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ORIGINAL ARTICLE

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Urinary endotoxin excretion and urinary tract infection following kidney transplantation

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Abstract Following kidney transplantation, urine endotoxin levels were measured among 44 patients and compared to bacterial cultures. Urine samples were collected either via transurethral catheters or – after removal of the catheter on postoperative day 4 – by midstream void. In a control group of ten healthy volunteers, urine endotoxin levels were measured daily for 10 days. Urinary endotoxin concentration was measured by means of a chromogenically modified *Limulus* amebocyte lysate (LAL) test. The levels among patients with positive bacteriological findings ($n = 21$) were always elevated (> 0.7 EU/ml). Furthermore, there was a marked, statistically significant difference in endotoxin values between samples with bacterial growth and samples with fungal or without any growth ($P < 0.001$). All 21 of the 44 patients with urinary tract infection (UTI) were endotoxin-positive. Seven more patients who received antibiotics had elevated urinary endotoxin levels, but no bacterial growth in the urine culture. No bacterial infection or significant urinary endotoxin was found in the control group. In summary, the detection of urinary endotoxin in samples obtained by either suprapubic/transurethral catheters or midstream void is an early, sensitive, and specific means of diagnosis that can be carried out even during antibiotic treatment.

Keywords Kidney transplantation · Endotoxin · *Limulus* amebocyte lysate test · Urinary tract infection

Abbreviations *LAL* Limulus amebocyte lysate · *UTI* Urinary tract infection

Introduction

Endotoxin is a major component of the membrane of gram-negative bacteria and has clinical relevance during gram-negative sepsis. Endotoxemia is well documented for occurrences after major abdominal surgery, cardiac surgery, and polytrauma, as well as during diagnostic procedures like colonoscopy [1, 2, 3, 9].

Endotoxin is the main pathogenic factor for gram-negative sepsis [3, 4, 8], and urinary tract infection (UTI) is mostly induced by gram-negative rods [5]. Diagnosis of UTI is based mainly on detection of bacteria in the urine of patients with clinical symptoms like fever and dysuria. However, during antibiotic therapy it is less likely that bacteria will grow in urine cultures; therefore, UTI is sometimes not determined. It can be hypothesized that the detection of endotoxin is more sensitive than that of bacterial cultures during antibiotic treatment since endotoxin is also released from dead bacteria [6, 7, 10].

After renal transplantation, patients receive immunosuppression and are usually treated with a urinary catheter for 4 days. A perioperative antibiotic prophylactic is usually administered. Furthermore, patients with catheters and reflux easily develop UTI, which is common after kidney transplantation. Early UTI is a

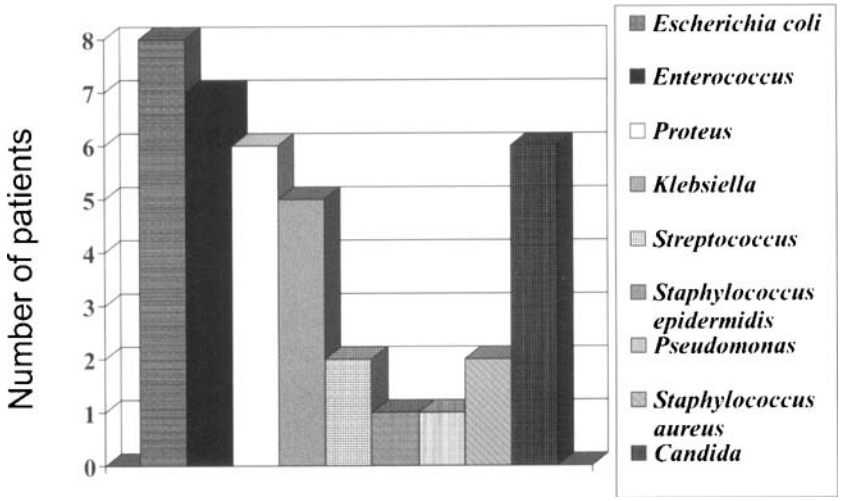

Fig. 1 Spectrum of microbiological agents that caused urinary tract infections within this study (double infections included)

risk factor for the transplanted kidney. However, a clinical study analyzing early postoperative endotoxinuria has not yet been undertaken. The aim of this study was to evaluate the incidence and significance of endotoxinuria after kidney transplantation.

Patients and methods

Following kidney transplantation, urinary endotoxin levels were determined and compared with urine cultures and reagent strips for leukocyte and nitrites for 10 days among 44 patients (20 females, 24 males; mean age 59 ± 12.5 years). Urine samples were collected first via transurethral catheters and, after removal of the catheter on day 4, by midstream void. In a control group of ten healthy volunteers (five females, five males; mean age 22 ± 6 years), samples were obtained by midstream void for 10 days, and urine endotoxin levels, urine sediment, and urine culture were investigated. This study was appoved by the local ethical comittee of the University of Ulm and all parents gave informed consent.

Urinary endotoxin concentration was measured by means of a chromogenically modified *Limulus* amebocyte lysate (LAL) test as described earlier [2]. In short, urine samples were diluted 1:10 with pyrogen-free water. No further inactivation was undertaken. The determination was performed as two-step-endpoint micromethod. The following solutions were used:

- *Solution A*: LAL by Pyroquant (Walldorf, Germany) was dissolved in pyrogen-free water, as recommended by the manufacturer
- *Solution B*: chromogenic substrate by LPS (Sinatal-Oberzell, Germany) dissolved in pyrogen-free water, 10 µmol/l
- *Solution C*: TRIS-HCl buffer, 0.05 mol/l, pH 9.0 (as determined at 24 °C), containing NaCl, 0.2 mol/l
- *Solution D*: 20% acetic acid

The respective diluted urine sample (50 µl) was incubated with 50 µl solution A for 23 min at 37 °C. The reaction was stopped by adding 100 µl solution D. The absorbance of released p-nitro-aniline was read at 405 nm in a spectrophotometer by SLT (Salzburg, Austria). The amount of endotoxin was quantified according to a simultaneously established standard curve in primarily pyrogen-free water using the EC-5 standard of Pyroquant. The endotoxin concentrations detected by the described method without further dilution ranged from 0.02 to 2.0 EU/ml.

Intra-assay variation coefficients of low (0.12 EU/ml) and high (1.35 EU/ml) urine endotoxin concentrations were below 10% as calculated from 25 determinations. Interassay variation coefficients for both samples were below 10% in 25 consecutive determinations.

The patients received triple-drug immunosuppression with cyclosporine, mycophenolate mofetil, and steroids. Perioperative single-shot antibiotic treatment with 1.5 g imipenem was given intravenously. A urine culture was considered to indicate UTI if more than 10^5 bacteria were detected. Statistical evaluation was performed by means of the χ^2-test.

Results

A positive urine culture was found in the case of 21 out of 44 patients (gram-negative or mixed cultures with levels of more than 10^5 bacteria, Fig. 1). Urine endotoxin concentrations among patients with positive bacteriological findings were greater than 0.7 EU/ml. There was a marked, statistically significant difference in endotoxin between samples with bacterial growth and samples with fungal or without any growth ($P < 0.001$). Endotoxin tests were positive in 28 of these samples. Among four patients receiving antibiotic therapy for other medical problems, endotoxinuria was detected despite sterile urine samples. Obviously the incidence of endotoxinuria is not influenced by antibiotics. Three patients were endotoxin-positive and showed pathological urine reagent strip results, but no growth in culture. All six patients with candiduria were negative for endotoxin (Table 1). Furthermore, endotoxin excretion in the urine preceded positive urine culture during the course of UTI (Fig. 2).

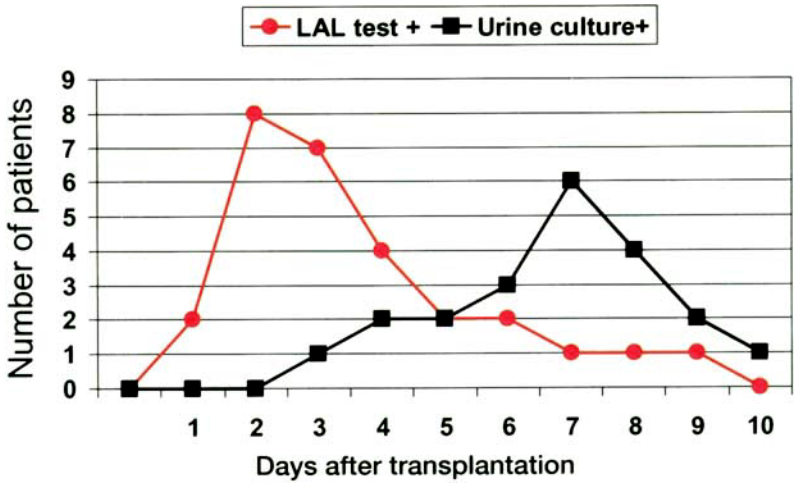

Fig. 2 Time point at which patients showed elevated urine endotoxin levels in the *Limulus* amebocyte lysate (*LAL*) test and positive urine cultures after kidney transplantation. The LAL test indicates urinary tract infections usually a few days earlier than urine cultures

Table 1 Sensitivity and specificity of the *Limulus* amebocyte lysate (*LAL*) test. All patients with positive urine culture had a positive LAL test. Patients with *Candida* in the urine had no false positive LAL test results ($P < 0.001$, χ^2-test)

	Urine culture +	Urine culture –	*Candida* +
LAL test +	21	7	0
LAL test –	0	10	6

In a control group of ten healthy volunteers, endotoxin content in the urine was below detection level (< 0.01 EU/ml). Urinalysis and urine culture showed no bacterial infection or contamination.

Discussion

This study was performed to investigate urinary endotoxin levels by means of the LAL test. The results of the LAL test were compared with bacteriological findings obtained by urinalysis with the help of reagent strips and urine cultures. The diagnosis of UTI is mainly based on the results of urinalysis and urine cultures. In this study, all patients with positive urine cultures had elevated urinary endotoxin concentrations. However, there were also patients with negative urine cultures who nevertheless had high endotoxinuria levels. These patients were treated with antibiotics, and their urinalysis results were pathological but unspecific, i. e., showing a mild leukocyturia with up to 90 cells/µl, which could be explained by the use of transurethral or suprapubic catheters.

In connection with kidney recipients, our data indicate for the first time that the LAL test is a sensitive method for detecting UTI. In 1991, UTI was already examined with the help of the LAL test among 64 patients in a study by Roskansky et al. [11]. Therein, all patients with urine samples contaminated by gram-negative rods had elevated endotoxin levels in the LAL test. The authors showed that antibiotic treatment could inhibit bacterial growth in the urine, but that endotoxin could be detected in the samples. Among patients with neurogenic bladder disorders and urinary bacterial growth, antibiotic treatment even stimulates endotoxin release in the urine [7]. In our study, patients undergoing kidney transplantation received a single-shot antibiotic treatment during surgery. All patients with negative urine cultures but elevated endotoxin levels in the urine had received antibiotic treatment before. The urinalysis of these patients revealed unspecific pathological findings that can be attributed to the use of indwelling catheters. Therefore, the detection of elevated endotoxin levels in the urine after questionable urinalysis can confirm the diagnosis of UTI despite negative urine cultures. Taken together, determination of endotoxin can serve as an early marker in identifying UTI with high sensitivity and specificity.

A technique for detecting fungal UTI was described in 1994 [13]. The conventional LAL test can be activated via two different pathways, by endotoxin and by β-D-glucan, a cell wall constituent of fungi [11, 12, 13]. By comparing in a retrospective fashion the results of two LAL tests, one responding to only the endotoxin-specific pathway, the other to both pathways, the quantification of β-D-glucan should be possible. Our results did not support this notion since all six samples contaminated with fungi were endotoxin-negative. Previous in vitro experiments studying the concentrations of β-D-glucan and endotoxin needed for stimulation of the cascades found that exceedingly high β-D-glucan concentrations are necessary to stimulate the LAL test [13]. Therefore, fungal UTI may not be detected by the LAL test because critical β-D-glucan concentrations are not achieved, at least not with our test system.

In conclusion, among kidney transplant patients the detection of urinary endotoxin in samples obtained by either suprapubic/transurethral catheters or midstream void is an early, sensitive, and specific marker for the identification of UTI in this high-risk group.

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