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Distinctive inflammatory profile between acute focal bacterial nephritis and acute pyelonephritis in children

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ABSTRACT

Background: Acute focal bacterial nephritis (AFBN) is a severe form of upper urinary tract infection (UTI) with neurological manifestations and focal renal mass lesions on computed tomography (CT). Prolonged antibiotic therapy may improve the renal outcome, but the early differential diagnosis of AFBN from acute pyelonephritis (APN) is challenging. We searched for effective biomarkers of AFBN based on the pathophysiology of upper UTIs.
Methods: Of 52 upper UTI cases treated at Yamaguchi University between 2009 and 2016, 38 pediatric patients with AFBN (n = 17) or APN (n = 21) who underwent ultrasonography and/or CT were enrolled. The clinical data and serum cytokine concentrations were analyzed to differentiate AFBN from APN.
Results: AFBN patients tended to be older, and have a higher body temperature, longer febrile period, more frequent neurological symptoms, higher immature neutrophil count, lower lymphocyte count, higher procalcitonin and urine β_2-microglobulin levels. AFBN patients showed higher serum levels of IFN-γ, IL-6, IL-10 and soluble TNF-receptor 1 (sTNFR1) (all $p < 0.05$). Although the cytokine levels were variably correlated among each other, multiple logistic regression analysis revealed that combination of IFN-γ and IL-6 levels were most relevant for distinguishing AFBN from APN. The discriminant power of the logistic equation was 0.86 in terms of the area under the curve by the ROC analysis.
Conclusions: Circulating 4 out of 7 cytokines in AFBN patients were at higher levels compared with those in APN patients. IFN-γ and IL-6 levels might most effectively distinguish AFBN from APN.

1. Introduction

Acute focal bacterial nephritis (AFBN) is a severe form of upper urinary tract infection (UTI) showing an inflammatory mass lesion but no abscess formation in the kidney [1]. The majority of cases develop in infants and children with abnormal urinary tracts, although adult cases rarely occur in immunocompromised patients. The diagnosis of AFBN depends on contrast-enhanced computed tomography (CT) and ultrasonography [2,3]. The morphology of the lesion exemplifies the intermediate stage of an upper UTI between acute pyelonephritis (APN) and renal abscess [4,5]. Of note, recently it is advocated that AFBN is divided into two forms, simple and complicated AFBN by the patterns of the AFBN lesions detected by contrast-enhanced CT. Simple AFBN appears as striated or wedge-shaped regions and can be regarded as severe APN. Complicated AFBN represents a relatively early stage of the development of a renal abscess, while APN or simple AFBN does not progress to a renal abscess [6,7].

AFBN and APN share septic symptoms, including a high fever and altered consciousness, although the former is more closely associated with a prolonged fever or convulsions and less with pyuria than the latter [8,9]. AFBN patients are at a high risk of treatment failure and renal scarring [10,11]. At present, a three-week regimen of antimicrobial therapy is the standard treatment for achieving a good renal outcome [12]. Contrast-enhanced CT has greater sensitivity and

Abbreviations: AFBN, acute focal bacterial nephritis; APN, acute pyelonephritis; UTI, urinary tract infection; CT, computed tomography; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; sTNFR1, soluble TNF-receptor 1; PCT, procalcitonin; CRP, C-reactive protein; α_1MG, α_1-microglobulin; β_2MG, β_2-microglobulin; L-FABP, liver-type fatty acid binding protein; NAG, N-acetyl-β-D-glucosaminidase; Cr, creatinine; ECLIA, electro-chemiluminescence immunoassay; CLEIA, chemiluminescent enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; AUC, area under the curve; ROC, receiver operating characteristics; MLRA, multiple logistic regression analysis; OR, odds ratio; CI, confidence interval; MERS, mild encephalopathy with reversible splenial lesion; SD, standard deviation; VUR, vesicoureteral reflux; LPS, lipopolysaccharide

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specificity for the diagnosis of AFBN than ultrasonography. However, the optimal timing of therapy and the risk of adverse events remain challenging. The irradiation associated with CT and the use of sedative agents to perform the scan pose risks for young infants.

Aside from imaging studies, there are no useful biomarkers to differentiate between AFBN and APN. The serum interferon (IFN)-γ levels of AFBN patients have been reported to be higher than those of healthy controls but were not compared with those of APN patients [13]. AFBN develops from blood-born and ascending routes of infection. However, there is little information regarding the underlying mechanisms responsible for the progressive inflammation in AFBN patients.

In the present study, we examined various inflammatory parameters of upper UTIs, such as immature neutrophils, lymphocytes, procalcitonin (PCT) and major cytokines including IFN-γ and interleukin (IL)-6 to clarify the pathogenesis and identify effective biomarkers to diagnose AFBN.

2. Materials and methods

2.1. Patients

Fifty-two patients (age, < 15 years) who were admitted to Yamaguchi University Hospital from January 2009 to April 2016 for the treatment of upper UTIs were enrolled in the present study (Fig. 1). The medical records were retrospectively reviewed to investigate the clinical and laboratory findings at the time of the diagnosis and throughout the treatment course. All of the patients met the diagnostic criteria for an upper UTI: fever (body temperature > 38.0 °C), positive urine culture, low back pain and the presence or absence of focal mass lesions in the kidney as assessed by screening echography (n = 52) on admission and/or before contrast-enhanced abdominal CT (n = 23). Positive urine cultures were defined by the detection of $\geq 5 \times 10^4$ colony-forming units/mL of a single organism in urine samples obtained by transurethral catheterization or midstream urine [14]. AFBN and APN were diagnosed based on imaging findings in 19 and 33 patients, respectively. After excluding the 14 patients who gave incomplete samples or who did not provide consent, 17 patients with definite AFBN (all underwent CT) and 21 patients with probable APN including 1 simple AFBN (4 underwent CT) and no patient with renal abscess were identified. All AFBN patients received intravenous antibiotics (cefmetazole or cefotaxime or piperacillin or meropenem) for two weeks and subsequent oral cefaclor or sulfamethoxazole-trimethoprim for one week. All APN patients were initially treated with intravenous antibiotics, and then treated with oral antibiotics for a total of two weeks. We also included a group of 12 children who suffered from sepsis (acute bacterial pneumonia; 6, cellulitis; 2, bacteremia; 1, acute bronchitis; 1, acute pharyngitis; 1, acute suppurative otitis media; (1) as a disease control. We compared the inflammatory parameters among the three groups. This study was approved by the Institutional Review Board of Yamaguchi University Hospital (No. 2013-205-3).

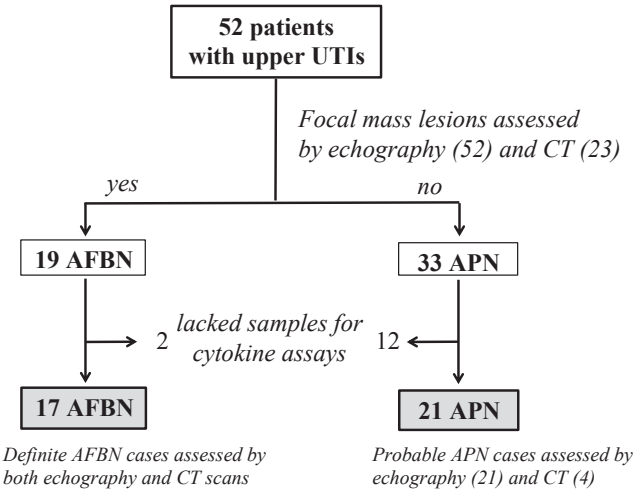

Fig. 1. Flowchart of the selection of AFBN and APN patients. All 52 patients underwent renal ultrasonography (n = 52) and partly enhanced CT (n = 23) at the time of the diagnosis of upper UTI. UTI: urinary tract infection.

2.2. Sample preparation and analysis

Blood and urine samples were collected after obtaining informed consent. The serum samples were separated promptly from the remaining part of blood samples and were stored at −20 °C until the time of analysis.

The blood cell counts including neutrophil, lymphocyte and platelet were measured on the Sysmex XN-3000 analyzer (Sysmex corporation, Kobe, Japan). The number of immature neutrophil was microscopically measured. C-reactive protein (CRP), ferritin, serum β_2-microglobulin (β_2MG), urine β_2MG and urine α_1-microglobulin (α_1MG) were measured using a latex coagulating nephelometry on a JCA-BM 6070 analyzer (Japan Electron Optics Laboratory, Akishima, Tokyo, Japan). D-dimer was also measured using a latex coagulating nephelometry on a Coapresta 2000 analyzer (Sekisui medical corporation, Chuo, Tokyo, Japan). PCT was measured using an electro-chemiluminescence immunoassay (ECLIA) on a Cobas 8000 analyzer (Roche Diagnostics K.K., Minato, Tokyo, Japan). Urine N-acetyl-β-D-glucosaminidase (NAG) was measured using an optical density method on a JCA-BM 6070 analyzer. Urine liver-type fatty acid binding protein (L-FABP) was measured using a chemiluminescent enzyme immunoassay (CLEIA) on Lumipulse® G1200 analyzer (Fujirebio corporation, Chuo, Tokyo, Japan). The urine protein concentrations were corrected for creatinine (Cr) levels, which were measured using an enzyme method on a JCA-BM 6070 analyzer. The reference values were as follows: CRP, < 0.15 mg/dL; PCT, < 0.05 ng/mL; ferritin, 6.0–80.0 ng/mL; serum β_2MG, < 2.0 mg/L; urine β_2MG, < 250 μg/L; urine α_1MG-, < 30,000 μg/L; urine Cr, < 0.68 g/L; urine NAG, 0.9–2.4 U/gCr; urine L-FABP, < 8.5 μg/gCr.

The serum concentrations of IL-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α and IFN-γ were measured using a BD™ Cytometric Bead Array Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Jose, CA, USA), while the serum concentrations of soluble TNF-receptor 1 (sTNFR1) were measured using a human sTNFR1/TNFRSF1A Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, MN, USA). The detection limits were as follows: IL-2 and IL-4, 2.6 pg/mL; IL-6, 3.0 pg/mL; IL-10 and TNF-α, 2.8 pg/mL; IFN-γ, 7.1 pg/mL; and sTNFR1, 1.2 pg/mL.

2.3. Imaging studies

Renal echography was performed to assess the length (standard deviation [SD] of the major axis) of the affected and non-affected kidneys based on the standards for Japanese children [15]. A voiding cystourethrogram obtained two weeks after the onset of the UTI was examined for vesicoureteral reflux (VUR). Kidney scintigraphy (^{99m}technetium dimercaptosuccinic acid renal scan) was conducted to assess the scarring at four months after the onset of the UTI. At this time, we assessed the stage of kidney scarring according to the classifications of the RN Forum Japan [16], and investigated the presence of a relative renal uptake of < 45% on 1 side, and the existence of defects.

2.4. Statistical analysis

We first analyzed the distribution of each laboratory tests and found that IFN-γ, IL-6, IL-10, PCT, and urine β_2MG/Cr showed skewed distributions with a tailing toward the higher values. Therefore, in the statistical analyses, the test results were first transformed logarithmically to amend the skewness. Since there were differences in age