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# Significance of urinary endotoxin concentration in patients with urinary tract infection

**T. Matsumoto, M. Tanaka, N. Ogata, Y. Mizunoe, K. Takahashi, and J. Kumazawa**

Department of Urology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

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**Summary.** Endotoxin is a component of the outer membrane of gram-negative rods (GNR). Since GNR are responsible for the majority of urinary tract infection (UTI), we measured the concentration of endotoxin in urine using chromogenic endotoxin-specific assay and examined its diagnostic utility in patients with suspected UTI. In all 18 urine samples with an endotoxin concentration exceeding 350 pg/ml and 2 samples with 10–350 pg/ml of endotoxin concentration, GNR were detected at a count of $10^4$ cfu/ml. Negative for endotoxin were 3 samples of culture positive for gram-positive cocci (GPC), 2 samples containing various bacterial contaminants and all 37 samples with no growth on culture. Two urine samples collected 5 h after antibiotic dosage showed negative culture for GNR but a significant concentration of endotoxin. In an in vitro experiment, a residual concentration of antibiotic in urine inhibited bacterial growth, leading to a false-negative culture. These results suggest that chromogenic endotoxin assay is a reliable method for diagnosing UTI caused by GNR and detecting false-negative culture of GNR.

**Key words:** Endotoxin – Urinary tract infection

Endotoxin is an essential component of the outer membrane of gram-negative bacteria. A high concentration of endotoxin is present during infection caused by gram-negative rods (GNR) and is one of the main causes of the accompanying fever and endotoxin shock. To measure endotoxin we have used the conventional limulus test, a coagulating system of *Limulus polyphemus* amebocytes [1]. This method is, however, not specific for endotoxin and cannot quantify its concentration. A new chromogenic endotoxin-specific assay, Endospecy (ES test: Seikagaku Kogyo, Japan), developed by Obayashi et al. [4], is now commercially available. This method uses recombined limulus coagulation enzymes, except for factor G, and a chromogenic assay system which is specific for endotoxin without the inclusion of (1, 3)-β-*D*-glucan, a component of the cell wall of fungi. Furthermore, the method is rapid and quantitative.

Since GNR are responsible for the majority of urinary tract infections (UTI), we measured the concentration of endotoxin in urine and examined its diagnostic usefulness in patients with suspected UTI compared with conventional culture methods.

## Materials and methods

### *Patients and urine samples*

We evaluated 64 patients who were attending the outpatient clinic of the Kyushu University Hospital for the diagnosis and treatment of a UTI. There were 49 males and 15 females 20 to 82 years of age. A clean midstream-voided urine specimen (10–50 ml) was collected from each patient for evaluation by the ES test and conventional culture. Each urine specimen was divided into two samples, one of which was transferred to an aluminum-capped glass test tube, which was heated at 250°C for 2 h and then stored at 4°C before evaluation by the ES test. The other sample was used for conventional bacterial culture using the dilution method or dip-slide technique (Uricult: Orion Diagnostica, Finland). A significant bacterial count was considered to be over $10^5$ colony forming unit (cfu)/ml, and a positive culture with less than $10^3$ cfu/ml was considered to be contaminated.

### *ES test*

The endotoxin-specific chromogenic assay was performed as previously described [4]. In brief, a factor G-free endotoxin assay kit containing a chromogenic substrate, Boc-Leu-Gly-Arg-pNA (ES-Test, Seikagaku Kogyo, Japan) was used. A volume of 0.1 ml of urine sample was added to 0.1 ml of the ES test dissolved in 0.2 mmol TRIS-HCL buffer, pH 8.0. This mixture was incubated at 37°C for 30 min. Absorbance was measured at 545 nm using a double-beam spectrophotometer following diazotrization to avoid interference by yellow pigment.

**Table 1.** Results of ES test and bacteriologic findings in urine samples from 64 patients with urinary tract infections

| Bacteriologic finding | Endotoxin concentration (pg/ml) | | | | Total |
|---|---|---|---|---|---|
| | >350 | 10–350 | <10 | 0 | |
| GNR ($10^4$ cfu/ml) | 18 | 2 | | | 20 |
| GPC ($10^4$ cfu/ml) | | | | 3 | 3 |
| Diverse contaminants ($10^3$-$10^9$ cfu/ml) | | | | 2 | 2 |
| No growth | 2 | | | 37 | 39 |

GNR, Gram-negative rods; GPC, gram-positive cocci

### *In vitro effect of antibiotic on the concentration of endotoxin in urine*

To evaluate the effect of a residual urinary concentration of antibiotic, an in vitro test was performed in which $10^7$ cfu/ml of bacteria was cultured in urine for 15 min with or without a graded concentration of ceftizoxime. The bacterial count and endotoxin concentration were then determined. Clinical isolates of *Escherichia coli* and *Serratia marcescens* were used as the test pathogens. The dilution method and dip-slide technique were used to determine the bacteria count.

### *Statistical method*

This was performed by the $\chi^2$ test according to the urinary endotoxin concentration in UTI patients.

## Results

### *Relationship between endotoxin concentration and bacterial count*

In all 18 urine samples with an endotoxin concentration exceeding 350 pg/ml and 2 samples with 10–350 pg/ml of endotoxin concentration, GNR were detected at a count of $10^4$ cfu/ml. Negative for endotoxin were 3 samples of culture-positive for gram-positive cocci (GPC), 2 samples containing various bacterial contaminants and all 37 samples with no growth on urine culture. However, 2 urine samples negative culture for GNR showed a significant concentration of endotoxin (>350 pg). Over 10 pg/ml of endotoxin concentration showed a significant positive culture of GNR ($P<0.001$) (Table 1). Two samples with significant endotoxin concentration and negative culture were collected from 2 patients with acute uncomplicated cystitis who had received antibiotic treatment for 3 days. Their urine samples were collected 5 h after the final dose of antibiotic (Table 2).

### *Effect of antibiotic concentration on bacterial count and endotoxin concentration*

To determine the effect of antibiotic concentration on the bacterial count and the concentration of endotoxin, an in vitro experiment was conducted using ceftizoxime and two pathogenic strains of *E. coli* and *S. marcescens*. When *E. coli* was cultured with more than 500 μg/ml of ceftizoxime for 15 min, a bacterial count of less than $10^2$ cfu/ml was detected by the dilution method. There was no growth

**Table 2.** Cases showing discrepancies between endotoxin concentration and bacteriuria

| Patient | Diagnosis | Drug | Endotoxin (pg/ml) | Bacteria (cfu/ml) | Time after final administration of drug |
|---|---|---|---|---|---|
| M. H. | AUC* | Loracarbef | ≧350 | Negative | 5 h |
| N. J. | AUC | Loracarbef | ≧350 | Negative | 4 h |

AUC, Acute uncomplicated cystitis

**Table 3.** Endotoxin concentration and bacterial count in vitro experiment with ceftizoxine

| Species | Ceftizoxim concentration (μg/ml) | Bacterial count (cfu/ml) | | Endotoxin concentration (pg/ml) |
|---|---|---|---|---|
| | | Dilution | Uricult | |
| *E. coli* | 0 | $>10^5$ | $10^7$ | 350 |
| | 500 | $6\times10^2$ | 0 | 350 |
| | 1,000 | $2.6\times10^2$ | 0 | 350 |
| | 2,000 | $2\times10^2$ | 0 | 350 |
| *S. marcescens* | 0 | $>10^5$ | $10^5$ | 350 |
| | 500 | $>10^5$ | 0 | 350 |
| | 1,000 | $10^4$ | 0 | 350 |
| | 2,000 | $10^4$ | 0 | 350 |

using the dip-slide method. Endotoxin was, however, detected at same level with and without the antibiotic. When *S. marcescens* was the test species, this tendency became more obvious, suggesting that a residual concentration of antibiotic in urine will inhibit bacterial growth, leading to a false-negative culture. The concentration of endotoxin directly reflected bacterial growth and/or the existence of bacterial products in urine (Table 3).

## Discussion

Several investigators have previously reported the clinical importance of the limulus assay for detecting a gram-negative infection using such samples as serum, cerebrospinal fluid and urine [2, 5, 6]. The limulus assay, however, was not as specific for endotoxin because of the existence of factor G activated by (1, 3)-β-*D*-glucan. Thus, the conventional limulus assay was unable to quantitate endotoxin. Recently, a new chromogenic endotoxin-specific assay was developed that was specific for endotoxin and capable of quantitating it. In this assay factor G is absent from the limulus coagulation factors, so endotoxin can be measured by this chromogenic method.

Nachum and Berzofsky [2] reported the usefulness of limulus assay for detecting endotoxin in gram-negative bacteriuria. Nurminen et al [3] also reported the detection of urinary endotoxin using the chromogenic assay and reported it to be a rapid and reliable method for diagnosing a gram-negative urinary tract infection when bacteriuria exceeded $10^5$ cfu/ml.

In this study we determined the urinary concentration of endotoxin using a highly specific chromogenic assay and evaluated the utility of the ES test in detecting endotoxin concentration and its reliability in diagnosing a gram-negative urinary tract infection. The ES test was able to show the results within 30 min and thus is a rapid diagnostic method. In addition, a false-negative urine culture in a patient being treated with an antimicrobial drug was positive by this method.

In conclusion, the ES test is a rapid and reliable method for diagnosing a urinary tract infection caused by gram-negative rods and detecting false-negative GNR cultures.

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Tetsuro Matsumoto, MD, PhD
Department of Urology
Faculty of Medicine
Kyushu University, 3-1-1, Maidashi
Higashi-ku, Fukuoka
812 Japan