Urine TREM-1 as a marker of urinary tract infection in children

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Abstract

Objective: Triggering receptor expressed on myeloid cells (TREM)-1 is a receptor that is thought to improve recognition of patients with true infection. In this study, we investigated whether Triggering receptor expressed on myeloid cells (TREM-1) is present in urine samples from children with urinary tract infection (UTI) and in samples from healthy children.

Methods: A total of 128 samples met the inclusion criteria for the study. Urine samples were processed for culture and urinalysis as a regular protocol for patients with UTI. Samples were classified according to culture and urinalysis results. TREM-1 protein expression was detected with flow cytometry and sTREM-1 was assessed by ELISA.

Results: Flow cytometry showed detectable expression of TREM-1 in 100% of samples, UTI and non-UTI groups ($p < 0.001$). Mean fluorescence intensity of TREM-1 was different between the groups ($p < 0.001$). Levels of sTREM-1 were detected in patients with UTI, but not in non-UTI patients.

Conclusions: All of our patients (healthy and diseased) showed TREM-1 expression. However, TREM-1 levels in patients with UTI tend to be higher and are associated with increased neutrophils and cytokine activity induced by bacteria.

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Introduction

The urinary tract represents a formidable mechanical barrier to infection. Of the numerous Gram-negative species of bacteria that can cause urinary tract infection (UTI), uropathogenic *Escherichia coli* is responsible for >80% of these UTIs in uncompromised patients.[1] UTI constitutes the second most common infectious disease in humans, following respiratory tract infection. The majority of patients with UTI are females, ranging in age from the early teen years to older people. A large proportion (up to 25%) of these patients will subsequently be afflicted by reoccurring or persistent infection. The presence of immune surveillance molecules is critical to any immune response mounted by the urinary tract. The Toll-like receptor (TLR) family is the best characterized of these molecules. TLR4 promotes vigorous cytokine and chemokine responses in the urinary tract to Gram-negative bacteria.[1] Interestingly, recent studies have shown that TLR4 plays a number of additional antimicrobial roles that appear to be specific for the urinary tract.[2] Polymorphonuclear neutrophil cells (PMN) play an important role in the progression of inflammation. They are the first cells to be recruited to the site of aggression and become highly activated by a wide array of ligands. Receptor-activated PMN release chemokines and cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-8, macrophage inflammatory protein 1 alpha (MIP)-1-α, and MIP-1β, among others. The products of activated PMN recruit more cells to the inflammation site and actively contribute to modulation of the adaptive immune response.[3] One novel molecule, the human triggering receptor expressed on myeloid cells (TREM)-1, is a 30-kDa glycoprotein of the immunoglobulin superfamily. *In vitro* and *in vivo* studies have shown that Triggering receptor expressed on myeloid cells (TREM-1) expression is strongly upregulated by extracellular bacteria, particularly the cell wall component, and by fungi, although not by mycobacteria. In contrast, TREM-1 is poorly expressed in noninfectious inflammatory diseases, such as psoriasis, ulcerative colitis, and vasculitis, and in granulomatous disorders, such as tuberculosis (TB) or foreign body granulomas. A soluble form of TREM-1 (sTREM-1) is released from activated phagocytes and can be found in body fluids.[4] The role of TREM-1 in UTI has been defined in some studies, but to the best of our knowledge, only the soluble form has been studied.[5]

We designed this prospective and analytical study to measure TREM-1 and sTREM-1 in myeloid cells in urine sediment to investigate their role in UTI.

Methods

Subjects

Our study comprised patients who were outpatients during 1 year at the Pediatric Urology Department of the Pediatric Hospital, Western National Medical Center, National Institute of Social Security (IMSS) in Jalisco, Mexico. The institutional review board approved the study (number: R-2010-1205-9). Patients and parents were informed about the study. Inclusion criteria were as follows: children between the ages of 1 and 16 years, both sexes, and those with clinical

symptoms of UTI (frequency, dysuria, and abdominal pain).

Children with the following specific risk factors for UTI were excluded: vesicoureteral reflux, urinary tract stone disease, chronic renal disease, TB, patients on antibiotics or immunosuppressor drugs (transplanted patients), and patients with immunological or neoplastic diseases. Urine samples were collected for urinalysis and urine culture in all of the patients. Diagnosis of UTI was performed according to American Academy of Pediatrics criteria.[6,7]

Patients were divided into two groups: UTI and non-UTI. Nearly 70% of children who were diagnosed UTI presented with urinary frequency, dysuria, and abdominal pain. All patients with UTI (>100,000 CFU/mL; urine cultures with greater than 100,000 colony forming units (CFU)/mL) were managed with antibiotics. Patients with fever and macroscopic haematuria were not included in the study. Patients with negative results were managed symptomatically. Patients with <100,000 CFU/ml or two or more different microorganisms were excluded. None of the patients included in the study required management as an inpatient.

Data collection

Demographic and disease data of patients included, sex, age, clinical history, urinalysis, urine culture, and sTREM-1 and TREM-1 levels. Results of TREM-1 and urinalysis were collected on the sampling day. Urine culture results were collected 72 h after sampling. With regard to sTREM-1, the samples were processed as soon as 40 of these were accumulated for the enzyme-linked immunosorbent assay (ELISA) plate.

Urine samples

For sampling, all of the patients underwent genital hygiene with soap and water in the laboratory. In the majority of patients, samples were able to be collected during the urinary midstream. Young children required placement of a collection bag. Urinalysis and urine culture samples were processed and the remainder (5–15 mL) of the urine was used for the next step (sTREM-1, TREM-1, and mean fluorescence intensity [MFI] measurements).

All samples were centrifuged at 2,000 rpm during 5 min. After centrifugation, 1 mL of supernatant was collected and stored at $-80°C$ until assessment of sTREM-1 by ELISA. The pellet was prepared and we subsequently analysed TREM-1 expression by flow cytometry (FC).

Assessment of TREM-1 expression by flow cytometry

The pellet was washed three times with 2 mL PBS and resuspended in 100 μL PBS buffer added to Fc Receptor Blocking Solution (BioLegend, San Diego, CA, USA). The cells were then incubated with antihuman TREM-1-phycoerythrin (R&D Systems, Minneapolis, MN, USA) for 30 min at room temperature. Subsequently, the cells were washed with PBS, fixed with 1% paraformaldehyde, and analysed by FC. An appropriate isotype control was used to adjust for discarding a non-specific background signal. The results are reported as the % of expression or as the geometric MFI. For each sample, at least 1,000 events were acquired in an EPICS XL MCL (Beckman Coulter, Fullerton, CA, USA). Data were processed with WimMDI ver. 2.8 free software.

Measurement of sTREM by ELISA

Supernatants from the groups were collected to determine sTREM-1 levels. The assay was performed as described by the manufacturer (Quantikine®; R&D Systems). After stop solution, optical density was determined

using a microplate reader (Synergy[TM] HT Multi-Mode Microplate Reader; BioTek Instruments, Inc., Winooski, VT, USA) that was set at 450 nm with a 570-nm wavelength correction. The concentration was expressed in pg/mL.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Comparison of mean values in both groups was conducted using the Student's *t* test. The chi-square and Pearson correlation tests were used to search for associations among the variables. The results were considered statistically significant if *p* was <0.05.

Results

Paediatric patients' characteristics

A total of 469 patients with a clinical diagnosis of UTI were seen by appointment. Only 128 patients complied with the inclusion criteria. We only included samples with appropriate characteristics for the study. Patients with insufficient urine samples were excluded from the study. The remaining 341 patients were excluded because they had other aggregated medical problems (infectious, oncological, immunological, cardiovascular, gastrointestinal, and endocrine). The medical centre where the research was carried out is a national tertiary care centre. Therefore, UTI associated with other diseases in children is the most common.

The collected samples corresponded to 75 girls (58.59%) and 53 boys (41.40%). The median age in both groups was 6 years (range, 1–15 years). Urine culture and urinalysis showed 62 (48.43%) cases of UTI and 66 (51.56%) cases of non-UTI. Table 1 shows the demographic characteristics of the study groups.

Upregulation of TREM-1 expression in UTI-infected paediatric patients

FC showed detectable TREM-1 expression in 100% of samples (UTI and non-UTI groups). Mean TREM-1 expression was significantly higher in the UTI group (43.49% ± 25.76%) than in the non-UTI group (11.19% ± 12.31%, $p < 0.001$).

A total of 92% of patients with UTI had TREM-1 levels of >10. Table 2 shows the proportions of patients in terms of TREM-1 cut-off points.

Upregulation of MFI in the UTI group

MIF measurement was performed in all samples (UTI and non-UTI). Mean MFI was significantly higher in the UTI group (45.77 ± 8.3) than in the non-UTI group (32.37 ± 4.5, Student *t* test, $p = 0.0001$).

sTREM-1 is secreted in the UTI group

A total of 80 samples were tested with the ELISA assay (40 patients with UTI and

Table 1. Demographic characteristics of paediatric patients with UTI and non-UTI.

	UTI n (%)	Non-UTI n (%)	Total n (%)
Female	38 (61)	37 (56)	75 (58.59)
Male	24 (39)	29 (44)	53 (41.40)
Total	62 (48.43)	66 (51.56)	128 (100%)

Table 2. Proportions of patients with UTI and non-UTI according to TREM-1 cut-off points.

	UTI	Non-UTI
TREM-1	n/%	n/%
≥10%	57/92	22/35
5 to 9%	3/4.8	16/25
<5%	2/3.2	25/40

The chi-square test was used to validate the statistical association of TREM-1 levels of >10 and UTI.

40 non-UTI patients). Levels of sTREM-1 were not detected in the non-UTI group. In the UTI group, sTREM-1 levels were only detected in 10 (25%) samples. The mean sTREM-1 value in these samples was 140.6 ± 253 pg/dL ($p = 0.007$ compared with the non-UTI group). Only 80 samples were processed because we encountered a technical problem during the thawing process in the remainder of the samples, which could alter the outcome.

Discussion

Neutrophils and monocytes/macrophages are the primary mediators of the innate immune response to bacterial infection, promoting the release of proinflammatory cytokines, such as TNF-α and IL-1β. When these cytokines are produced in excess, they contribute to end-organ dysfunction and overwhelming sepsis. TREM-1, which is part of the immunoglobulin superfamily, is upregulated in response to bacteria or fungi. When TREM-1 is bound to a ligand, it stimulates the release of such cytokines via the signal transduction molecule DAP12. The soluble form of TREM-1 is shed from the membranes of activated phagocytic cells and can be quantified in human body fluids.[8] Several studies have investigated the use of TREM-1 as a diagnostic biomarker and have shown it to be more sensitive and specific than C-reactive protein (CRP) and procalcitonin (PCT).[8] Gibot et al.[9] conducted a study in 76 patients in whom sepsis was suspected. They found that plasma sTREM-1 levels were highly accurate (8.6 LR +; sensitivity, 96%; specificity, 89%, at a cut-off level of ±60 ng/mL) for distinguishing systemic inflammatory response syndrome (SIRS) from sepsis or septic shock. The soluble form of TREM-1 was a superior diagnostic marker to PCT and CRP in their study. Our study was not designed with the aim of determining TREM-1 sensitivity and specificity in urine. However, in

urine, we found that 92% of patients with UTI presented TREM-1 percentages of >10%, while in the non-infected group, only 35% presented similar levels. These data suggest the usefulness of TREM-1 in urinary sediment cells as a potential diagnostic test.

Su and colleagues reported changes in the dynamics of sCD163, sTREM-1, PCT, and CRP during the course of sepsis, as well as their prediction of outcome in a group with SIRS (30 patients) and in a group with sepsis (100 patients). Based on 28-day survival, sepsis was further divided into survivor and non-survivor groups. Serum markers and the white blood cell (WBC) count were examined on days 1, 3, 5, 7, 10, and 14. On admission to the intensive care unit (ICU), the sepsis group showed higher levels of sTREM-1, sCD163, PTC, and CRP than did the SIRS group ($p < 0.05$). Although PCT and sTREM-1 are good markers for identifying severity, sTREM-1 is more reliable, and it is a risk factor related to sepsis. During a 14-day observation in previous studies, sCD163, sTREM-1 levels, PCT levels, and Sepsis-related Organ Failure Assessment scores continued to rise among non-survivors, while their WBC and CRP levels decreased. The authors concluded that sTREM-1 is more ideal for diagnosis of sepsis and determining severity, and constitutes a risk factor.[10,11]

Van Bremen et al.[12] examined the expression of TREM-1, TLR2, TLR4, CD14, and human leukocyte antigen-D related in blood monocytes and neutrophils using FC in 22 patients with *E. coli* sepsis and six healthy controls. The authors found significantly higher expression of TREM-1 and TLR2 in monocytes and neutrophils in patients compared with the controls. TREM-1 expression tended to be higher ($p = 0.07$) in monocytes and lower in neutrophils of patients with severe sepsis compared with controls. TREM-1 expression in neutrophils was associated with IL-10

(Lipopolysaccharide: $r = 0.61$; $p < 0.02$) and TNF-α inducibility (Lipopolysaccharide: $r = 0.78$; $p < 0.002$). The authors concluded that there was an association of TREM-1 expression in neutrophils of patients with *E. coli* sepsis.

The diagnostic value of urinary sTREM-1 was studied by Long and co-workers in terms of identification of early sepsis, severity, and prognosis, as well as secondary acute kidney injury (AKI). The authors compared sTREM-1 with WBC, CRP, PCT, urine output, creatinine clearance (CCr), serum creatinine (SCr), and blood urea nitrogen (BUN). This study enrolled 104 patients who had been admitted to the ICU as follows: 16 patients with SIRS, 35 with sepsis, and 53 with severe sepsis. Results for urinary sTREM-1 levels, WBC count, serum CRP levels, and PTC levels were recorded on days 1, 3, 5, 7, 10, and 14. For the 17 patients with sepsis who were diagnosed with associated AKI, comparisons between their urine sTREM-1 levels, urine output, CCr, SCr levels, and BUN levels at diagnosis and 48 h prior to diagnosis were carried out. On the day of admission to the ICU, the sepsis group had higher urine sTREM-1 levels and Acute Physiologic Assessment and Chronic Health Evaluation scores compared with the SIRS group ($p < 0.05$). The areas under the curve as determined by the scores were 0.797 (95% confidence interval [95% CI], 0.711–0.884) and 0.722 (95% CI, 0.586–0.858), respectively. On days 1, 3, 5, 7, 10, and 14, urine sTREM-1 levels, serum PCT levels, and the WBC count were higher in the severe sepsis group compared with the sepsis group ($p < 0.05$). Urine sTREM-1 and serum PCT levels continuously increased among non-survivors, while the WBC count and serum CRP levels in both groups declined. For the 17 patients with AKI, urine sTREM-1, SCr, and BUN levels at 48 h prior to diagnosis of AKI were higher, and the CCr was lower than those in non-AKI patients ($p < 0.05$).

The authors concluded that urine sTREM-1 testing is more sensitive than WBC, and serum CRP and PCT testing for early diagnosis of sepsis, as well as for dynamic assessment of severity and prognosis.[13] Other authors, such as Derive and colleagues, consider that urinary sTREM-1 could become a new biomarker for sepsis-associated AKI.[14] We found that sTREM-1 analysed by ELISA had a frequency of 25% in infected patients and 0% in non-infected patients. The strength of this study lies in determination of the TREM-1 fraction, which is joined with the cellular membrane, as well as determination of the MFI. These variables have not been previously related to urinary infections in the paediatric population.

A meta-analysis conducted by Jiyong et al. evaluated the accuracy of sTREM-1 for diagnosis of bacterial infection. Studies for inclusion in the meta-analysis were required to report an accepted test for sTREM-1 (ELISA). The analysis included 13 studies (980 patients: 557 patients with bacterial infection and 423 with non-bacterial infection). The global prevalence of UTI was 56.8%. Global sensitivity was 0.82 (95% CI, 0.68–0.90) and specificity was 0.86 (95% CI, 0.77–0.91). The positive likelihood ratio was 5.66 (95% CI, 3.41–9.38), the negative likelihood ratio was 0.21 (95% CI, 0.12–0.40), and the diagnostic odds ratio was 26.35 (95% CI, 0.77–0.91), with a Q point value of 0.84. The sensitivity of the sTREM-1 assay for diagnosis of UTI was low (0.18; 95% CI, 0.05–0.51). The authors concluded that sTREM-1 may be not a sufficient biological marker for UTI because of its low sensitivity.[15] None of the studies described in this meta-analysis reported determination of TREM-1 in urinary sediment cells or the MFI.

Jiyong et al.[15] included only one experiment in which sTREM was measured in urine. In Determann et al.'s study, urine samples were collected from patients who

presented at the emergency room with symptoms of lower or upper UTI. For inclusion in the study, infection had to comprise the presence of leukocytes (>10 leukocytes per high-power field) and bacteria in the urine sediment. Samples were centrifuged at $1,500 \times g$ for 10 min at $4°C$ and the supernatant was stored at $-80°C$. Levels of sTREM-1 were measured by ELISA (recovery, 95–100% in urine; detection limit, 20 pg/ml). Samples from 70 patients with UTI were analysed. Urine samples of 10 healthy controls and of nine patients with asymptomatic bacteriuria served as controls. Patients with UTI were 49 ± 23 years old and 31 (44%) were male. Culture was performed in 39 (56%) patients and was positive ($\geq 10^5$ colony-forming units per ml) in all patients. Fifty-five patients presented with lower UTI and 15 patients with pyelonephritis/urosepsis. Nineteen patients were immunocompromised because of diabetes mellitus ($n = 5$), use of immunosuppressive drugs ($n = 15$), or haematological disease ($n = 2$). Urinary levels of sTREM-1 were detectable only in 8/55 (15%) patients with lower UTI and in 5/15 (33%) patients with pyelonephritis/urosepsis. Levels of sTREM-1 ranged from undetectable to 68 pg/ml in patients with upper UTI. The authors calculated a specificity of 89% and a positive predictive value of 93% for discrimination between UTI and asymptomatic bacteriuria. However, sensitivity (19%) and negative predictive value (12%) were low. sTREM-1 levels did not correlate with plasma PCR levels, plasma or urine leukocyte counts, or bacterial species cultured from urine. Five (26%) immunocompromised patients had detectable sTREM-1 levels, but these levels were not different when compared with those of non-immunocompromised patients ($p = 0.13$). The authors concluded that sTREM-1 urine levels were low to undetectable.[15]

In our study, notably, measurement of sTREM-1 was performed in two similar groups (infected and non-infected). The mean sTREM-1 level was $140.6 \pm$ pg/ml compared with 68 pg/ml reported by Determann et al.[15] The sensitivity in our study was similar to that found by Determann et al. 15 (25% vs 19%). Regarding our results, the probability of the test correctly identifying noninfected subjects was 100%. However, our research has made a very important point. Using FC, we measured TREM-1 levels and MFI in urine, and obtained significant differences ($p < 0.001$).

Patients with and without UTI have the presence of TREM-1 in urine. Urine TREM-1 levels in patients with UTI tend to be higher than those without UTI. This finding might be associated with increased neutrophil and cytokine activity induced by bacteria. TREM-1 has been studied in several types of infections to determine its usefulness in diagnosis and prognosis of UTI. The use of TREM-1 and MFI as biomarkers in UTI should be further evaluated in clinical and experimental studies.

Declaration of Conflicting Interests

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