Detection of PCT and urinary β₂-MG enhances the accuracy for localization diagnosing pediatric urinary tract infection

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Objective: The purpose of this article was to investigate whether the combination of urinary beta 2 microglobulin (urinary β₂-MG) and procalcitonin (PCT) diagnosis could enhance the localization diagnostic precision of pediatric urinary tract infection comparing with single diagnosis.

Methods: A study was conducted in the Nephrology Department of Wuhan women and children’s health care centre. This study incorporated 85 participants, including 35 children who were diagnosed as upper urinary tract infection (UUTI) with the symptom of fever and 50 children who conducted lower urinary tract infection (LUTI). Levels of PCT and urinary β₂-MG in both UUTI and LUTI patients were measured and compared.

Results: The level of PCT and β₂-MG were both significantly higher in UUTI group compared with in LUTI group. AUC of urinary β₂-MG ROC (sensitivity of 71.4%, specificity of 90.0%) was significantly smaller than that of PCT ROC (sensitivity of 77.1%, specificity of 96.0%) in the single diagnosis. Although in the combined diagnosis, the sensitivity and specificity increased to 88.6% and 98%, respectively.

Conclusions: Both PCT and β₂-MG could be used to localize the UTI. Introducing urinary β₂-MG into PCT diagnosis could increase the sensitivity and specificity of UTI lesion diagnosis in clinical practice.

KEYWORDS
children, localization diagnosis, procalcitonin, urinary tract infection, urinary β₂-MG

INTRODUCTION

Urinary tract infections (UTIs) commonly occur in young febrile children and they trigger a wide range of bacterial infections.¹ For children under the age of 6 years old, the prevalence of male and female pediatric UTI was nearly 2% and 7%, respectively.² Since UTI symptoms are non-typical among febrile newborns and children, distinguishing upper UTI from lower UTI is challenging. Apart from that, UTI may result in renal scarring which is characterized by many different forms including hypertension, poor renal growth, and end-stage renal failure. Many studies have been conducted to assess the appropriateness of diagnostic approaches and treatments for UTI.³,⁴ Conventional serum markers for diagnosing UTI include total white blood cell count (WBC), C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR), and interleukin (IL)-6.⁵ Procalcitonin (PCT) generates a relatively desirable diagnostic accuracy compared with other serum markers.

Procalcitonin (PCT) is a 116-amino acid propeptide of calcitonin, which could be an indicator of bacterial infections. PCT has been reported to be a reliable diagnostic biomarker of bacterial infections due to its specificity, short time of induction and long half-life.⁶⁻⁸ Common biomarkers for UTI includes serum leukocyte or neutrophil counts, C-reactive protein (CRP) and interleukins, compared with which, PCT has been proved to be more specific in terms of
differentiating acute pyelonephritis from LUTIs. PCT is also specific in terms of differentiation of viral and bacterial infections particularly in children. In addition, PCT can be detected in serum 2 hours after the infections and the PCT levels, thereafter, rises and reaches a plateau 12 hours after the infections. Serum PCT concentration is lower than 0.5 μg/L under normal circumstances in healthy individuals. However, the association between PCT production and bacterial infection still remains not elucidated. It is believed that the CALC1 gene is overexpressed in liver and peripheral blood mononuclear cells in response to the bacterial infections and thereafter produces calcitonin precursors. Recent researches have demonstrated a correlation between high concentrations of serum PCT and renal parenchymal inflammation (RPI) and renal scarring in UTI patients. Children with higher PCT levels (>0.5 ng/mL) could have a high risk of renal damage and acute RPI.

β-2-microglobulin (β2-MG) is a small protein, approximately 100 amino acids long, which is found on the surface of all nucleated cells. It is identified as the light chain of the major histocompatibility complex I. Urinary β2-MG is the indicator of glomerular filtration rate and tubular reabsorption. Comparisons of β2-MG to other serum biomarkers such as creatinine and cystatin C levels have been generally used to estimate kidney functions and prognose the outcomes of chronic kidney disease. The abnormal urinary and serum β2-MG levels were associated with post infallament in urinary tract. High β2-MG levels were correlated with a high grade of vesicoureteral reflux. Combined use of β2-MG and enzymuria could increase the diagnosis sensitivity up to 75%, however, the levels of β2-MG and enzymuria were too variable to be a stable diagnostic indicator. In addition, serum and urine β2-MG have been reported to be elevated in the both acute and chronic renal transplant rejection.

The following study was designed to explore the potential value of the combination of PCT and urinary β2-MG in localization diagnosing paediatric urinary tract infections. Conclusions based on this study may be generalized to clinical practice.

2 | MATERIALS AND METHODS

2.1 | Study objects

A total of 85 children aged between 1 and 13 years old were consecutively enrolled into this study. These children were selected from patients who were admitted in Wuhan Women and Children’s Health Care Center due to febrile urinary infection from August 2014 to September 2015. They were divided into two groups as UUTI and LUTI on the basis of UTI diagnosis treatment guidelines. The exclusion criteria were specified as: children with severe liver damage or incomplete kidney functions; children with congenital immunodeficiency; children with severe congenital heart disease; children who had received antibiotics in the last week before study commencement; children with recurrent urinary infections. This study was approved by the Ethics Committee of Wuhan Women and Children’s Health Care Center and agreed by all participants.

2.2 | Sample collection and the detection of PCT and β2-MG

Five milliliter urine and 5 mL venous blood samples were collected and stored in tubes before antibiotics were applied. 2 mL blood was then centrifuged and preserved at temperature ranging from −20 to −70°C. Then enzyme-linked fluorescent analysis was carried out, in which the relevant kits were purchased from Bio Mérieux, Lyons, France. Quantitative measurement was conducted using Mini VIDAS automatic quantitative fluorescence immunoassay analyzer also purchased from Bio Mérieux. The results were considered positive when the PCT concentration was higher than 0.5 ng/mL. Urinary β2-MG levels were detected using DPC1000 automatic chemiluminescence meter (DPC company, Omaha, NE). The relevant kits were purchased from DPC company and the instructions were strictly followed.

2.3 | Statistical analysis

Statistical analysis was performed using SPSS 21.0 program (IBM, Armonk, NY). Simple descriptive statistics were used to describe participants’ characteristics. Continuous biomarkers in two groups were compared using student t-test. Counted data were expressed in the form of frequency and percentage. Differences in counted data were analyzed using the chi-square test. Diagnostic accuracy of makers was evaluated using the ROC curve analysis.

Logistic regression analysis was conducted to determine the correlation between the dependent variable (UUTI) and independent variables (PCT and β2-MG).

3 | RESULTS

3.1 | Demographic features of participants

Ten boys and 25 girls were enrolled in the UUTI group (age <1 year: n=19, age between 1 and 3 years: n=13, age between 4 and 7 years: n=2, age between 8 and 13 years: n=1). The average age in the UUTI group was 1.55±1.28 years old. The LUTI group consisted of 18 boys and 32 girls (age of <1 year: n=16, age between 1 and 3 years: n=16, age between 4 and 7 years: n=12, age between 8 and 13 years: n=6) with an average age of 3.59±1.81 years old. The age distribution and average age showed significant differences between two groups (P<0.01). There were significant differences in clinical symptom of fever between two groups (P<0.02), while there were no significant differences in other clinical symptoms (P>0.05). The demographic characteristics were shown in Table 1.

3.2 | The level and distribution of PCT and urinary β2-MG in UUTI and LUTI groups

The mean level of PCT in the UUTI group was 1.37±0.67 ng/mL, whereas that in the LUTI group was 0.53±0.23 ng/mL. The mean levels of urinary β2-MG in the UUTI and LUTI groups were 1.08±0.73 and 0.39±0.21 μg/mL, respectively. The average levels of serum PCT and
3.4 | The optimal cut-off values of PCT and urinary β₂-MG for diagnosis of UTI

As suggested by ROC curves, the optimal cut-off points of PCT and urinary β₂-MG were 0.91 and 0.64 ng/mL, respectively (Figure 2). The sensitivity of PCT and urinary β₂-MG associated with the corresponding cut-off points were 77.1% and 71.4%, respectively, whereas the specificity were 96.0% and 90.0%, respectively (Table 3).

3.5 | Diagnostic value of incorporating PCT with urinary β₂-MG

The logistic regression analysis was conducted to predict the relationship between the dependent variable (UUTI) and independent variables (PCT and β₂-MG), and a strong association was found. Regression coefficients of PCT and urinary β₂-MG are 16.39314 (P=.0001), -12.4977 (P=.00070), respectively. The establishment logistic regression model is as follows log (UUTI)=16.39314×PCT−12.4977×β₂-MG.

The ROC curves of single as well as combined diagnosis was shown in Figure 2. ROC curve of PCT combined with urinary β₂-MG had an AUC of 0.943, bigger than PCT or urinary β₂-MG single diagnosis (P<.05).

4 | DISCUSSION

Early identification and diagnosis of UTI is important, yet remains challenging because the symptoms of UTI are usually neither apparent nor disease-specific during infancy and early childhood.28,29 The baseline characteristics analysis showed that urinary irritation and gross hematuria were not significantly different between UUTI and LUTI groups whereas fever and average age demonstrated significant group difference.30 However, fever is such a common symptom in many infection diseases, thus loses its value in specificity for diagnosis of UTI. The analysis of baseline characteristics in this study further explained the difficulties to distinguish UUTI from LUTI among children through identification of just symptoms.

The misdiagnosis rate of UTI is still unexpectedly high due to the limitation of current diagnostic methods. At present, the urine bacteria

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**TABLE 1** The basic information of individuals in UUTI group and LUTI group

|         | UUTI | LUTI | P value |
|---------|------|------|---------|
| Gender  |      |      |         |
| Male    | 10   | 18   | .47     |
| Female  | 25   | 32   |         |
| Age     |      |      |         |
| <1 year | 19   | 16   | .03     |
| 1-3 years | 13 | 16   |         |
| 4-7 years | 2  | 12   |         |
| 8-13 years | 1 | 6    |         |
| Mean age (y) | 1.55±1.28 | 3.59±1.81 | <.01 |
| Symptoms|      |      |         |
| Fever   | 5    | 19   | .02     |
| Urinary irritation | 12 | 8    | .05     |
| Gross haematuria | 12 | 8    | .05     |
| Turbid urine | 3 | 5    | .82     |
| Back pain or abdominal discomfort | 6 | 5    | .33     |
| Emesis  | 1    | 3    | .50     |
| Hypoevolutism | 0 | 1    | .40     |

**TABLE 2** The expression level of PCT and urinary β₂-MG inside the participants from UUTI group and LUTI group

|         | UUTI | LUTI | P value |
|---------|------|------|---------|
| PCT (ng/mL) | 1.37±0.67 | 0.53±0.23 | <.01     |
| Urinary β₂-MG (μg/mL) | 1.08±0.73 | 0.39±0.21 | <.01     |

PCT, procalcitonin; urinary β₂-MG, urinary β₂ microglobulin.

urinary β₂-MG in the UUTI group were significantly higher than those in the LUTI group, respectively (P<.05) (Table 2). The distribution of PCT and urinary β₂-MG were both left-skewed without extreme values (Figure 1).

3.3 | ROC curve of urinary β₂-MG and PCT for diagnosing UTI

ROC curves of PCT and urinary β₂-MG for diagnosis of UTI were displayed in Figure 2. The AUC of PCT was 0.902 (95% CI=0.818-0.956; P<.01) whereas that of urinary β₂-MG was 0.830 (95% CI=0.733-0.903; P<.01). AUC of PCT was significantly higher than that of urinary β₂-MG.
AUC, area under the curve of ROC.

The value of PCT and urinary β2-MG in the localization diagnosis of UTI in children along

| Index       | AUC | Cut-off point | Sensitivity % | Specificity % |
|-------------|-----|---------------|---------------|---------------|
| PCT         | 0.902 | 0.91 | 77.1          | 96.0          |
| Urinary β2-MG | 0.830 | 0.64 | 71.4          | 90.0          |
| Combined    | 0.943 | 6.53 | 88.6          | 98.0          |

AUC, area under the curve of ROC.

culture is the most precise method to identify urinary tract infection, but is time-consuming.\cite{13} The 99mTc-dimercaptosuccinic acid (DMSA) scintigraphy is a wide-applied sensitive technology for UTI diagnosis, but is too expensive to be popularized nation-wide. In addition, there have been some traditional biomarkers for UTI diagnosis, but few have appropriate specificities.\cite{6,16} Therefore, it is necessary to develop quick, economical, and specific diagnosis approaches for pediatric UTI. Novel molecules such as PCR, CRP, and β2-MG, etc. have been identified as biomarkers in diagnosing UTI.\cite{13,30,31}

For localizing acute UTI, proximal tubular dysfunction testing was thought to be more clinically valuable.\cite{25} In addition, β2-MG has been reported to be increased significantly in UUTI patients but normal in patients with cystitis, which indicated that β2-MG could be used to distinguish UUTI from LUTI.\cite{13} Sgardijn et al. long ago pointed out the possibility of applying the urinary β2-MG to localization diagnosis of UTI, unless patients simultaneously suffer from pre-existing tubular damage.\cite{30} Similarly, our study revealed that β2-MG showed higher concentrations in UUTI urine samples than in LUTI urine samples, indicating that β2-MG could be used to distinguish UUTI from LUTI. The ROC analysis also demonstrated that urinary β2-MG had good diagnostic accuracy with an AUC value of 0.830, though a little inferior to that of PCT.

Various studies have been done to investigate the diagnostic value of PCT as a biomarker for infection. For instance, Mahajan et al. suggested PCT a more accurate biomarker compared with other indexes such as white blood cell (WBC) count, absolute neutrophil count (ANC), absolute band count.\cite{32} Leroy et al. conducted a meta-analysis, in which they asserted that PCT concentration was an independent predictor of UTI and vesicoureteral reflux (VUR).\cite{12,33} García de Guadiana-Romualdo et al. suggested the usefulness of PCT as a diagnostic marker for infection in patients with febrile neutropenia.\cite{34} Pecile et al. identified serum PCT as a sensitive indicator for early diagnosis of febrile UTIs in children.\cite{6} Simon et al. performed a meta-analysis and reported that the diagnostic accuracy of bacterial infection in urinary tract with PCT as the marker was higher than that with CPR marker.\cite{13} Likewise, Kotoula et al. also indicated that PCT was a more reliable marker for UTI recognition.\cite{16} PCT test was reported to show a higher likelihood ratio than CRP test in UUTI diagnosis.\cite{10} In addition, the serum PCT level has been recommended to differentiate and predict acute pyelonephritis (assessed by DMSA) from LUTI.\cite{25} Consistently, Smolkin et al. found that the serum PCT level showed excellent diagnosis accuracy and specificity compared with CRP in terms of acute pyelonephritis diagnosis.\cite{15} Likewise, Xu et al. has reported that PCT demonstrated better specificity than CRP in terms of distinguishing acute pyelonephritis from LUTI.\cite{26} Moreover, Sheu et al. discovered that PCT could be an independent risk factor for renal involvement in urinary tract infection.\cite{37}

Our data showed that both serum PCT and urinary β2-MG in patients with UUTI were significantly higher than those in patients with LUTI, indicating that both of them could be used in localization diagnosis of UTI. Despite of the good performance of clinical indexes such as WBC count and the demonstrated good prognosis outcomes of single diagnosis of PCT or β2-MG, we have not been completely satisfied. Giorgetti et al. have showed that the combined use of urinary β2-MG and N-acetyl-beta-D-glucosaminidase (NAG) could raise the sensitivity of diagnosing the acute UTI in contrast with the single application of β2-MG or NAG. We thus investigated the diagnosis accuracy, sensitivity, and specificity of innovative combination diagnosis method i.e., the combination of PCT and β2-MG. The ROC analysis suggested that the combination of serum PCT and urinary β2-MG diagnosis outperformed the two single diagnoses in terms of accuracy, sensitivity, and specificity.

5 CONCLUSION

In summary, the combination of urinary β2-MG and serum PCT could increase the localization diagnosis accuracy, specificity, and sensitivity of paediatric UTI. Nonetheless, we consider a further study conducted within a larger paediatric population is required.

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