, Iron profile and serum Ferritin**), the second group included 18 children with CKD who didn't** develop any infectious complications & attended Pediatric Nephrology Unit for dialysis and routine care

All the cases satisfied the inclusion criteria: Children between (2-18 years) diagnosed with Chronic Renal Failure (CRF) [Stage 4 & 5 CKD (GFR<30ml/min/1.73m^2)] in pediatric department in Zagazig University. **Exclusion criteria:** Cases with dehydration, malignancy, end-stage liver disese, immuno-compromised patients, coagulopathy and chronic lung disease, also cases with infectious complications starting antibiotics for more than 48 hours were excluded.

Data collection

Age, sex, height, weight, virology, duration of dialysis and drugs (Hypertensive drugs, iron, calcium and vitamin D supplementation) were recorded.

Thorough clinical examination to detect anthropometric data (weight for age SDS), vital data with stress on body temperature, heart rate, blood pressure, signs of anemia (pallor), Presence of focus of infection .Systemic inflammatory response syndrome (SIRS), Septicemia and Septic Shock.

Serum samples:

Fasting blood samples were extracted from each subject. Supernatants were obtained after centrifugation (4°C, 3000 r/min, 10 min) and stored at –80°C. Routine blood testing was conducted by laboratory physicians at our hospital. Microparticle enzyme immunoassay was used to test the serum levels of PCT and Dako Cytomation immunoturbidimetric assay was used for PSP analysis.

Also, All cases underwent radiological investigations, urine analysis ,urine culture , Complete blood count (CBC),Serum C-reactive protein (CRP), Iron profile,Serum urea, creatinine, albumin , Ferritin, Serum Na, K, Ca, Ph ,parathormone (PTH) and blood culture sampled by skin puncture.

Ethical Clearance: Written Informal consent was obtained from the patient parents to engage in the study. The approval for the study was received from the Pediatrics Departments of Zagazig University Hospitals after the approval of the Institutional Review Board (IRB). The research was carried out in compliance with the Code of Ethics of the World Medical Association (Decleration of Helsinki) for studies involving humans.

Statistical Analysis:

Data was analyzed using Microsoft Excel software. Data was then imported into the Research Software Statistical Package for Social Sciences (SPSS version 20.0) (Statistics Package for Social Sciences). Quantitative continuous group represents the mean ± SD, the Chi square test (X2) was used for the study of categorical variables. Differences between quantitative independent groups by t or Mann Whitney, Pearson's correlation or Spearman's correlation. P value was set at < 0.05 for small results and < 0.001 for high significant results. ROC analysis was used to test the value of PCT, PSP and CRP in early diagnosis of infection in Children with chronic renal failure.

RESULTS

Temperature and heart rate were significantly higher among infected group. WBCs was significantly higher in infected group (**Table 1)**.Serum ferritin level was significantly higher among infected group **(Table 2).** Pus cells and RBCs in urine were significantly higher among infected group **(Table 3).** Serum levels of CRP, PCT and PSP were significantly higher among infected group **(Table 4).** Significant area under curve for three parameters and suggested cutoffs were >0.62 ng/ml for PCT, >22.8 ng/ml for PSP and >6.5mg/L for CRP with sensitivity 88.9%, 81.8% and 83.3% respectively and specificity 95.6%, 72.2% and 94.4% respectively **(Table 5).**

Serum levels of PCT and PSP were significantly positive correlated with each other and with CRP, Temp, HR, pus cells and RBCs in urine, serum level of PSP was significantly positive correlated with Ferritin and serum level of PCT was significantly positive correlated with Ca **(Figure 1).** ROC curve for detection of PCT, PSP and CRP regard infection **(Figure 2).**.

Table (1): Comparison between studied groups regarding vital data and Laboratory findings:

	cCKD	iCKD	T	P
SBP	**118.33±16.8**	**116.11±15.7**	**0.408**	**0.686**
DBP	**79.44±11.09**	**80.27±9.15**	**-0.246**	**0.807**
Temp	**36.93±0.22**	**39.14±0.6**	**-14.430**	**0.00****
HR	**83.33±9.07**	**110.55±13.4**	**-7.103**	**0.00****
WBCs	**7.52±1.7**	**12.28±4.1**	**-2.454**	**0.019***
Hb	**9.63±1.27**	**9.32±1.01**	**0.808**	**0.424**
Plt	**236.38±49.7**	**290.22±98.4**	**-1.459**	**0.154**
Urea	**53.66±11.2**	**53.14±15.6**	**0.115**	**0.909**
Creatinine	**7.39±2.26**	**6.81±2.01**	**0.816**	**0.420**
Albumin	**4.07±0.47**	**3.99±0.45**	**0.540**	**0.593**
PTH	**377.22±122.6**	**349.0±87.6**	**1.021**	**0.314**
Ca	**9.07±1.24**	**8.88±1.15**	**0.468**	**0.643**
Ph	**5.48±1.51**	**5.56±1.55**	**-0.152**	**0.880**
Na	**137.55±3.16**	**136.77±3.2**	**0.732**	**0.469**
K	**5.64±1.17**	**5.42±1.17**	**0.554**	**0.583**

iCKD:infected(case) group, cCKD:control group ,T:T value ,P:p value,
SBP: Systolic blood pressure, DBP: Diastolic Blood Pressure, Temp: Temperature and HR: Heart Rate, Hb: hemoglobin level/dl ,Plt: platelet number/dl, Urea:serum urea level/dl,Creatinine:serum creatinine leve/dl, albumin:serum albumin level/dl, PTH: serum parathyroid horomone level/dl, ca:serum calcium level/dl,ph:serum phosphate level/dl,Na:serum sodium level/dl and k:serumpotassium level/dl

Table (2):Comparison between studied groups regarding serum Iron and ferritin:

	cCKD	iCKD	Mann Whitney	P
Iron	**91.55±48.5** **80.5 (22-216)**	**112.8±63.7** **79 (42-405)**	**-0.916**	**0.366**
Ferritin	**839.72±476.1** **781.5 (131-2000)**	**1279.9±621.2** **1244.5 (66-2000)**	**-2.387**	**0.023***

iCKD:infected(case) group, cCKD:control group, Iron:serum iron level/dl
Ferritin :serum ferritin level/dl

Table (3):Comparison between studied groups regarding urine analysis :

			cCKD	iCKD	Mann Whitney/X2	P
Pus cells (/HPF)			**5.77±1.7** **6 (3-9)**	**25.88±21.37** **22.5 (4-70)**	**-3.979**	**0.00****
RBCs (/HPF)			**4.16±1.65** **4 (2-8)**	**11.1±5.8** **8.5 (3-30)**	**-3.756**	**0.001****
Urate (/HPF)	**---**	**N**	**5**	**3**		
		%	**27.8%**	**16.7%**		
	+	**N**	**8**	**7**		
		%	**44.4%**	**38.9%**		
	++	**N**	**5**	**6**	**2.65**	**0.44**
		%	**27.8%**	**33.3%**		
	+++	**N**	**0**	**2**		
		%	**0.0%**	**11.1%**		
Oxalate (/HPF)	**---**	**N**	**15**	**11**		
		%	**83.3%**	**61.1%**		
	+	**N**	**3**	**4**	**3.75**	**0.15**
		%	**16.7%**	**22.2%**		

			cCKD	iCKD	Mann Whitney/X2	P
	++	N	0	3		
		%	0.0%	16.7%		
Phosphate (/HPF)	---	N	11	16		
		%	61.1%	88.9%		
	+	N	5	1		
		%	27.8%	5.6%		
	++	N	1	0	4.59	0.204
		%	5.6%	0.0%		
	+++	N	1	1		
		%	5.6%	5.6%		
Total		N	18	18		
		%	100.0%	100.0%		

iCKD:infected(case) group, cCKD:control group, HPF:high power field

Table (4): Comparison between studied groups regarding serum levels of CRP, PCT and PSP:

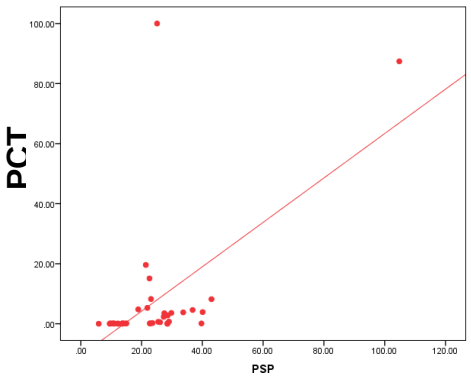
	cCKD	iCKD	Mann Whitney/X2	P
CRP	2.47±1.99 1.9 (0.1-10)mg/L	26.28±19.7 20 (6-77) mg/L	-4.785	0.00**
PCT	0.14±0.11 0.09 (0.01-10)ng/ml	13.25 ± 15.22 14.25 (0.1-100) ng/ml	-2.202	0.035*
PSP	16.27±7.35 13.6 (5.9-28.5) ng/ml	33.12±19.25 28.1 (18.9-104.8) ng/ml	-3.469	0.001**

iCKD:infected(case) group, cCKD:control group,CRP:serum C_reative protein level/L,PCT:serum procalcitonin level/ml, PSP:serum pancreatic stone protein level/ml

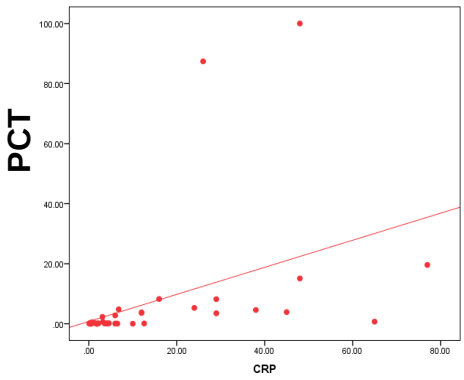
Table (5): Area under curve, cutoff and validity of PCT, PSP and CRP:

Variables	Cut off point	AUC	P-value	95% Confidence Interval		Sensitivity	Specificity	PPV	NPV
				Lower Bound	Upper Bound				
PCT	>0.62	0.944	0.0001	0.866	1.000	88.9	95.6	90.0	96.0
PSP	>22.8	0.870	0.0001	0.757	0.984	81.8	72.2	71.0	84.0
CRP	>6.5	0.931	0.0001	0.729	0.859	83.33	94.44	93.7	85.0

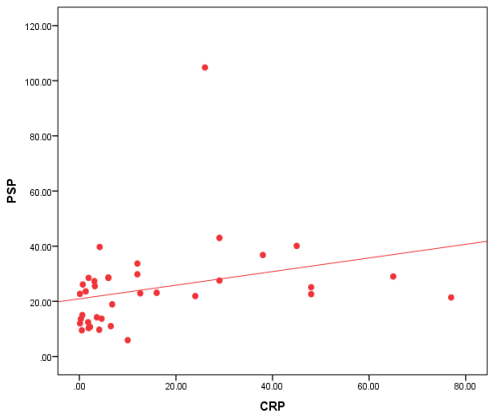
PCT: procalcitonin, PSP: pancreatic stone protein ,CRP :C_reative protein ,AUC:area under curve, PPV: Positive predictive value and NPV: negative predictive value .



PCT and PSP



PCT and CRP



PSP and CRP

Figure 1: Correlations between PCT and PSP and other parameters

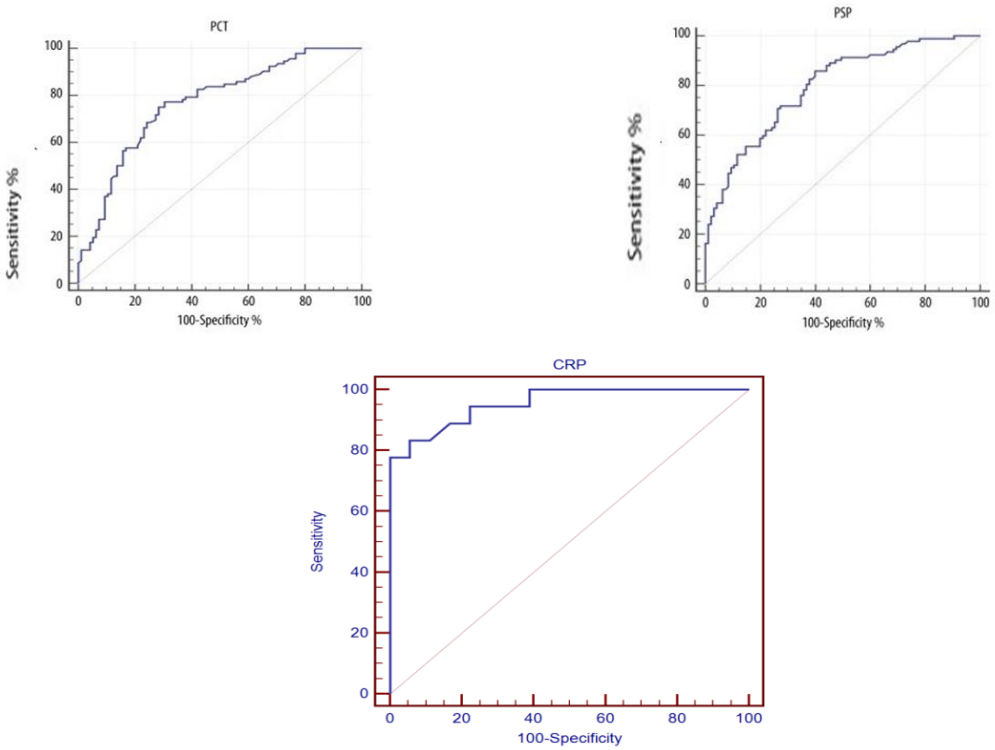


Figure (2): ROC curve for detection of PCT, PSP and CRP regard infection

DISCUSSION

Rapid identification and management of systemic bacterial infections is important in children with CKD. Nonetheless, postponing treatment for serious bacterial infections can have a bad outcome **[6] .**

Scientists are therefore interested in researching markers that can predict and identify early infectious complications in children with CRF. The following criteria should be used for a strong marker for bacterial infection; early diagnosis, of prognostic interest and helpful in decision taking for antimicrobial therapy **[7].**

Procalcitonin (PCT), the precursor molecule of calcitonin, is a 116-amino acid peptide with a molecular weight of 13 KDa with no known hormonal properties **[6].** For healthy individuals, PCT circulating levels are very low, usually below 0.1 ng /mL**[8] .**

PCT increases faster than other markers that increase rapidly and intermittently in serious bacterial infections such as CRP (the increase in serum CRP begins between 6 and 12 hours and only reaches a peak of 24 to 48 hours) **[9] .**

In this analysis, the mean serum PCT levels in the case group were significantly higher than in the control group in children with CRF. The level in patients with CKD was 13.25±15.22 ng/mL for the infected group and 0.11±0.14 for the control group. ROC curve analysis was used to test the ability of PCT rates to predict the existence of infection, which showed a large area under the curve of 0.94 with a sensitivity, accuracy, positive predictive value and negative predictive value of 88.9 percent, 95.6 percent, 90.0 percent and 96 percent respectively.

The PCT cut-off value to be differentiated between case & control group of CRF children was > 0.62 ng/ml. However, the sensitivity, precision, positive predictive value and negative predictive value for CRP > 6.5 mg / l were 83.33 %, 94.44 %, 93.7 per cent and 85%. We may therefore conclude that our data indicate that PCT is not only a strong marker for bacterial infection, but also more sensitive and accurate than CRP, in accordance with other studies. The drastic and rapid rise in serum PCT in response to bacterial infection makes it more robust and specific to children than CRP as a marker for bacterial infection **[10] .**

Aljabi et al. [11] found that Procalcitonin was the most sensitive and specific marker for the identification of surgical site infection in the immediate postoperative period with a sensitivity and specificity of 100 per cent and 95.2 per cent respectively and a cut-off level of 0.5 ng / ml compared to CRP with a sensitivity of 100 per cent and a specificity of 91.7 per cent.

In addition, **Herget-Rosenthal et al. [12]** used serum PCT to diagnose severe infection or septicemia. PCT was measured before dialysis and its sensitivity and specificity were 89% and 81%, respectively.

Analogously, **Lee et al. [13]** noticed that the mean serum PCT concentration of the iESRD group was significantly higher than in the cESRD group (2.95±3.67 ng/mL vs. 0.50±0.49 ng/mL, p = 0.006), &suggested a cutoff level of 0.75 ng/ml with a sensitivity and specificity of 76.2% and 80.0% for infection respectively.

Furthermore, **Maharajan et al., [14]** found plasma PCT as a diagnostic marker for acute osteomyelitis with a cutoff point of 0.4 ng / ml, a sensitivity of 85.2 per cent and a specificity of 87.3 per cent for diagnosis of septic arthritis and acute osteomyelitis.

On contrast, **Cui et al., [15]** concluded that PCT is a weak diagnostic aid in pediatric acute osteomyelitis. AUC value of PCT was 0.767 (95% CI, 0.700–0.826) and PCT showed sufficient sensitivity 77.17% , specificity 69.47% and at a cutoff value of 3.56 ng/mL . Some studies conclude that PCT was less useful than CRP, or of little value **[16].**

Besides, **Becker et al., [17]** showed that PCT levels were elevated in patients suffering from neuroendocrine tumors (i.e., medullary thyroid cancer) and small cell lung carcinoid tumors that could be associated with false positive symptoms of infection and inadequate care.

In this study, we found that PCT is positively correlated with PSP, CRP, WBCs, pus cells in urine& positive blood cultures. PCT has a highly significant positive correlation with urine culture and, to a lesser extent, with blood culture.

Pancreatic Stone Protein (PSP), Often known as regenerating protein, pancreatic acinar cells and subsets of intestinal and gastric cells are constitutively secreted. Protecting function was demonstrated by encouraging cell proliferation during beta-cell regenerative processes and epithelial repair **[18]**.

Recent focus is on the role of PSP in infectious diseases, such as sepsis. **Palmiere et al., [19]** PSP was studied in postmortem serum in a series of sepsis-related and non-septic deaths and found that PSP was positively associated with PCT, the serum concentrations of which were substantially lower in non-sepsis patients. They used a cut-off value of 1.0 mg / ml (much lower than the cut-off values suggested in clinical trials involving adult septic patients), PSP / reg showed both a satisfactory sensitivity (90 %) and a precision (90 per cent).

In this study, we measured serum PSP levels in 36 children with CKD and found that PSP, which was

positively correlated with PCT, is a similar diagnostic marker of infection in CRF Children. The AUC value of PSP was 0.870 (95% CI, 0.757–0.984) and its sensitivity and specificity were 81.8 per cent and 72.2 per cent, respectively, and the suggested cut-off level was 22.8 (ng / mL) which is constitutive of **Cui et al., [15]** which indicated that the PSP cut-off level should be 26.49 (ng / mL); the AUC value of PSP was 0.796 (95% CI, 0.731–0.851) and its sensitivity and specificity were 85.87% and 60.00% respectively.

A recent study revealed by **Wu et al. [20]** also showed that PSP was an independent risk factor for pediatric sepsis and was a promising biomarker for risk stratification of pediatric sepsis. **Wu et al. [20]** found that there was a statistically significant differences in serum PSP/reg concentrations between sepsis and control cases with a cutoff level of 256ng/ml, AUC 0.73 with sensitivity and specificity 79.7% and 57.7% respectively.

In this study, we observed a significant positive correlation between PSP, PCT and WBCs, as well as CRP, Temp, HR, pus cells and RBCs in urine .PSP has a highly significant correlation with urine culture, but with no significant correlation with blood culture or throat swab culture.

In this study, usual signs of infections such as fever, Tachycardia ,WBCs & CRP are significantly higher among case group than control group in consistent with other studies in **[21].**

The prevalence of urinary tract infection (UTI) observed in this study was 27.7% which was lower than the finding of 34.5% by **Dash et al. [22]**, 36.6% by **Mehta et al. [23]** and higher to 6.87% by **Nazme et al. [24]** & 4.7% by **Mohanty et al. [25]**. The key etiological agents of UTI have modified significantly in the last few decades of **Wu et al. [26]**. In the present analysis: E. Coli, Proteus and Klebsiella have been isolated as the most common pathogens. E. Coli was still the dominant etiological agent of UTI, accounting for 60 per cent of all cases, which is consistent with other researchers **Erol et al., [27]**; **Mirsoleymani et al. [28]**; **Coban et al. [29]** who reported that E. coli represented 74.1%, 65.5% and 68.4% of all cases, respectively. The disparity of results in UTI patients between studies is mostly either due to changes in environmental factors or due to variations in sample size.

Limitations of our study: Further studies of large samples are required for further assessment of the diagnostic accuracy of PSP for early detection of infections in children with CKD.

CONCLUSION

Serum Procalcitonin (PCT) is the best diagnostic test for acute infections in children with CRF and its diagnosis of sepsis was substantially higher than CRP and PSP. Serum PCT threshold of 0.62 ng/mL is more suitable for diagnosis of infection in patients with Chronic Kidney Disease. Serum Pancreatic stone protein(PSP) is a promising new biomarker of infection in CRF Children, but its diagnostic value is less than CRP. Cut off level of 22.8ng/mL can be useful for early detection of infectious complication in these children.

RECOMMENDATIONS

Serum Procalcitonin is a strong indicator of early detection of infections in children with Chronic Renal Failure and has to be placed in routine investigation of sepsis in chronic kidney disease patients undergoing continuous renal replacement therapy (CRRT).

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