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Research Article

Comparing Interleukin-6, C-Reactive Protein, Erythrocyte Sedimentation Rate, and White Blood Cell Count for the Early Diagnosis of Acute Pyelonephritis in Children

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Abstract

Background: Urinary tract infection (UTI) is common in children. Differentiating lower UTI from acute pyelonephritis (APN) is of great importance in children since early diagnosis and timely management can prevent kidney scarring and associated complications.

Objectives: We aimed to compare the diagnostic performance of serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count with interleukin-6 (IL-6) for the diagnosis of APN in children.

Methods: This cross-sectional study included 38 children with APN (based on clinical findings and positive urine culture) aged 1 month to 15 years admitted to Bandar Abbas Pediatric Hospital, Bandar Abbas, Iran, during 2019-2020. Serum CRP, WBC, IL-6, and WBC count were measured in all children. Age and sex of the participants were also recorded.

Results: The mean ± SD age of the children was 65.82 ± 46.67 months, of whom 23 (60.5%) were girls. The sensitivity of WBC, ESR, CRP, and IL-6 for the early diagnosis of APN was 39.5%, 71.1%, 60.5%, and 71.1%, respectively. Taking the results of ESR, CRP, and WBC into account as the basis of diagnosis, altogether 9.98 pg/mL was the best cut-off of serum IL-6 for the diagnosis of APN with 70%-80% sensitivity and 56.5%-73.7% specificity. A significant correlation was found between serum IL-6 and CRP, ESR, and WBC with the strongest correlation between IL-6 and CRP ($r = 0.451$, $P = 0.004$). Age and sex did not affect these correlations. The sensitivity of serum IL-6 at the 5 pg/mL cut-off for the diagnosis of APN was higher in boys and children younger than 6 years.

Conclusion: IL-6 can diagnose APN in children aged 1 month to 15 years with 71.1% sensitivity. Its sensitivity is superior to CRP and WBC and comparable with ESR in this regard.

Keywords: Acute pyelonephritis, Interleukin-6, Children, C-reactive protein, Erythrocyte sedimentation rate, Inflammation

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Background

With an annual incidence of approximately 3%, urinary tract infection (UTI) is regarded as one of the most common childhood infections (1). Cystitis or lower UTI occurs when only the bladder is involved; however, in some children, the bacteria from the bladder ascent to the kidneys causing upper UTI or acute pyelonephritis (APN). Differentiating cystitis from APN is very important since children with APN are at risk of long-term renal complications such as hypertension, renal parenchymal injury, also called renal scarring, and chronic kidney disease (2, 3). Moreover, children with APN may require stronger or longer courses of antibiotics (4). Therefore, researchers have explored various biomarkers to distinguish between cystitis and APN.

Interleukin-6 (IL-6) is an inflammation mediator produced in response to bacterial infections. IL-6 is a cytokine that regulates multiple body functions, including organ development, acute phase response, and inflammation. Macrophages, T-helper cells, podocytes, hepatocytes, and neutrophils express the receptor to which IL-6 directly binds (5, 6). Studies have reported elevated serum IL-6 levels in UTI; nevertheless, the role of IL-6 as a biomarker for the site of infection is a matter of debate (7, 8). C-reactive protein (CRP) is an acute phase reactant whose value in pediatric UTI is well documented (8, 9). In addition, both CRP and erythrocyte sedimentation rate (ESR) have been reported to be sensitive but not specific for the prediction APN (1). White blood cell (WBC) count can also suggest renal involvement, but with variable sensitivity and specificity (10, 11).

Objectives

We aimed to compare IL-6, CRP, ESR, and WBC count for the early diagnosis of APN in children.

Methods

Participants

This cross-sectional study included children with APN (based on clinical findings and positive urine culture) aged 1 month to 15 years admitted to Bandar Abbas Pediatric Hospital, Bandar Abbas, Iran in from March 21, 2019 to March 20, 2020. Patients who had received any antibiotics within one week prior to admission, as well as those with any comorbidities, confirmed vesicourethral reflux, or renal dysfunction were excluded from the study. Sample size was calculated as at least 38 participants based on the correlation coefficient in a previous study ($\alpha = 0.05$, $\beta = 0.1$) (1), using MedCalc statistical software, version 14.0.

Study Design

After obtaining written informed consent from the parents or guardians of the children, their age and sex were recorded. A complete medical history was taken from the patients and they were all thoroughly examined by an expert pediatric nephrologist.

Body temperature was measured using a standard tympanic thermometer and fever was defined as >38°C in children < 3 years, >37.8°C in children 3-11 years, and >37.6°C in children >11 years. Accordingly, all children in the study were febrile. Urinalysis and urine culture were performed for all participants and those with a positive urine culture were included.

Random venous blood samples were collected from all patients before the initiation of any treatments. WBC count was determined using an automated blood cell counter. ESR was measured using an ESR reader. CRP was measured quantitatively using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran) and a biochemistry autoanalyzer (Technicon RA-1000, Technicon Instruments Corp., TerryTown, NY, USA) with the immunoturbidity method. Serum IL-6 was measured using the Roche kit (Roche Diagnostics, Germany) and the electrochemiluminescence immunoassay (ECLIA) method (by Roche's Elecsys® cobas e 411 analyzer for immunoassay tests). ESR >10 mm/h, CRP >10 mg/L, IL-6 >5 pg/mL, and WBC count >10 000/μL were regarded as elevated.

Data analysis

We used the Statistical Package for the Social Sciences (SPSS) software (version 25.0, Armonk, NY: IBM Corp.) for data analysis. For the description of quantitative variables, means and standard deviations were used and for qualitative variables, frequencies and percentages were used. Based on the results of Kolmogorov-Smirnov normality test for age, Spearman's correlation test was used to evaluate the association between IL-6 and other inflammatory markers. By taking the results of ESR, CRP, and WBC count as reference, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) of IL-6 >5 pg/mL was calculated. Also, receiver operating characteristic (ROC) curves of serum IL-6 were drawn for the diagnosis of APN. The area under the curve (AUC) was calculated for every curve and the optimal IL-6 cut-off was determined. $P \leq 0.05$ was regarded as statistically significant.

Results

From the 38 children included in this study with a mean ± SD age of 65.82 ± 46.67 months, 23 (60.5%) were girls and 15 (39.5%) were boys. General characteristics and laboratory finding of the participants are demonstrated in Table 1. Elevated ESR, CRP, WBC count, and serum IL-6 were observed in 71.1%, 60.5%, 39.5%, and 71.1% of the children, respectively. Since APN was diagnosed in all the children, these figures are also reflective of the sensitivity of each marker for the diagnosis of APN.

Serum IL-6 was significantly correlated with CRP ($P = 0.004$), ESR ($P = 0.018$), and WBC count ($P = 0.043$); however, when controlled for age and sex, the correlations were no longer statistically significant (Table 2). Table 3

Table 1. General Characteristics and Laboratory Findings of the Study Population

Variables	Results
Age (months) mean ± SD	65.82 ± 46.67
Gender, No. (%)	
Male	15 (29.5)
Female	23 (60.5)
ESR (mm/h), mean ± SD	28.37 ± 27.27
CRP (mg/L), mean ± SD	22.45 ± 22.88
WBC count (/μL), mean ± SD	10297.37 ± 3452.57
Serum IL-6 (pg/mL) mean ± SD	38.18 ± 90.68
ESR, No. (%)	
Normal	11 (28.9)
Elevated	27 (71.1)
CRP, No. (%)	
Normal	15 (39.5)
Elevated	23 (60.5)
WBC count, No. (%)	
Normal	23 (60.5)
Elevated	15 (39.5)
Serum IL-6, No. (%)	
Normal	11 (28.9)
Elevated	27 (71.1)

Abbreviations: n, number; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; IL-6, interleukin-6.

Table 2. Correlation of Serum IL-6 With Other Inflammatory Markers by Age and Gender

Variable	CRP (CC)	*P* Value*	ESR (CC)	*P* Value*	WBC (CC)	*P* Value*
Serum IL-6	0.451	0.004	0.383	0.018	0.330	0.043
Age < 6 years (n = 19)	0.208	0.394	0.111	0.651	0.222	0.361
Age 6-15 years (n = 19)	0.401	0.089	0.355	0.136	0.396	0.093
Male gender (n = 15)	0.503	0.056	0.500	0.058	0.374	0.170
Female gender (n = 23)	0.375	0.077	0.322	0.135	0.371	0.081

Abbreviations: CC, correlation coefficient; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; IL-6, interleukin-6.
*Analyzed by Spearman's correlation test.

Table 3. Comparison of Serum IL-6 with CRP, ESR, and WBC Count for the Diagnosis of APN

Serum IL-6	CRP		ESR		WBC Count	
	Normal	Elevated	Normal	Elevated	Normal	Elevated
Normal	8	3	5	6	9	2
Elevated	7	20	6	21	14	13

Abbreviations: CC, correlation coefficient; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; IL-6, interleukin-6.

Table 4. Diagnostic Value of IL-6 at the 5 pg/mL Cut-off for the Diagnosis of APN by Age and Gender

Variables	TP (N)	TN (N)	Sensitivity
Age < 6 years (n = 19)	16	3	84.2%
Age 6-15 years (n = 19)	11	8	57.9%
Male gender (n = 15)	11	4	73.3%
Female sex (n = 23)	16	7	69.6%

Abbreviations: N, number; APN, acute pyelonephritis, TP, true positive; TN, true negative.

shows the comparison of serum IL-6 results at the 5 pg/mL cut-off, ESR at the 10 mm/h cut-off, CRP at the 10 mg/L cut-off, and WBC count at the 10 000/μL cut-off. Accordingly, sensitivity, specificity, PPV, NPV, and DA of IL-6 for the diagnosis of APN based on CRP results were 86.9%, 53.3%, 74.1%, 72.7%, and 73.7%, respectively. The corresponding diagnostic indices of IL-6 based on ESR and WBC count results were 77.8%, 45.5%, 77.8%, 45.5%, and 68.4% as well as 86.7%, 39.1%, 48.1%, 81.8%, and 57.9%, respectively.

Elevated serum IL-6 (>5 pg/mL) was found in 11 boys and 16 girls as well as 16 patients < 6 years of age and 11 aged 6-15 years. Since all patients in the current study had APN, the sensitivity of serum IL-6 was 73.3% in boys, 69.6% in girls, 84.2% in children aged < 6 years, and 57.9% in children aged 6-15 years (Table 4).

The ROC curve of IL-6 for the diagnosis of APN is shown in Figure 1, taking CRP >10 mg/L as reference. Consistently, the AUC was calculated as 0.772 with an optimal cut-off of 9.98 pg/mL for serum IL-6, having 78% sensitivity and 73.3% specificity. Figure 2 shows the ROC curve of IL-6 for the diagnosis of APN, taking ESR>10 mm/h as reference. Appropriately, the AUC was calculated as 0.702 with an optimal cut-off of 9.98 pg/mL for serum IL-6, having 70% sensitivity and 72.7% specificity. Figure 3 demonstrated the ROC curve of IL-6 for the diagnosis of APN, taking WBC count >10 000 /μL as reference. The AUC was 0.623 with an optimal cut-off of 9.98 pg/mL for serum IL-6, having 80% sensitivity and 56.5% specificity.

Discussion

In the current study we found 71.1% sensitivity for serum IL-6 for the diagnosis of APN in children aged 1 month to 15 years. We also found a significant correlation between serum IL-6 and ESR, CRP, and WBC count in children with APN. The sensitivity of serum IL-6 was comparable with ESR (71.1%) but superior to CRP (60.5%) and WBC count (39.5%) for the diagnosis of APN. On the other hand, the sensitivity of IL-6 >5 pg/mL for the diagnosis of APN was higher in boys compared to girls and in children < 6 years of age compared to 6-15 years of age.

Multiple studies have assessed serum and urine biomarkers for the differentiation of upper from lower UTI with contradictory results. In the most recent systematic review by Shaikh and colleagues, performed on 25 studies, the average sensitivity of CRP at the 20 mg/L cut-off and ESR at the 30 mm/h cut-off for this purpose were 93% and 83%, respectively. They concluded that ESR is not sufficient for distinguishing between cystitis and APN in children, while CRP < 20 mg/L decreased the possibility of APN to lower than 20% (1). The foremost reason for the discrepancy between their study and ours is that Shaikh and colleagues used the results of dimercaptosuccinic acid (DMSA) scanning as reference (1). Moreover, the larger sample size, the sensitivity of diagnostic kits and measurement tools, as well as demographic characteristics of the participants might have contributed to this discrepancy. Also, they evaluated CRP and ESR at different cut-offs compared with our study.

Contrary to our findings, one study showed that IL-6 is not an appropriate marker for the diagnosis of UTI and the differentiation of upper from lower UTI. They explained the wide range of IL-6 values and its inappropriateness by the variety of APN symptoms onset, sex distribution, and the virulence of the responsible organisms (12). While serum IL-6, especially at the 10 pg/mL cut-off, had a relatively suitable sensitivity for the diagnosis of APN in our study. However, our findings have to be confirmed in larger studies. In another study, Sheu et al found that

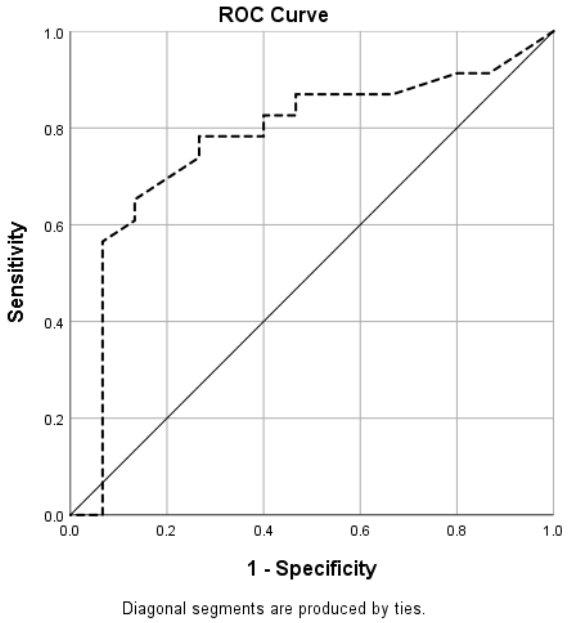

Figure 1. ROC curve of IL6- for the Diagnosis of APN Taking CRP >10 mg/L as Reference.

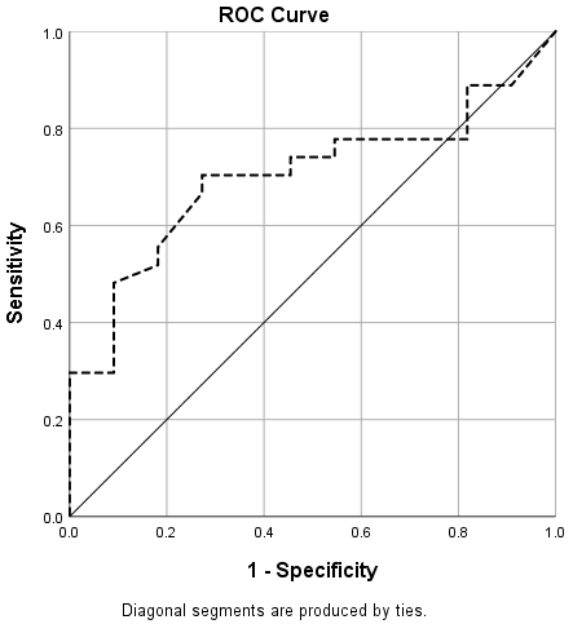

Figure 2. ROC Curve of IL6- for the Diagnosis of APN Taking ESR >10 mm/h as Reference.

in smaller children with APN and high fever, an acute elevation in serum and urine IL-6 increases the risk of renal scarring (13). This is quite consistent with our findings, as we also found a higher sensitivity for serum IL-6 in children < 6 years of age compared to those age 6-15 years. The results of an older study showed the utility of serum and urine IL-6 for the early diagnosis of APN in febrile children (7).

In other studies on the evaluation of biomarkers to differentiate upper from lower UTI, procalcitonin has been proposed as a useful inflammatory marker. For

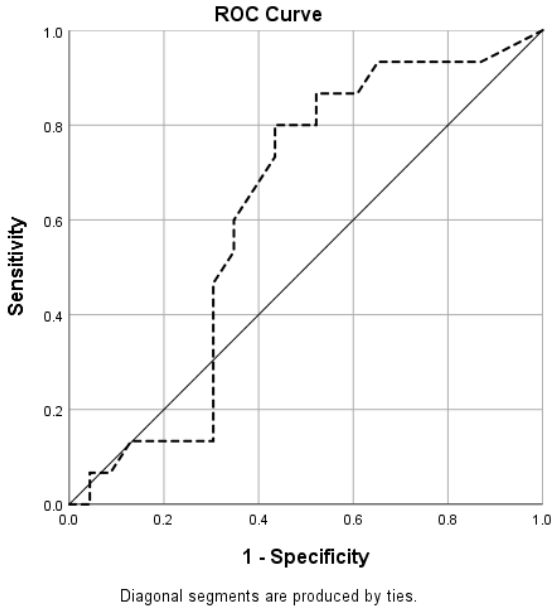

Figure 3. ROC Curve of IL6- for the Diagnosis of APN Taking WBC Count >10000 /µL as Reference.

instance, one study showed a higher sensitivity and specificity for procalcitonin, compared to CRP, for the early diagnosis of APN. Additionally, they showed a significant correlation between procalcitonin values and the degree of renal parenchymal involvement (14). This shows that other biomarkers such as procalcitonin should be investigated along with other markers evaluated in our study, for the simultaneous comparison of their utility in the early diagnosis of APN. Furthermore, serum CRP is an effective and valuable marker for the diagnosis of APN which can help prevent APN-associated complications (9). Other researchers found that CRP, ESR, and WBC count are not sensitive or specific enough for the diagnosis of UTI and determination of the infection location and that where advanced laboratory facilities are available, other laboratory tests should also be performed along with CRP, ESR and WBC count (15). Their findings question what we did to ascertain the diagnostic value of serum IL-6. We took the results of CRP, ESR, and WBC count as reference. A major limitation of the current study was that because of limited resources, patients did not undergo DMSA scan, which is the gold standard for the diagnosis of renal parenchymal involvement, indicative of APN. Therefore, we calculated diagnostic indices based on ESR, CRP, and WBC count results, which may not have been as sensitive or specific as DMSA scanning for the diagnosis of APN.

Conclusion

In the current study, we found that the sensitivity of WBC count, ESR, CRP, and serum IL-6 was 39.5%, 71.1%, 60.5%, and 71.1% for the diagnosis of APN in children. We also showed that by taking the results of ESR, CRP, and WBC count as reference, serum IL-6 levels higher

than 9.98 pg/mL has 70-80% sensitivity and 56.5-73.7% specificity for APN. Overall, serum IL-6 showed promising results; however, we should bear in mind that aside from acceptable diagnostic performance, a test should be cost-effective and available.

Authors' Contribution

Conceptualization and study validation: KG. Study supervision: ME. Implementation, data analysis and interpretation: MAA. Writing and reviewing: KG. All the authors have read and approved the manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interests

The authors declare that they have no competing interests.

Ethical Approval

The study received ethics approval from the Ethics Committee of Hormozgan University of Medical Sciences (ethics code: IR.HUMS.REC.1399.147) and it complies with the statements of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

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