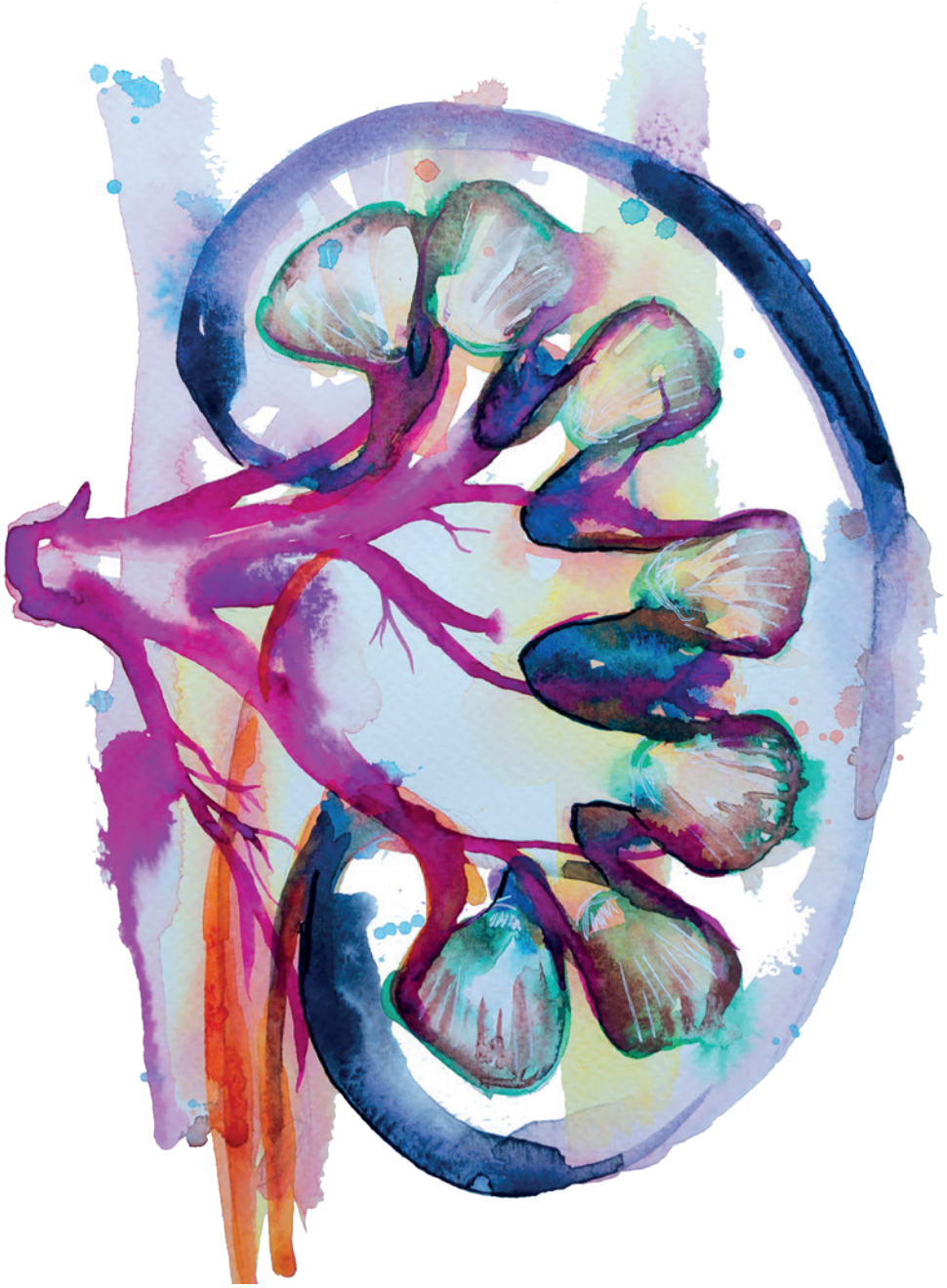
TREATMENT DURATION AND PROGNOSTICS IN FEBRILE URINARY TRACT INFECTION



WILLIZE VAN DER STARRE

TREATMENT DURATION AND PROGNOSTICS IN FEBRILE URINARY TRACT INFECTION

Willize van der Starre

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Treatment duration and prognostics in febrile urinary tract infection

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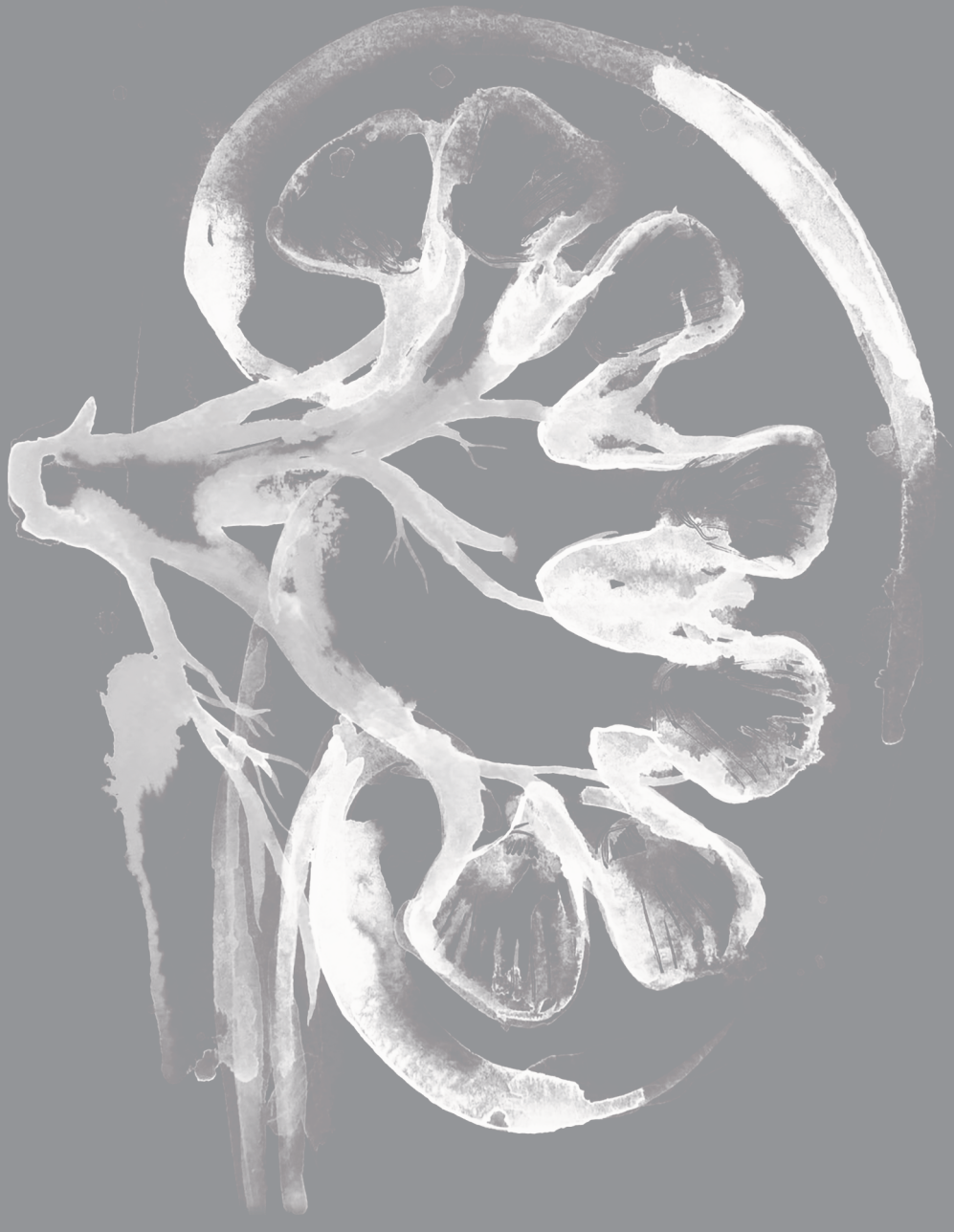
Contents

General introduction		7
Chapter 1	Risk factors for fluoroquinolone-resistant *Escherichia coli* in adults with community-onset febrile urinary tract infection	23
Chapter 2	Treatment duration of febrile urinary tract infections	41
Chapter 3	Ciprofloxacin for 7 days versus 14 days in febrile urinary tract infection: a randomized, double-blind, placebo-controlled non-inferiority trial in men and women	59
Chapter 4	Diabetes and the course of febrile urinary tract infection	79
Chapter 5	Prognostic value of pro-adrenomedullin, procalcitonin and C-reactive protein in predicting outcome of febrile urinary tract infection	95
Chapter 6	Urinary proteins, vitamin D and genetic polymorphisms as risk factors for febrile urinary tract infection and relation with bacteremia: a case control study	111
Chapter 7	Procoagulant tissue factor activity on microparticles is associated with disease severity and bacteremia in febrile urinary tract infections	135
Summary and general discussion		151
Nederlandse samenvatting		171
Dankwoord		181
Curriculum vitae		183
List of publications		185
Lijst van deelnemende centra		187



General introduction

Introduction

Urinary tract infection (UTI) is a common bacterial infection with substantial impact on quality of life and considerable health care expenditures across all ages.[1;2] Approximately half of all women will once experience an UTI during their lifetime. Moreover, elderly and patients with underlying illnesses like diabetes mellitus or urologic abnormalities are at increased risk.[3;4] It is expected that the incidence of UTI will continue to rise, given the increase in life expectancy and prevalence of diabetes mellitus in the next decades.

Classification

In the literature, various classifications of UTIs have been used, based on location of the infection, presence of fever and/or presence of complicating patient factors, which make the terminology and classification rather complex.

Firstly, based on the location, UTIs can be classified into 'lower UTI' and 'upper UTI'. When UTI is limited to the lower urinary tract (urethra and bladder), symptoms may include dysuria, urgency, frequency, hematuria and/or suprapubic pain, reflecting the presence of bladder infection (lower UTI or cystitis). If symptoms of lower UTI are accompanied by fever or chills, flank pain, nausea or vomiting, the upper urinary tract is also involved (upper UTI, kidney infection or pyelonephritis). In men, those symptoms may also reflect infection of the prostate (prostatitis). (Figure 1)

Another way of classifying UTIs is labelling UTI as 'uncomplicated' or 'complicated' by taking into account certain patient characteristics. The term 'uncomplicated' is usually restricted to premenopausal, non-pregnant women with no known anatomical or functional abnormalities of the urinary tract or other comorbidities.[5-7] However, some authors advocate to consider UTIs in women with well controlled diabetes mellitus or postmenopausal women without urological sequelae to be 'uncomplicated' too.[8] All other UTIs are by definition 'complicated', e.g. men and patients with urological abnormalities. To make it a some more complex, international guidelines use a combination of the above mentioned classifications: acute uncomplicated cystitis, acute uncomplicated pyelonephritis, complicated UTI and acute complicated pyelonephritis, and in addition for men several categories of prostatitis are distinguished.[6;8-13]

Given the broad range of classifications of UTIs in the literature, it makes sense to classify UTI patients uniformly according to their clinical presenta-

tion, in which fever is the main determinative clinical factor. Fever in UTI indicates that the infection is not limited to the bladder mucosa, but invasive and spread locally of systemically. Although an exact anatomical distinction can often not be made only on clinical symptoms, febrile UTI thus reflects the presence of a parenchymal inflammation of the urinary tract and the ensuing host response, in which the bladder, prostate, kidney, blood circulation, lymph nodes of the pelvis or a combination of those can be involved. In this thesis, we will use febrile UTI as the clinical syndrome of interest, because this is how patients present, and fever mainly determines the appropriate clinical approach. According to the discussed classifications, febrile UTI includes complicated UTI with fever, acute (un-)complicated pyelonephritis, acute prostatitis and urosepsis.

Pathogenesis

The majority of UTIs is caused by bacteria from the intestine that ascend through the urethra to the bladder and kidneys. *Escherichia coli* is by far the most common uropathogen, causing up to 80% of UTIs, followed by other bacteria such as *Klebsiella* species, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Rarely, viruses and fungi are involved.[15;16]

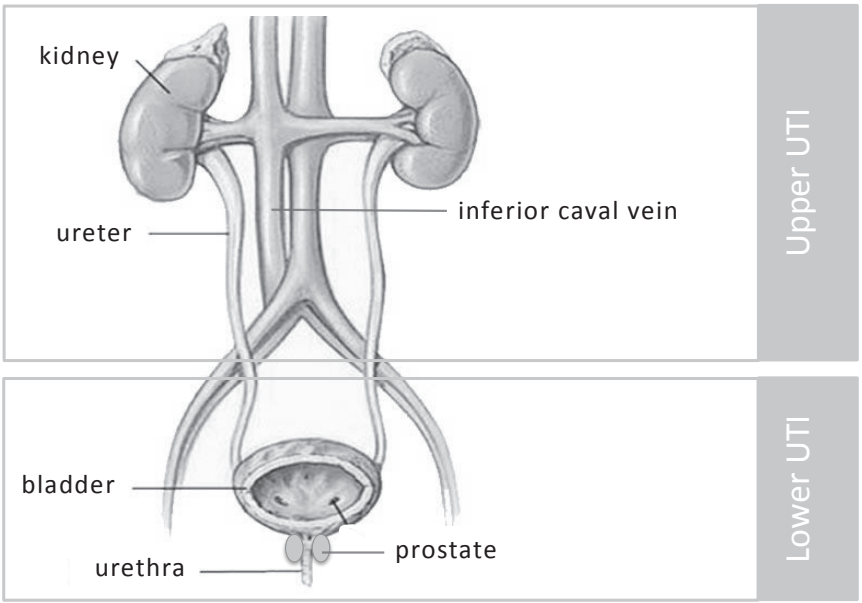


Figure 1. Lower UTI is limited to the urethra and bladder. Uropathogens may translocate to the prostate in men (prostatitis) and/or ascend to the kidney (acute pyelonephritis) and blood stream (urosepsis) causing a febrile upper UTI (adapted from [14]).

Periurethral colonization with a uropathogen usually is one of the critical initial steps in the pathogenesis of UTI. Most uropathogens originate from the rectal microbiota and enter the bladder via the urethra.[17] The urinary tract is normally well protected against microbial invasion by a variety of host defense mechanisms. The main defense mechanisms of the host against colonization of the bladder are dilution and micturition. However, several behavioural and environmental risk factors can facilitate the colonization and influence the susceptibility of the host. Anatomical or functional abnormalities that prevent complete emptying of the bladder, like urethral stenosis, benign prostate hypertrophia may increase the chance of colonization with uropathogens. Behavioural risk factors associated include voiding dysfunction, bladder catheterization and sexual intercourse.[18-20] After colonization, the next step in pathogenesis is the adhesion of uropathogens to the epithelial bladder and kidney cells and invasion into the tissue. After adherence, uropathogens are protected against wash-out by urine flow. Whether subsequent UTI occurs is the result of a dynamic interaction between the host and uropathogen.

Uropathogens have proven themselves to be a formidable opponent to the human host defense, capable of survival and rapid adaption in the relatively hostile environment of the urinary tract due to virulence factors. Virulence factors enable bacteria to overcome local host defences, enter and multiply within the urinary tract and invade the uroepithelium. Examples of such virulence factors, which have been studied in particular in *Escherichia coli* strains, include adhesins like fimbriae, siderophores, polysaccharide coatings, proteases and toxins.[21;22] Fimbriae, hair-like organelles, play a major role in the adhesion of *Escherichia coli* to the uroepithelial cells. The most important are type-1 fimbriae and P-fimbriae. Type-1 fimbriae cause adhesion to epithelial cells of the vagina, urethra and bladder, whereas P-fimbriae (type-2 fimbriae) adhere to kidney cells. In more than 70% of all *E. coli* strains of patients with acute pyelonephritis, such P-fimbriae are found.[15]

Despite the discussed first line of host defense, uropathogens may thus sometimes persist in the urinary tract due to effective virulence characteristics. Then, antimicrobial properties of the uroepithelium and the innate immune system of the urinary tract become critical to the host response to uropathogens. The uroepithelium produces several antimicrobial peptides, amongst others cathelicidin LL37, β-defensins and uromodulin, as part of the innate immune response.[23;24] Such antimicrobial peptides possess a direct

toxic activity. In addition, defensins e.g. can enhance innate responses by causing mast cell degranulation and by promoting neutrophil chemotaxis. Uromodulin adheres to fimbriae of uropathogenic *E. coli*, thus preventing its attachment to the epithelium. Uromodulin also has an immunomodulatory function.[25] If the antimicrobial peptides fail to completely kill and wash-out the invading pathogens, the innate immune response is initiated in order to eliminate the invading microbes. Toll-like receptors (TLR) are key initial molecules in the pathogenic cascade of UTI.[26] Bacterial recognition leads to cytokine (like IL-6, IL-8) and chemokine responses and recruitment of inflammatory cells.
The role of adaptive immunity in UTI remains controversial, but does not seem to play a primary role in the protection against bacteria, as is also reflected in the frequent recurrent UTIs observable especially in young women.[25;27]

Both antimicrobial properties of the urinary tract and the innate immune response to the presence of bacteria in the kidney may be influenced by genetic predisposition, as has been shown recently.[28-32]

Treatment

In the last decade, treatment of UTI has become more and more complicated by rising antimicrobial resistance of Enterobacteriaceae, the most common uropathogens of UTI. In the Netherlands, fluoroquinolones, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole are yet the preferred agents for oral treatment of febrile UTI. Fluoroquinolones are recommended as first choice empirical agent, particularly because there is a relatively low rate of antimicrobial resistance.[5;33-35] However, the extensive use of antimicrobials in veterinary and human health care practice has resulted in the emergence and dissemination of resistant pathogens in the community, endangering the efficacy of (oral) treatment options.[36;37] In the Netherlands, a country known for its restrictive use of antibiotics and overall low rates of antimicrobial resistance, *Escherichia coli* resistance to fluoroquinolones increased from 3% in 2001 to 10-17% in 2014 with even higher rates at urology services.[38;39] In order to select the most appropriate empirical antimicrobial oral therapy, it is very important to know which patients are at particularly risk for e.g. fluoroquinolone-resistant uropathogens. However, data on host-related and/or environmental risk factors for fluoroquinolone resistance in community-acquired febrile UTI are limited.

With a lack of new antimicrobial classes in development,[40] it is increasingly important to develop strategies to maintain and even increase effectiveness of available antimicrobials. Optimization of treatment duration represents one such important strategy, because the development and spread of antimicrobial resistance is closely related to the total amount of antimicrobials used in countries.[41] The duration of antimicrobial therapy exerts differential selecting pressure on gut flora which leads to selection of resistant strains and reduction of resident commensal bacteria paving the road for e.g. *Clostridium difficile* infection.[42] Moreover, the potential adverse effects of unnecessary extended treatment periods reach beyond the individual treated: the longer antimicrobials are taken and excreted into the environment, the more pressure is exerted on the ecological balance of bacteria outside the human gut.[43]

Although febrile UTI is a relatively common and potentially severe infection, yet the optimal duration of treatment has not been established with the exception of uncomplicated cystitis and acute pyelonephritis in otherwise healthy women. Young women without comorbidities can be treated for febrile UTI with a 1-week regimen of fluoroquinolones provided a low level of fluoroquinolone resistance,[44] or, if proven susceptible, with a 2-week course of trimethoprim-sulfamethoxazole.[35;45-47] In contrast, there is less conformity on therapeutic approaches for other patient categories, as most randomized trials have excluded male patients, the elderly, and those with urinary tract abnormalities or underlying systemic illnesses.
Because of a lack of randomized treatment duration trials, current guidelines usually recommend antimicrobials for 7-14 days, with longer durations in special circumstances such as chronic bacterial prostatitis.[5;48-50]

It can be concluded that, despite the importance of minimal yet optimally efficacious duration of treatment, the evidence on optimal treatment duration of febrile UTI is scarce. We therefore initiated the FUTIRST- (*Febrile Urinary Tract Infection Randomized Short Treatment*) trial, a randomized, placebo-controlled double-blind non-inferiority study including consecutive adults presenting with a community-acquired febrile UTI. Patients were recruited at 35 primary care centers and 8 affiliating regional emergency departments in the Leiden-The Hague region (Figure 2). They were randomly assigned to receive ciprofloxacin 500 mg orally twice daily for 7 days or for 14 days. The aim of this study was to determine whether the efficacy and safety of a 7-day antimicrobial regimen (short treatment) is similar to a 14-day regimen

(standard treatment) in a unselected population with community-acquired febrile UTI.[51]

Apart from that, we performed a prospective observational multicentre cohort study. The aim of this study is to clarify the clinical relevance of urine and blood biomarkers, as well as genetic markers, for the prediction of severity and course of febrile UTI. Although several markers of febrile UTI (e.g. MR-proADM) have been identified, little is known of clinical relevance and specificity of biomarkers for disease progression and prognosis of the patient. The benefit of addition of urine and/or blood (bedside) biomarkers to readily-available clinical information will be evaluated with respect to patient's prognosis and management. Moreover, although selective genetic control of innate immunity (e.g. IL-6, IL-8) is presently being unravelled, little is known of the clinical relevance of such combined underlying genetic mechanisms.
To obtain these data, the participating patients were thoroughly followed clinically, and microbiological data and blood and urine samples were collected at different time points (Table 1).

Patients were recruited both in primary care and at regional emergency departments (Figure 2), reflecting a real-life, full spectrum of invasive UTI. The prognostic value of a certain biomarker measured in a febrile UTI patient at e.g. the emergency department is not one-to-one applicable to a febrile UTI patient presenting at a primary care center. The inclusion of both patients with a relatively mild illness as well as those with a life-threatening condition would allow us to investigate the prognostic value of biomarkers in various subgroups separately.

Table 1. Flowchart of data collection in febrile UTI patients (adapted from [51])

	Days after enrollment			
Assessment	**0**	**3-4**	**24-32**	**84-92**
Enrollment	x			
Demography	x			
Clinical data	x	x	x	x
Urine culture	x		x	
Blood culture	x			
Plasma sample	x	x	x	
Urine sample	x	x	x	
DNA sample	x			
Adverse events	x	x	x	x
Survival		x	x	x
Contact - in person	x	x	x	
Contact - by phone				x

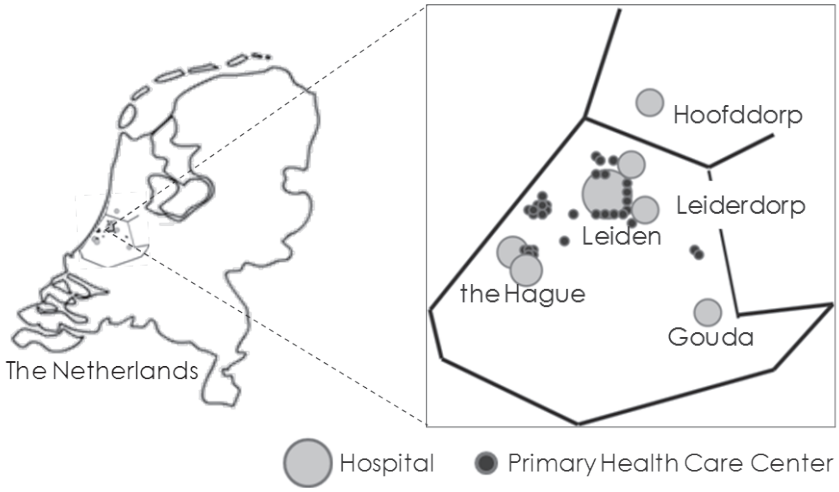


Figure 2. Participating study sites (adapted from [51])

Outline of the thesis

The aim of this thesis is to clarify the clinical relevance of urine and blood biomarkers, as well as genetic markers, for the prediction of severity and course of febrile UTI. Furthermore, this thesis focuses on the optimal duration of antimicrobial treatment.

In **Chapter 1**, we assess potential risk factors for resistance to fluoroquinolones in patients with febrile *Escherichia coli* UTI. Both host-related risk factors and environmental risk factors like contact with health care workers and animals were included.[52]
The review in **Chapter 2** highlights the main evidence from randomized trials on the optimal treatment duration of community-acquired febrile UTI.[53]
Chapter 3 describes the FUTIRST-trial, a randomized, placebo-controlled non-inferiority multicentre trial on treatment duration of febrile UTI.[51] In this trial, short treatment duration (7 days) is compared to standard (14 days) duration of oral ciprofloxacin with respect to clinical and microbiological cure both in primary care and in admitted patients.
The cohort study in **Chapter 4** evaluates the effect of pre-existing diabetes on presentation and microbiological and clinical outcome of febrile UTI. The effect of diabetes and concurrent illnesses is assessed separately.[54] Do diabetic patients with febrile UTI indeed have a complicated course or not?
Although several markers of febrile UTI (e.g., procalcitonin) have been identified, little is known of clinical relevance and specificity of biomarkers for disease progression and prognosis of the patient. **Chapter 5** evaluates the prognostic value of the plasma biomarker midregional pro-adrenomedullin (MR-proADM) in predicting bacteraemia, need for hospital admission and a complicated course of the infection. Moreover, this study compares the prognostic value of MR-proADM with more conventional biomarkers like C-reactive protein (CRP), leucocyte count and procalcitonin (PCT).[55]
Chapter 6 focuses on the host defense of febrile UTI, especially on its relation with the occurrence of bacteraemia. The study investigates the urinary production of cytokines (IL-6, IL-8) and antimicrobial peptides (cathelicidin LL37, β-defensin 2 and uromodulin) in febrile UTI. Moreover, the influence of plasma vitamin D level and genetic variations in host defense on the production of these urinary proteins and the clinical course is assessed.[56]
Sepsis is associated with activation of the coagulation cascade. Microparticles expressing procoagulant tissue factor (MP-TF) are released in blood concurrently with markers of inflammation and coagulation, and could play a

major role in the onset of sepsis-related morbidity. In **Chapter 7**, we evaluate whether the release of MP-TF into blood is accompanied by inflammatory and procoagulant changes in febrile *E. coli* UTI.[57]

Finally, in the general discussion, the major findings of all studies are summarized and remaining questions and the implications for future research are discussed.

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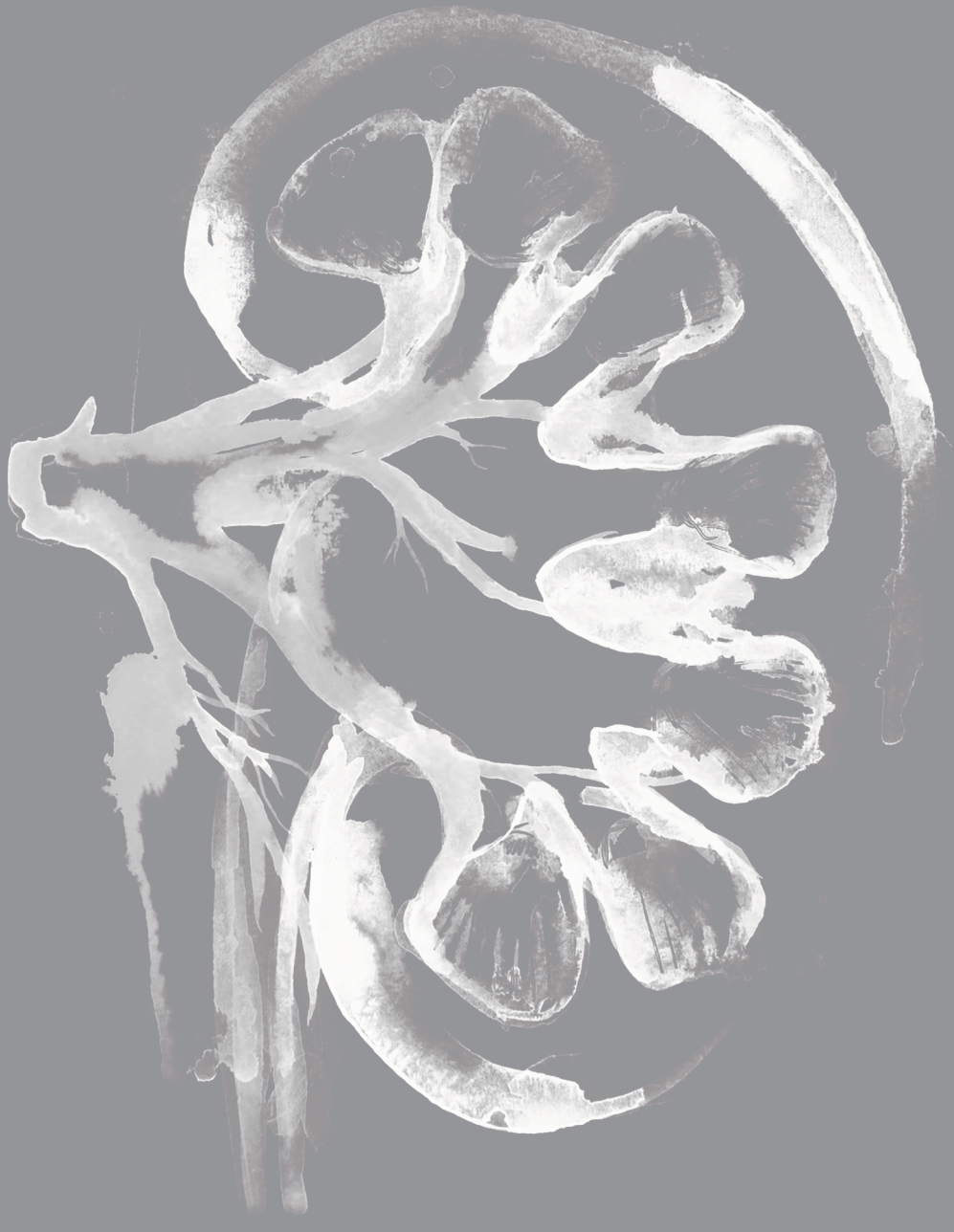

Chapter 1

Risk factors for fluoroquinolone-resistant *Escherichia coli* in adults with community-onset febrile urinary tract infection

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Abstract

Objectives

To assess risk factors for fluoroquinolone resistance in community-onset febrile *Escherichia coli* urinary tract infection (UTI).

Methods

A nested case-control study within a cohort of consecutive adults with febrile UTI presenting at primary health care centres or emergency departments during January 2004 through December 2009. Resistance was defined using EUCAST criteria (MIC ciprofloxacin > 1.0 mg/L). Cases were subjects with fluoroquinolone-resistant *E. coli,* and controls those with fluoroquinolone-susceptible isolates. Multivariable logistic regression analysis was used to identify potential risk factors for fluoroquinolone resistance.

Results

Of 787 consecutive patients, 420 had *E. coli* positive urine cultures. Of these, 51 (12%) were fluoroquinolone resistant. Independent risk factors for fluoroquinolone resistance were urinary catheter (OR 3.1; 95% CI: 0.9-11.6), recent hospitalization (OR 2.0; 95% CI: 1.0-4.3) and fluoroquinolone use in the past six months (OR 17.5; 95% CI: 6.0-50.7). Environmental factors (e.g. contact with animals or hospitalized household members) were not associated with fluoroquinolone resistance. Of fluoroquinolone-resistant strains, 33% were resistant to amoxicillin/clavulanate and 65% to trimethoprim/sulfamethoxazole; 14% were ESBL-positive compared to <1% in fluoroquinolone-susceptible isolates.

Conclusions

Recent hospitalization, urinary catheter and fluoroquinolone use in the past six months were independent risk factors for fluoroquinolone resistance in community-onset febrile *E. coli* UTI. Contact with animals or hospitalized household members was not associated with fluoroquinolone resistance. Fluoroquinolone resistance may be a marker of broader resistance, including ESBL-positivity.

Introduction

Fluoroquinolones and trimethoprim/sulfamethoxazole are the preferred agents for oral treatment of febrile UTI. Fluoroquinolones are recommended to be the first choice, particularly because there is a relative low rate of antimicrobial resistance[1-4]. However, the emergence of fluoroquinolone-resistant *Escherichia coli* in the community may limit oral treatment options[5]. Reported rates of *E. coli* resistance to ciprofloxacin in UTI vary widely over the years and between countries, ranging from <1% to 38%[6,7]. In The Netherlands, a country known for its restrictive usage of antimicrobials and overall low rates of antimicrobial resistance, *E. coli* resistance to ciprofloxacin increased from 3% in 2001 to 11% in 2008 with even higher rates in patients at urology services[8,9]. Moreover, fluoroquinolone resistance in *E. coli* isolates is frequently associated with resistance to other classes of antibiotics[10]. Therefore, there is a need for knowledge of risk factors for fluoroquinolone-resistant *E. coli* in patients presenting with febrile UTI in order to better select the most appropriate empirical antimicrobial oral treatment.

Previous studies on fluoroquinolone-resistant *E. coli* primarily have focused on host-related risk factors such as older age, prior fluoroquinolone usage, urinary tract disorders and hospitalization[7,11-16]. Others have studied the emergence of *E. coli* resistance in the environment and found household members, pets and livestock colonized with resistant *E. coli* strains to be possible sources of human infection[17-20]. To our knowledge, these potential environmental risk factors for fluoroquinolone resistance have not been assessed in a general population presenting with community-onset febrile UTI or acute pyelonephritis.

We therefore conducted a multicentre nested case-control study to identify host-related and environmental risk factors for fluoroquinolone resistance in adults presenting with community-onset febrile UTI. In addition, the relation with extended spectrum beta-lactamase (ESBL)-positivity.

Patients and methods

We conducted a nested case-control study from a prospective multicentre cohort study. Participating centres were 35 primary health care centres and emergency departments of 7 hospitals, all clustered into one single area of the Netherlands. From January 2004 till December 2009, consecutive patients who presented with febrile UTI were considered for enrolment in the

study. The local ethics committees approved the study and all participants provided written informed consent.
Inclusion criteria were age of 18 years or above, fever (≥ 38.0 °C) and/or a history of fever and chills within 24 h before presentation, at least one symptom of UTI (dysuria, frequency, urgency, perineal pain, flank pain or costovertebral tenderness) and a positive nitrite dipstick test or leukocyturia as defined by a positive leukocyte esterase dipstick test or the presence of more than 5 leukocytes per high-power field (pyuria) in a centrifuged sediment. Exclusion criteria were current treatment for urolithiasis or hydronephrosis, pregnancy, hemo- or peritoneal dialysis, a history of kidney transplantation or known presence of polycystic kidney disease. Patients were only included once in the study.
Cases were eligible patients with urine culture-confirmed febrile UTI caused by fluoroquinolone-resistant *E. coli*. Patients with febrile UTI due to fluoroquinolone-susceptible *E. coli* served as controls.

Procedures

Demographic, clinical and microbiological data were collected within 24-48 h upon notification. This was done by qualified research nurses or the clinical investigators (CvN, WEvdS) by reviewing the medical record completed with an interview by telephone or in person using a standardized questionnaire including host-related variables. All patients were empirically treated with antibiotics according to local policy (oral ciprofloxacin 500 mg twice daily for outpatients and for inpatients cefuroxime ± gentamicin intravenously). Based on the culture results, hospitalized patients were subsequently switched to oral antibiotic treatment (first choice ciprofloxacin 500 mg twice daily).
As data on environmental exposures were initially not collected, we contacted patients for a second time in March 2010. All cases were selected for additional interview and for each case, two controls were selected matched by centre and date of inclusion. A standardized questionnaire was used containing the following dichotomous items present within 3 months before initial inclusion: household member with UTI, recent hospitalization, working in health care facility, ownership and/or contact with pets or livestock and receipt of home health care support. The interviewer was blinded to the antimicrobial susceptibility outcome of the isolated *E. coli* strains.

Definitions

Recurrent UTI was defined as two or more episodes in the last six months or three or more episodes of UTI in the last year. A urinary tract disorder

was defined as the presence of any functional or anatomical abnormality of the urinary tract excluding the presence of a urinary catheter or history of nephrolithiasis. These two latter variables were analyzed separately. Data regarding recurrent UTI and antibiotic use in the past 6 months were missing in 5 and 13 patients respectively. Missing values of these categorical variables were considered to indicate the absence of that characteristic.

Microbiological analysis

Clean midstream-catch urine cultures were obtained before starting antimicrobial therapy and were analyzed using local standard microbiological methods. In case of a urinary catheter the urine sample was collected from the port of the catheter. A positive urine culture was defined as bacterial growth over 10^3 cfu/mL urine or a bacterial monoculture over 10^2 cfu/mL urine in the presence of pyuria[21]. Urine cultures revealing growth of 2 or more different bacterial species reflecting mixed skin or gut flora, were considered to indicate contamination[21]. Susceptibility tests were done from the selective media using the Vitek2 system (bioMerieux). MIC breakpoints for resistance were based on EUCAST criteria (www.eucast.org). *E. coli* isolates with ciprofloxacin MIC values > 1 mg/L were considered to be fluoroquinolone-resistant. In 16 *E. coli* isolates ciprofloxacin susceptibility was not specifically tested. Fifteen of these were norfloxacin susceptible and thus considered fluoroquinolone susceptible; one was resistant to norfloxacin and considered fluoroquinolone resistant.
ESBL production was phenotypically detected by double-disk diffusion test using ceftazidime/ceftazidime clavulanate and cefotaxime/cefotaxime clavulanate or by E-test.

Statistical analysis

Descriptive analysis included means or percentages with 95% confidence intervals (CIs) or medians and ranges, as appropriate. Univariate analysis was performed using the Mann-Whitney *U*-test for continuous variables and Chi-square tests for categorical variables. All variables associated with ciprofloxacin resistance in univariate analysis with $p < 0.2$ were included in a multiple logistic regression model using backward selection method with conditional tests. Interactions between paired variables were tested. A two-tailed P-value <0.05 was considered to indicate statistical significance. All analysis were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA).

Results

During the study period, 787 patients with febrile UTI were enrolled. *E. coli* was the most frequent causal uropathogen, present in 420 (53%) of the patients. Additional causative organisms were *Klebsiella* spp (4.1%) and *Enterococcus faecalis* (1.6%) and others (Figure 1). In 199 (25%) patients, the urine culture showed either no significant bacteriuria or mixed flora; 52% of them could be explained by antibiotic pretreatment.

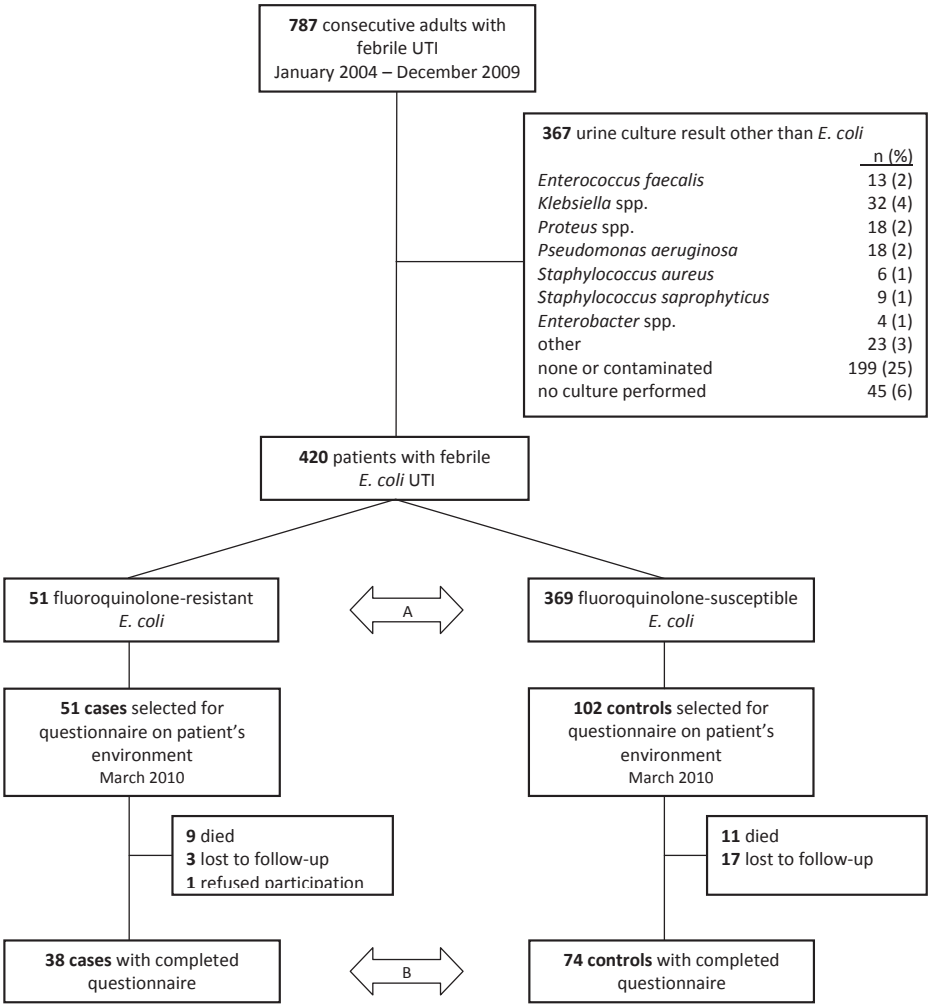

Figure 1. Flowchart of participants in the study
A: analysis of host-related risk factors for fluoroquinolone resistance,
B: analysis of environmental risk factors for fluoroquinolone resistance.

Of 420 patients with *E. coli* positive urine cultures, 51 (12%) had a culture with a fluoroquinolone-resistant isolate (designated as cases) and 369 with a fluoroquinolone-susceptible isolate (designated as controls). The median age was 66 years [IQR: 45-78], 137 (33%) were men and 224 (53%) had co-morbidity. Baseline characteristics of the study population are summarized in Table 1.

Out of the 369 controls, 102 were matched by centre and date of inclusion to the 51 cases for additional interview on environmental issues, but otherwise selected randomly. These 102 selected controls were comparable to the remaining 267 controls with respect to gender, age and comorbidity, except for diabetes mellitus that was more frequent in the selected controls (19% versus 11%, $p = 0.047$). During follow-up till March 2010, 9 cases and 11 controls died. Of the remaining 42 cases and 93 controls, 38 cases (response rate 90%) and 74 controls (response rate 80%) participated (Figure 1).

Risk factors for fluoroquinolone-resistant *E. coli*

Univariate and multivariate potential risk factors for fluoroquinolone-resistant *E. coli* are listed in Table 1. Significant univariable host-related risk factors were the presence of a urinary catheter (OR 6.0; 95% CI: 2.0-18.1), underlying urinary tract disorder (OR 2.3; 95% CI: 1.2-4.4), recurrent UTI (OR 2.2; 95% CI: 1.2-4.1), hospitalization past six months (OR 2.3; 95% CI 1.2-4.4) and fluoroquinolone usage past six months (OR 18.6; 95% CI: 6.6-52.4). None of the environmental characteristics were significantly associated with fluoroquinolone resistance, with ORs all around 1.

Independent risk factors for fluoroquinolone-resistant *E. coli* in the multivariate analysis were the presence of an urinary catheter (OR 3.1; 95% CI: 0.9-11.6), recent hospitalization (OR 2.0; 95% CI: 1.0-4.3) and fluoroquinolone use in the past six months (OR 17.5; 95% CI: 6.0-50.7). Potential interactions between variables (e.g. urinary tract disorder and presence of a urinary catheter), were additionally tested, but they did not significantly change the model. In total, 90 (21%) of the patients had at least one of those three risk factors accompanied with a 26.7% risk to have fluoroquinolone-resistant *E. coli* compared to 330 patients with no risk factor who had a 8.2% risk to have fluoroquinolone-resistant *E. coli*.

Microbiological outcome

Among 420 *E. coli* isolates tested, 12% were resistant to ciprofloxacin; 51% to amoxicillin; 11% to amoxicillin/clavulanate; 30% to trimethoprim/sulfamethoxazole; 5% to cefuroxime and 6% to gentamicin. Fluoroquinolone-

Table 1. Baseline characteristics of 420 patients presenting with febrile UTI due to *E. coli*

Patient characteristics	All n = 420	Cases n = 51 (12%)	Controls n = 369	Univariate OR (95% CI)	p	Multivariate[a] OR (95% CI)
Age, years, median [IQR]	66 [45 – 78]	71 [54 - 80]	66 [44 – 78]		0.115	
≥ 65 years	216 (51)	30 (59)	186 (50)	1.41 [0.78-2.54]	0.260	
Male sex	137 (33)	18 (35)	119 (32)	1.15 [0.62-2.12]	0.664	
Co-morbidity						
Any	224 (53)	33 (65)	191 (52)	1.71 [0.93-3.14]	0.082	
Urinary catheter	14 (3)	6 (12)	8 (2)	6.02 [2.00-18.1]	< 0.001	3.14 [0.85-11.60]
Urinary tract disorder[b]	83 (20)	17 (33)	66 (18)	2.30 [1.21-4.35]	0.009	
History of nephrolithiasis	38 (9)	5 (10)	33 (9)	1.11 [0.41-2.98]	0.841	
Diabetes mellitus	59 (14)	11 (22)	48 (13)	1.84 [0.88-3.83]	0.099	
Malignancy	34 (8)	6 (12)	28 (8)	1.62 [0.64-4.14]	0.305	
Cerebrovascular disease	57 (14)	7 (14)	50 (14)	1.02 [0.43-2.38]	0.973	
COPD	52 (12)	7 (14)	45 (12)	1.15 [0.49-2.70]	0.756	
Immunocompromised state	44 (11)	4 (8)	40 (11)	0.70 [0.24-2.05]	0.512	
Recurrent UTI[c]	109 (26)	21 (41)	88 (24)	2.24 [1.22-4.10]	0.008	
Hospitalization in past 6 months	72 (17)	15 (29)	57 (15)	2.28 [1.17-4.44]	0.013	2.03 [0.96-4.31]
Residence in nursing home	16 (4)	4 (8)	12 (3)	2.53 [0.78-8.17]	0.108	
Antibiotic treatment in past 6 months	140/407 (34)	23/49 (47)	117/358 (33)	1.82 [1.00-3.33]	0.049	
Fluoroquinolones	18 (4)	12 (24)	6 (2)	18.6 [6.62-52.4]	< 0.001	17.5 [6.0-50.7]
β-lactams	30 (7)	4 (8)	26 (7)	1.12 [0.38-3.36]	0.836	
Trimethoprim/sulfonamide	14 (3)	2 (4)	12 (3)	1.21 [0.26-5.59]	0.803	
Nitrofurantoin	16 (4)	2 (4)	14 (4)	1.04 [0.23-4.70]	0.964	
Patient environment characteristics[d]	n = 112	n = 38	n = 74			
Household member with UTI	3 (3)	0	3 (4)	-	0.214	
Daily contact with pets[e]	28 (25)	10 (26)	18(24)	1.12 [0.45-2.72]	0.818	
Daily contact with livestock	1(1)	1(3)	0 (0)	-	0.161	
Household healthcare employee	9 (8)	3 (8)	6 (8)	0.97 [0.23-4.12]	0.969	
Home care medical support	19 (17)	7 (18)	12 (16)	1.17 [0.42-3.26]	0.768	

Data are presented as n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease

[a] multivariate OR, adjusted for sex, obtained by backward regression analysis using conditional tests and selecting all variables with $p < 0.2$ in univariate analysis as independent covariates. [b] defined as the presence of any functional or anatomical abnormality of the urinary tract except urinary catheter and history of nephrolithiasis. [c] defined as three or more UTIs in the past 12 months or two or more UTIs in the past 6 months. [d] environmental characteristics evaluated in 112 patients completing questionnaire, see Figure 1. [e] dogs and/or cats.

resistant *E. coli* strains were frequently resistant to other antibiotic classes used for treatment of febrile UTI: 33% to amoxicillin/clavulanate and 65% to trimethoprim/sulfamethoxazole. The distribution of cross-resistance of the oral antibiotics used for febrile UTI is illustrated in Figure 2. The prevalence of ESBL-producing *E. coli* was low (2%) but differed significantly between cases and controls: 7 (14%) versus 1 (<1%) respectively ($p < 0.001$). Of the 8 patients with ESBL-positive *E. coli,* 6 fulfilled the questionnaire; none of them had contact with animals. There were no statistically significant differences in the frequency of fluoroquinolone-resistant *E. coli* in between the years 2004 through 2009 and there was no trend towards a gradual increase (data not shown).

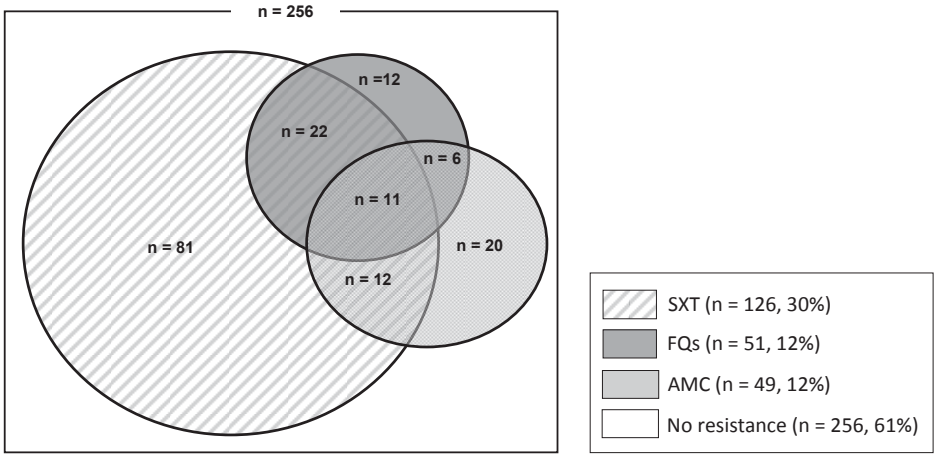

Figure 2. Distribution of resistance to oral antibiotics in 420 patients with febrile *E. coli* UTI. SXT: trimethoprim/sulfamethoxazole, FQs: fluoroquinolones, AMC: amoxicillin/clavulanate.

Clinical outcome

Among the 51 patients with fluoroquinolone-resistant *E. coli* febrile UTI, 16 (31%) were empirically treated with an inappropriate antibiotic, including 10 patients who were treated with ciprofloxacin (Table 2). Median fever duration in patients receiving ciprofloxacin was 2 days [IQR 1-4]; 70% of those switched to another antibiotic after a median of 6 days [IQR 2-7]. Patients treated with cefuroxime plus gentamicin had slightly longer fever duration (median 3 days [IQR 2-4]) and switched in 71% to another antibiotic after a median of 6.5 days [IQR 5.3-8.0] (Table 2).

Table 2. Empiric antimicrobial treatment and outcome of 51 patients with febrile UTI due to fluoroquinolone-resistant *E. coli*

Treatment			Outcome		
Empiric antibiotic(s)	**n**	**Inappropriate[a] n (%)**	**Fever duration**	**No. of patients switched to other antibiotic (%)**	**Days until antibiotic switch**
Ciprofloxacin	10	10 (100)	2.0 [1.0-4.0]	7 (70)	6.0 [2.0-7.0]
Cefuroxime	19	3 (16)	2.0 [1.0-4.0]	17 (90)	5.0 [4.0-6.0]
Cefuroxime + gentamicin	14	1 (7)	3.0 [2.0-4.0]	10 (71)	6.5 [5.3-8.0]
Amoxicillin/clavulanate	5	2 (40)	2.5 [1.3-3.8]	3 (60)	3.0 [3.0-3.5]
Other[b]	3	NA	NA	NA	NA

NA: not applicable

Data are presented as median [IQR] unless otherwise stated

[a] Inappropriate empirical antibiotic treatment defined as *E. coli* resistant to the antibiotic given.

[b] Trimethoprim/sulfamethoxazole (n=1), ceftazidime (n=1), meropenem (n=1)

Discussion

In this study, we evaluated host-related and environmental risk factors for fluoroquinolone resistance in adults with community-onset febrile *E. coli* UTI. We identified recent hospitalization, the presence of a urinary catheter and fluoroquinolone usage in the past six months as independent host-related risk factors for resistance. Environmental dynamics, like contact with pets, livestock or hospitalized household members, were not identified as risk factors. To our knowledge, this is the first prospective study evaluating a combination of those risk factors for fluoroquinolone-resistant *E. coli* among adults with community-onset febrile UTI or acute pyelonephritis. These data suggest that development of fluoroquinolone resistance in a general population at risk for febrile UTI is driven by individual fluoroquinolone usage rather than by within-household or animal-human transmission of resistant *E. coli.* However, this study does not exclude the suggested possibility of an animal origin of fluoroquinolone resistance via foodborne transmission[22,23].

The strengths of this study are its prospective design and the broad population of interest, reflecting daily practice of patients presenting with febrile UTI or acute pyelonephritis, as both primary health care centres and emergency departments participated.

There are however also some limitations. Our study had a relative small sample size of cases with fluoroquinolone resistance. However, to our knowledge this study is the largest prospective study on patients with fluoroquinolone-resistant *E. coli* febrile UTI so far, as most previous studies were retrospective chart reviews of microbiological laboratory databases[7,11-16]. Such studies

may overestimate the prevalence of resistance among uropathogens from patients with community-onset UTIs. One study at US emergency departments had a similar prospective design including 1271 patients with acute pyelonephritis of which 689 were caused by *E. coli* [4]. Yet, the prevalence of fluroquinolone-resistant *E. coli* in this study was 3-5% and too low to evaluate risk factors for fluoroquinolone resistance. In our study the prevalence of fluoroquinolone resistance in *E. coli* was remarkably higher (12%) but consistent with a recent survey in The Netherlands [8].

We used a MIC breakpoint for ciprofloxacin resistance of > 1 mg/L according to EUCAST criteria. As to date different laboratories over the world use different clinical MIC breakpoints for resistance, it is of interest that we found no differences in outcome of the patients with fluoroquinolone-resistant *E. coli* who were empirically treated with ciprofloxacin compared to those treated with appropriate antibiotics (Table 2). Moreover, the majority of patients recovered on ciprofloxacin as their fever resolved before the outcome of the urine culture became available and antibiotic treatment was subsequently switched. This may indicate that febrile UTI is to some extent a self-limiting disease or possibly ciprofloxacin treatment may be still effective in ranges of MICs > 1 mg/L. We could not explore this hypothesis further as we do not have results of the actual MICs of the fluoroquinolone-resistant isolates.
Several studies also found recent hospitalization[14,15], urinary catheter[11,13] and fluoroquinolone usage[7,11-16] to be related with fluoroquinolone resistance. In addition, other risk factors were discovered like previous invasive procedures[14], recurrent UTI[12,14,15], older age[7,11], presence of complicated UTI[7], underlying chronic disease[15,16] and urinary tract abnormalities[11,15]. All these risk factors for fluoroquinolone resistance seem biologically plausible and the differences in outcome of these studies likely reflect differences in study population. However, it should be noted that like our study, a recent meta-analysis demonstrated that in a general population individual antibiotic usage is the driving force for resistance of urinary bacteria[24]. Though some studies identified foreign travel to be a risk factor for infections with an antimicrobial-resistant uropathogen, in particular a trimethoprim-sulfamethoxazole-resistant strain, this was not found for infections with fluoroquinolone-resistant *E. coli*.[25-28] We did not systematically collect data on foreign travel to explore this issue in our study.
Compared to previous studies we used an additional questionnaire to evaluate potential environmental risk factors for fluoroquinolone resistance. This was done retrospectively holding a risk for observer, recall and selection bias. Yet,

several measures were taken to minimize this. First of all, the interviewer was blinded to the data with respect to fluoroquinolone susceptibility making observer bias unlikely. Secondly, when obtaining the questionnaire the patients were not specifically informed whether they had fluoroquinolone-resistant *E. coli*. Furthermore, cases and controls had comparable response rates. Thus recall bias is unlikely. Finally, the selected controls were comparable with the non-selected as they were randomly selected and matched only by centre and date of presentation with febrile UTI.

We did not find environmental risk factors for fluoroquinolone resistance. Thus our findings do not support the concern for an animal or human reservoir of fluoroquinolone resistance. This may contrast previous findings but it should be emphasized that the evidence for animal-human and human-human transmission of fluoroquinolone-resistant *E. coli* in UTI is limited to specific strains[17,18,20,29]. As each strain could have its specific mode and likelihood of transmission, our data do not contradict these studies. At least it suggests that to date such clones have not played a major role in a general Dutch community setting of patients at risk for febrile UTI. Further surveillance studies should include the genetic characterization of *E. coli* strains to confirm or refute the hypothesis that fluoroquinolone resistance in the community is driven by the introduction of clonal *E. coli* groups[30]. Furthermore, it must be emphasized that our study does not exclude a possible 2-hit mechanism for fluoroquinolone resistance, with an initial input of fluoroquinolone-resistant strains from food supply of colonized animals into the population followed by selection at the individual level by personal fluoroquinolone use. Further studies are urgently warranted to explore this hypothesis, particularly as the relation between animal food supply and fluoroquinolone-resistant *E. coli* in humans revealed conflicting results but at least indicate this is might be a major concern for the community.[23,28,31,32]

In case of the isolation of fluoroquinolone-resistant *E. coli,* we found accompanying high rates of resistance to other antibiotics: 33% to amoxicillin/clavulanate and 65% to trimethoprim/sulfamethoxazole. Similar multidrug resistance rates were found in a large study in North America[10]. Moreover, 14% of fluoroquinolone-resistant *E. coli* isolates in our study were ESBL-positive compared to less than 1% in fluoroquinolone-susceptible isolates. This supports a previous finding that fluoroquinolone susceptibility in *E. coli* makes the presence of ESBL-positivity unlikely[33]. In this respect, this highlights the importance of risk factors for fluoroquinolone resistance as these may also be risk factors for ESBL production.

The extent to which antibiotic resistance risk stratification could guide empirical therapy for febrile UTI is unknown. This study demonstrates that the absolute risk of fluoroquinolone resistance increases by about 20% in patients with at least one of the three risk factors we identified, but even with no risk factor there was a 8% risk for fluoroquinolone resistance. Further studies are therefore be required in order to better stratify fluoroquinolone resistance risk in patients with febrile UTI.

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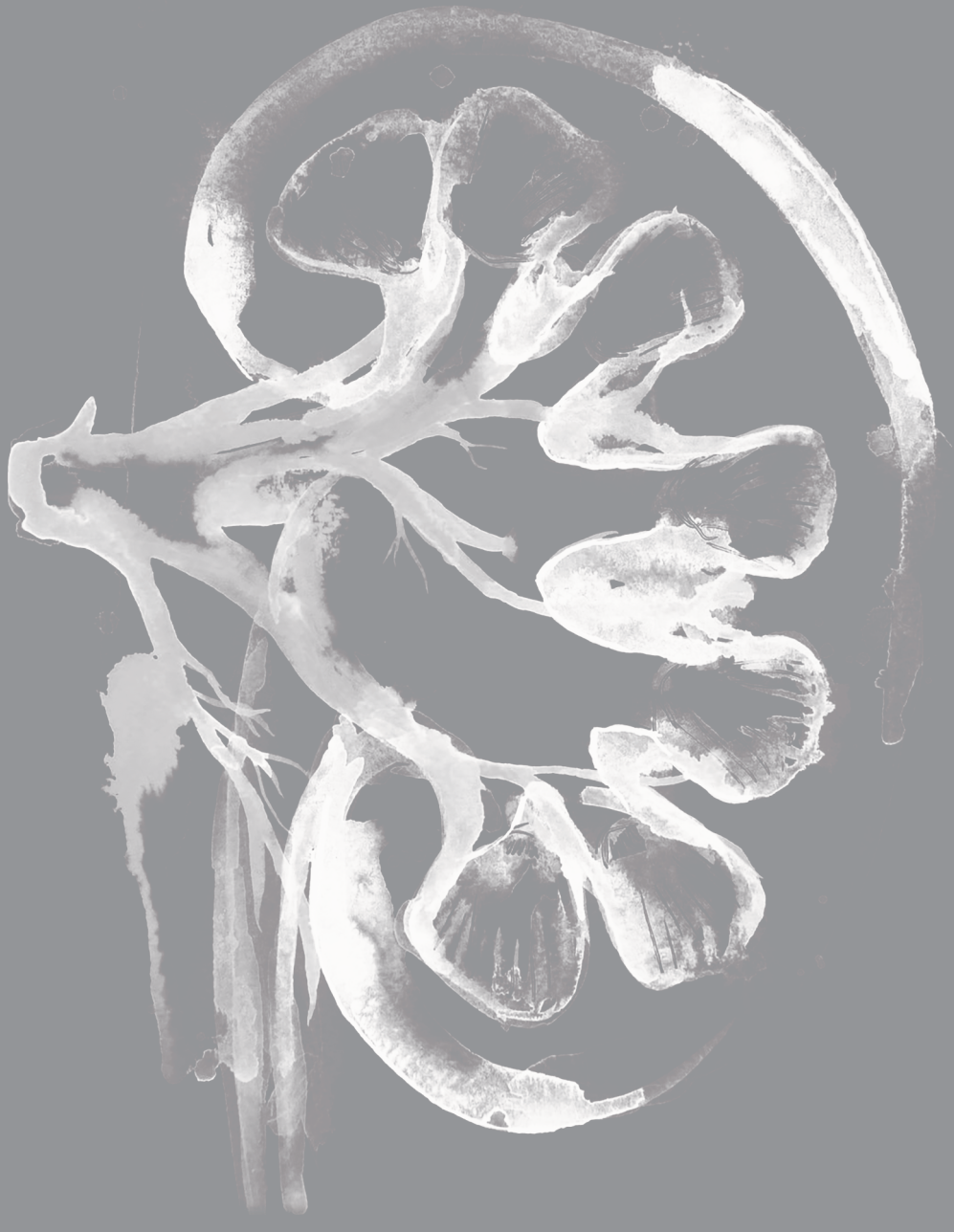

Chapter 2

Treatment duration of febrile urinary tract infections

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Abstract

Although febrile urinary tract infections (UTIs) are relatively common in adults, data on optimal treatment duration are limited. Randomized controlled trials specifically addressing the elderly and patients with comorbidities have not been performed. This review highlights current available evidence. Premenopausal, non-pregnant women without comorbidities can be treated with a 5-7 days regimen of fluoroquinolones in countries with low-level of fluoroquinolone resistance, or, if proven susceptible, with 14 days of trimethoprim-sulfamethoxazole. Oral β-lactams are less effective compared with fluoroquinolones and trimethoprim-sulfamethoxazole. In men with mild to moderate febrile UTI, a 2-week regimen of an oral fluoroquinolone is likely sufficient. Although data are limited, this possibly holds even in the elderly, patients with comorbidities or bacteremia.

Introduction

Urinary tract infections (UTIs) are among the most commonly encountered bacterial infections. In adults, UTIs can be classified into acute uncomplicated cystitis, acute uncomplicated pyelonephritis, complicated UTI, acute complicated pyelonephritis and for men several categories of prostatitis are distinguished.[1-3] The term 'uncomplicated' usually reflects UTIs in premenopausal, non-pregnant women with no known anatomical or functional urological abnormalities or other comorbidities.[4] Some authors advocate to consider UTIs in postmenopausal women or women with well-controlled diabetes mellitus without urological sequelae also to be 'uncomplicated'.[2] Given this various classifications of UTIs in the literature, it likely makes more sense to classify these patients uniformly according to their presentation in which fever reflects whether there is a parenchymal inflammation or not. From a scientific point of view, it is interesting to know whether the UTI involves the kidney, prostate, bladder, blood circulation, lymph nodes of the pelvis or a combination of those, but from a clinical point of view febrile UTI should be considered as tissue inflammation of the urinary tract whereas an exact anatomical distinction on clinical grounds can often not be made. In this article we will use febrile UTI as the clinical syndrome of interest because this is how patients present and fever mainly determines the appropriate treatment. According to the above mentioned classifications, febrile UTI includes complicated UTI with fever, acute prostatitis, acute (un-)complicated pyelonephritis and, as has been suggested, the urosepsis syndrome.[5]

The optimal treatment duration of febrile UTI has not been established yet, but current strategies recommend antimicrobial treatment for about 14 days in most patients.[4, 6-10] With the paucity of new antimicrobial classes in development, it is increasingly important to develop strategies to maintain or even increase the effectiveness of the available agents. Dose and regimen optimization represents one such strategy. Antimicrobials are associated with considerable side effects (e.g. *Clostridium difficile* infection with the use of fluoroquinolones).[11-13] Moreover, longer duration of antimicrobial therapy with consequently selecting pressure on gut flora might lead to an enhanced risk of the selection of resistant strains.[14] In this respect, it is questionable whether the benefit of antimicrobial therapy for at least 14 days at the end of treatment still outweighs its potential side effects.

This review highlights the main research findings on treatment duration of community-acquired febrile UTI in non-pregnant adults. A summary of the

pivotal studies among antimicrobial treatment duration of adults with febrile UTI is given in Table 1.

Febrile UTI in women

The majority of UTIs in women are community-acquired uncomplicated cystitis that usually responds to a 3-day course of empiric antimicrobials. However, if UTIs are accompanied by fever, another treatment regimen will be required. Treatment guidelines for febrile UTI often only discuss acute uncomplicated pyelonephritis, defined as acute pyelonephritis in premenopausal, non-pregnant and otherwise healthy women without any comorbidity.[4] Although febrile UTI is a relatively common and potentially serious infection even in young women, few controlled trials have been conducted to define optimal therapy directly comparing the same drug given for different durations of therapy, though a number of publications compared various treatment durations between different antimicrobial agents. In one open label, single center randomized trial comparing two versus six weeks of oral therapy with trimethoprim-sulfamethoxazole or ampicillin in 60 women with acute uncomplicated pyelonephritis, 2-week therapy was as effective as 6-week therapy with either drug. However, shorter duration of treatment resulted in fewer adverse effects, less frequent selection of resistant strains, and lower costs.[15] Trimethoprim-sulfamethoxazole was more effective than ampicillin due to more ampicillin-resistant strains and increased recurrence rate in ampicillin treated patients even with susceptible strains. With ampicillin resistance in *E. coli* exceeding 50% in many countries worldwide, ampicillin should not be used as empirical treatment of febrile UTI.
Nitrofurantoin should not be used for the treatment of febrile UTI because it does not achieve reliable tissue or serum levels.[16]

Trimethoprim-sulfamethoxazole and fluoroquinolones

Talan and colleagues showed that even therapy duration less than 14 days is effective in young, healthy women.[17] This double-blind, multicenter randomized controlled trial compared a 7-day regimen of oral ciprofloxacin 500 mg twice daily with a 14-day regimen of trimethoprim-sulfamethoxazole 160/800mg twice daily for treatment of otherwise healthy women with mild to moderate pyelonephritis. An initial intravenous 1-g dose of ceftriaxone in the trimethoprim-sulfamethoxazole group or a 400-mg intravenous dose of ciprofloxacin in the ciprofloxacin group was allowed at the discretion of the doctor. At the 4-11 days post-therapy visit, ciprofloxacin had significantly higher microbiological (99% vs 89%, respectively) and clinical (96% vs

83%, respectively) cure rates, regardless of whether an initial intravenous dose of ciprofloxacin was given. Among trimethoprim-sulfamethoxazole-treated women, microbiological eradication and clinical cure rates were significantly lower in women with a trimethoprim-sulfamethoxazole-resistant strain compared with those with a susceptible one. However, an initial intravenous dose of ceftriaxone significantly improved microbiological cure rate and moderately improved clinical cure rate in women with a trimethoprim-sulfamethoxazole-resistant uropathogen. Bacteremia (all *E. coli*) was present in 5.5% of the patients; 2/10 trimethoprim-sulfamethoxazole-treated subjects had bacteriologic persistence, all four ciprofloxacin-treated subjects achieved bacteriologic cure.

Additional evidence for a one week regimen of fluoroquinolones as effective and safe treatment for healthy young women was provided by two other articles, describing the same study.[18, 19] This double blind, randomized multicenter trial included both men and women with complicated UTI (without fever) and acute pyelonephritis (20-30%). Subgroup analysis of data about acute pyelonephritis[18] lend additional support that an oral 5-day regimen of a once-daily fluoroquinolone (levofloxacin 750 mg) or a 10-day regimen of ciprofloxacin 500mg twice daily (in 6.1% after initial intravenous ciprofloxacin 400 mg twice daily) might be effective for mild to moderate febrile UTI, even in those with bacteremia or complicating factors like obstruction or presence of an urinary catheter. Another pyelonephritis study in hospitalized men and women,[20] prospectively comparing 10 days of norfloxacin 400 mg twice daily with 10 days of ceftibuten 200 mg twice daily, after receiving intravenous cefuroxime for 2-4 days in each group prior to randomization to the study drug, showed excellent clinical and bacteriological cure rates in both groups and lower bacterial relapse rates in patients treated with norfloxacin. The finding that a one week regimen of fluoroquinolones is both efficacious and safe for treatment of mild to moderate febrile UTI was further supported by a randomized comparative study demonstrating similar outcomes in comparing levofloxacin 250 mg once daily, ciprofloxacin 500 mg twice daily and lomefloxacin 400 mg once daily, although in severe, invasive infections such a low dose of levofloxacin may result in marginal tissue and blood concentrations.[21] So, in regions with an acceptable low level of resistance to fluoroquinolones, this class of antimicrobials represents the preferred agents for empirical oral treatment of febrile UTI. With increasing rate of fluoroquinolone resistance in the last decade, the question raises at which threshold of resistance prevalence we should switch to an alternative agent for empirical treatment. Since clinical and bacterial outcomes with

different levels of resistance are not well studied, the recommended threshold of 10% fluoroquinolone resistance prevalence is mainly based on expert opinion.[4] For some areas in the world, the prevalence of fluoroquinolone resistance is >10%.[22, 23] Some experts advocate giving an initial dose of a long-acting parenteral antimicrobial, such as a 1-g dose of ceftriaxone or a consolidated 24-h dose of an aminoglycoside (e.g. one 5-7 mg/kg dose of gentamicin) in such situation.[4]

Oral β-lactams in women with febrile UTI

The data from Cronberg et al.[20] suggest inferior microbiological efficacy of oral cephalosporins compared with the fluoroquinolones, consistent with another Swedish study.[24] To our knowledge, there are no published studies on the efficacy of amoxicillin-clavulanate in the treatment of febrile UTI compared to trimethoprim-sulfamethoxazole or a fluoroquinolone. However, a single-blind randomized trial in women with acute uncomplicated cystitis comparing a 3-day oral regimen of amoxicillin-clavulanate 500/125 mg twice daily with ciprofloxacin 250 mg twice daily showed remarkable inferior clinical (60% and 77%, respectively) and microbiological (76% and 95%, respectively) cure rates in the amoxicillin-clavulanate-treated group, even in women infected with susceptible strains (60% in resistant versus 77% in susceptible strains). Most likely, the inferiority of β-lactams in the treatment of UTI is related to the lower rate of eradication of *E. coli* from the vagina in the amoxicillin-clavulanate group, maintaining a vaginal reservoir for infection.[25] Although not proven, these data are suggestive of an inferior efficacy of amoxicillin-clavulanate in febrile UTI.

Optimal treatment duration in young, otherwise healthy women

To summarize the above data regarding treatment duration of febrile UTI in young, non-pregnant women without comorbidities, a 5-7 day oral regimen of a fluoroquinolone is both efficacious and safe in mild to moderate febrile UTI given their superior clinical and bacteriological cure rates.[17-19] However, emerging fluoroquinolone resistance will possibly necessitate an initial dose of long-acting parenteral antimicrobial, such as a 1-g dose of ceftriaxone or a consolidated 24-h dose of an aminoglycoside.[4] Trimethoprim-sulfamethoxazole is also highly efficacious in febrile UTI if caused by a trimethoprim-sulfamethoxaxole-susceptible uropathogen[15] but current high rates of resistance to trimethoprim-sulfamethoxazole in many countries with corresponding failure rates for resistant strains make this agent an inferior choice for empirical therapy. When used empirically, combination with an

initial intravenous dose of ceftriaxone resulted in improved bacteriological and clinical cure rates.[17] When the uropathogen is susceptible, current evidence support a 14-day regimen;[17] shorter treatment duration has never been well investigated. Oral β-lactams are likely less effective compared with fluoroquinolones and trimethoprim-sulfamethoxazole.[15, 20, 24, 25] When used, a total course of 10-14 days of therapy is recommended.[4, 9, 10, 26] Probably, the same recommendations for treatment duration apply when the antimicrobial therapy was initially started intravenously (e.g. in case of severe sepsis or inability to take oral medication).

What do the guidelines say?

The above findings and treatment recommendations are in line with the recently updated IDSA-guideline.[4] The European Association of Urology (EAU) guideline recommends treating febrile UTI in premenopausal, otherwise healthy women with levofloxacin 750 mg once daily for 5 days or ciprofloxacin 500 mg twice daily for 7-10 days; a 14-day regimen is advised for all other oral antimicrobials and in all other febrile UTI patients.[10] However, the guideline of the American College of Obstetricians and Gynaecologists (ACOG) recommends treating febrile UTI in all women (both pre- and postmenopausal) for 14 days; fluoroquinolones are considered first line oral therapy and, in areas where resistance rates are 'low', trimethoprim-sulfamethoxazole is an acceptable alternative.[9] The guideline of the Dutch College of General Practitioners on the management of UTI recommends for outpatient treatment of adult patients with signs of tissue invasion including fever an oral regimen of either amoxicillin-clavulanate 500/125 mg three times a day, trimethoprim-sulfamethoxazole 160/800mg twice daily, or a fluoroquinolone (e.g. norfloxacin 400 mg twice daily or ciprofloxacin 500 mg twice daily) for 10 days.[26]

Febrile UTI in 'complicated' patients

While treatment recommendations of febrile UTI are quite straightforward in young, healthy, non-pregnant women with short oral antimicrobial courses on an outpatient basis (provided they are able to take oral medication and have no severe sepsis), there is a lack of conformity on therapeutic approaches for other (sometimes referred as 'complicated') categories of febrile UTI patients, like men, postmenopausal women, the elderly or patients with urological abnormalities or indwelling urinary catheter. Oral antimicrobial treatment regimens and optimal treatment duration have hardly been studied in the latter categories. Most studies focus exclusively

on febrile UTI in young, healthy women, or consist of a very heterogeneous study population with both women and men of all ages and a broad range of comorbidities, reducing the power and making subgroup analysis sometimes hardly to perform. There are no published trials specifically conceived to delineate optimal treatment duration in those categories, except for men. Currently, a randomized placebo-controlled study comparing a 7 to 14 day of antimicrobial therapy in consecutive patients with febrile UTI is ongoing to provide evidence within this category of 'complicated'.[27] The results of this study are expected to become available in 2012.

Febrile UTI in men

UTI is very uncommon in otherwise healthy, young and middle-aged men. A Norwegian study reported an estimated annual incidence of 6-8 UTIs per 10,000 men aged 21-50 years. Incidence increases with age because of urological abnormalities and instrumentation.[28] Since more than 90% of men with febrile UTI have a concomitant infection of the prostate, as measured by transient increases in serum PSA and prostate volume,[29] the goal of treatment is not only to sterilize the urine but also to reach sufficient antimicrobial concentration in the prostate. Therefore, antimicrobials reaching free concentrations in prostatic fluid and prostatic tissue that exceed the minimum inhibitory concentrations of most common causative bacteria should be chosen for therapy. Fluoroquinolones have such favourable pharmacokinetic properties and antibacterial spectra.[30] Trimethoprim also achieves adequate concentrations in the prostate and is an alternative to fluoroquinolones provided the bacteria are susceptible to this antimicrobial.[31] As described earlier in this article and demonstrated in some mixed trials with a minority of men, therapy with β-lactams may result in lower cure rates in men with febrile UTI.[20, 24]

There is an apparent lack of studies on optimal treatment duration of febrile UTI in men. We only found one study directly comparing different treatment durations in men.[32] In this open, prospective and randomized trial, 72 men with community-acquired febrile UTI (without a chronic indwelling catheter) were treated with ciprofloxacin 500 mg twice daily for two or four weeks. All responded successfully with resolution of fever and symptoms. There was no significant difference in bacteriological cure rate 2 weeks post-treatment between patients treated for 2 or 4 weeks (89% versus 97%, 95% CI for difference in proportions –3% to 19%), nor after 1 year (59% versus 76%, 95% CI –5% to 39%). The cumulative clinical cure rate after 1 year was 72% and 82%, respectively (95% CI –10% to 30%). Recurrences after 1

year comprised asymptomatic bacteriuria (48%), symptomatic lower UTI (23%) and another episode of febrile UTI (29%). A tendency towards more recurrences in the 2-week group could be attributed to a larger proportion of men with urological lesions requiring surgical interventions (26% versus 12%) in that group. The results should be interpreted with some caution given the wide confidence interval for the differences in cure rate, but this study suggests a 2-week course of ciprofloxacin 500 mg twice daily may be an adequate treatment for febrile UTI in men.

Another Swedish study lent additional support for a 2-week regimen of oral fluoroquinolones in men.[24] In this randomized, double-blind trial, adult men and women with a presumptive diagnosis of acute pyelonephritis (defined as febrile UTI) were randomly assigned to receive a 14-day course of oral treatment with either norfloxacin 400 mg twice daily or cefadroxil 1g twice daily. Of 197 patients enrolled, 16 (29.5%) men were treated with norfloxacin and 12 (21.1%) with cefadroxil. In this subgroup, a 14-day regimen of norfloxacin was highly effective, regardless of the presence of bacteremia or complicating factors such as diabetes mellitus or urinary tract abnormalities, with significantly higher bacteriological cure rate than with cefadroxil, both at 3-10 days (100% versus 73%, respectively) and up to 2 months after cessation of treatment (88% versus 75%, respectively).

The same results in men were obtained from a third Swedish trial which used step-down treatment; initial intravenous treatment with cefuroxime was followed by either norfloxacin 400 mg twice daily or ceftibuten 200 mg twice daily for 10 days.[20] Thus, these studies provided evidence that men with mild to moderate febrile UTI may be safely treated at home with a 14 days regimen of an oral fluoroquinolone.

Febrile UTI in the elderly and patients with comorbidities

Oral ciprofloxacin has a high bioavailability and a broad spectrum of activity against uropathogens. Therefore, Mombelli and colleagues analyzed the efficacy of ciprofloxacin in the empirical management of severe febrile UTIs.[33] In a multicenter prospective randomized study patients with serious (including febrile) UTI were randomized in the hospital setting to empirical antibiotic treatment with ciprofloxacin received either orally or intravenously. Excluded were patients with severe sepsis, inability to take oral medication or renal obstructive disease. In this study, 141 patients participated; 39% were men, 42 had co-morbidity (e.g. diabetes mellitus) and 35% had bacteremia. There were no infection-related deaths and no patients required an early change of antibiotics because of worsening clinical status during the initial empiri-

cal phase of treatment. The rates of microbiological failure (3% in the oral versus 2% in the intravenous treatment group) and of unsatisfactory clinical response (4% oral versus 3% intravenous) were low. A treatment change was eventually required in 14% of the patients assigned to the oral and 7% of the patients assigned to the intravenous regimen, mainly because of the isolation of enterococci or ciprofloxacin-resistant organisms in pretherapy urine specimens. There were no differences in outcome between the two regimens in premenopausal versus postmenopausal women. Unfortunately, treatment duration in both groups was not stated. The authors concluded that in the hospital setting oral ciprofloxacin is as effective as the intravenous regimen in the initial empirical treatment of serious UTI, including the bacteremic form. They hypothesize the oral regimen can be used for outpatient treatment even in serious UTI. Our own study among primary health care centers (PHCs) and emergency departments (EDs) supported this hypothesis.[34] We performed a prospective observational cohort study including consecutive non-pregnant adults with febrile UTI. Of 395 evaluable patients, 153 were recruited by their GP and treated as outpatients; 146 (95%) patients received oral ciprofloxacin 500 mg twice daily for 10-14 days. The remaining 242 patients were recruited at EDs of which 35 (14%) were treated as outpatients with oral ciprofloxacin and 207 (86%) were admitted and empirically treated with cefuroxime ± gentamicin. Median age was 63 years [IQR 42-77], 34% were male and 58% had comorbidity, all characteristics comparable between both groups. Bacteremia was present in 10% of the outpatients and 27% of the inpatients. During follow-up, 8 (5%) of PHC-group were hospitalized because of suspected deteriorating sepsis, progressive illness or persistent symptoms; none of them required ICU-admission nor were there any attributable deaths. Clinical cure rates at 30 days were high in both groups (90% in PHC and 89% in ED-group, respectively) and persistent at least until 3 months follow-up. Bacteriological outcomes were not reported. Thus, the outcome of patients treated with oral ciprofloxacin on an outpatient basis suggests that among selected adults with febrile UTI many can be safely treated at home using a 10-14 day regimen of oral fluoroquinolones, including men, the elderly and patients with comorbidity or bacteremia.

Besides this observational study, there is remarkably little evidence in the literature regarding the optimal treatment duration of febrile UTI in the elderly or patients with comorbidity like diabetes mellitus or urological abnormalities, despite high prevalence, since most randomized trials have excluded patients with underlying systemic illnesses or urinary tract abnor-

malities. Current treatment durations are mainly based on expert opinion; recommendations often do not differ from that in non-diabetic, young and otherwise healthy patients.[4, 9, 10, 35] Therefore, randomized therapeutic trials to define the optimal treatment duration in these patient categories are urgently needed.

Cather-related febrile UTI

A substantial part of the UTIs in elderly is catheter-related, but also in patient of any age with catheter-related febrile UTI it is not well known what the optimal duration of treatment is. We found one trial prospectively comparing treatment with either a parenteral aminoglycoside or oral ciprofloxacin 500 mg twice daily for 7-10 days in catheterized patients in a chronic care facility with complicated UTI (58.5% febrile UTI) and urinary tract abnormalities (e.g. neurogenic bladder, obstruction), the majority being men.[36] Whether the urinary catheter has been replaced before start of treatment was not described. Clinical cure 5-9 days post-therapy was about 80% in both groups, and 69% versus 58% 28-30 days post-therapy in the ciprofloxacin and aminoglycoside group, respectively. Bacteriological cure, defined as sterile urine culture, was achieved in the ciprofloxacin and aminoglycoside group in 63% and 15% 2-5 days after first dose and in 21% and 23% 5-9 days post-therapy, respectively. This trial, which is quite difficult to interpret because of uncertainty about catheter replacement before start treatment, suggests that ciprofloxacin 7-10 days at least clinically might be sufficient for treating catheter-related febrile UTI in men but more studies are needed to further explore this observation.

Conclusion

Based on the current evidence in the literature (Table 1) we conclude that premenopausal, non-pregnant and otherwise healthy women can be treated orally with 5-7 days of adequately dosed fluoroquinolones or, if proven susceptible to the urinary isolate, with 14 days of trimethoprim-sulfamethoxazole. Oral β-lactams are probably less effective in comparison with fluoroquinolones and trimethoprim-sulfamethoxazole. In all other patients with febrile UTI, optimal treatment duration is still unknown and should, awaiting randomized controlled trials, probably be at least 14 days. Although approximately 10% of patients with uncomplicated febrile UTI have bacteremia, there is no evidence that bacteremia has prognostic significance

or warrants longer therapy in an otherwise healthy individual when using an antibiotic regimen ensuring adequate tissue and blood concentrations.

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Table 1. Clinical trials comparing different antibiotic treatment strategies for febrile UTI in adults

Design				Outcomes				
				Short-term (4-9 days post)		Long-term (3-6 weeks post)		Comments
Study	**Design**	**Population**	**Regimens**	**Clin**	**Bact**	**Clin**	**Bact**	
Cronberg, 2001[20]	Placebo-controlled RCT	men: 42%; median age: women: 55y (19-90), men: 68y (23-90)	Norfloxacin 400 mg bid po, 10 days (n=83) Ceftibuten 200 mg bid po, 10 days (n=85)	96.0 89.0	89.0 75.0	84.0* 63.0*		after cefuroxime 0.75-1.5g tid iv 2-4 days
Fang, 1991[35]	Open label RCT	Mean age (range): 72y (20-95); men: 97%	Ciprofloxacin 500 mg bid po, 7-10 days (n=37) Aminoglycoside 1-1.7 mg/kg tid 7-10 days (n=28)	81.0 82.0	63.0 15.0	69.0 58.0	21.0 23.0	51% indwelling urinary catheter, 100% urinary tract abnormality
Klausner, 2007[18]	Placebo-controlled RCT	Mean age ± SD: 39 ± 17y men: 4%	Levofloxacin 750 mg od po, 5 days (n=146) Ciprofloxacin 500 mg bid po, 10 days (n=165)	93.8[a] 88.2[a]	91.3[a] 86.8[a]	92.5[a] 89.5[a]	92.5[a] 93.4[a]	6-7% initially iv treatment (levofloxacin or ciprofloxacin)
Mombelli, 1999[32]	Open label RCT	Median age: 66y (18-96); men: 41%	Ciprofloxacin 500 mg bid po, duration ns (n=72) Ciprofloxacin 200 mg bid iv, duration ns (n=69)	95.8 97.1	97.2 98.6	NS NS	NS NS	29.8% comorbidity; 23% postmenopausal women
van Nieuwkoop, 2010[33]	Prospective observational cohort study	Median age [IQR]: 63y [43-77]; men: 34%	Ciprofloxacin 500 mg bid po, 10-14 days (n=146) Cefuroxime ± gentamicin 2-4 days → ciprofloxacin 500 mg bid po, 6-12 days (n=242)	NS NS	NS NS	90.0 89.0	NS NS	- outpatient treatment - initially hospitalized, then first choice ciprofloxacin po
Richard, 1998[21]	Placebo-controlled and open label RCT	Mean age (range): 41y (18-91); men: 13%	Levofloxacin 250 mg od po, 7-10 days (n=89) Ciprofloxacin 500 mg bid po, 10 days (n=58) Lomefloxacin 400 mg od po, 14 days (n=39)	93.2 94.8 94.9	94.4 93.8 93.8	NS NS NS	87.3 93.5 93.5	
Sandberg, 1990[24]	Placebo-controlled RCT	Mean age (range): 50y (16-87); men:25%	Norfloxacin 400 mg bid po, 14 days (n=99) Cefadroxil 1 g bid po, 14 days (n=98)	97.0 97.0	98.0 65.0	88.0 47.0	87.0[b] 48.0[b]	25% 'complicated'
Stamm, 1987[15]	Open label RCT	Median age: NS (pre- and postmenopausal); men: 0%	Ampicillin 2 g od, 2 weeks (n=17) Ampicillin 2 g od, 6 weeks (n=10) TMP/SMX 160/800 mg bid, 2 weeks (n=21) TMP/SMX 160/800 mg bid, 6 weeks (n=12)	NS NS NS NS	NS NS NS NS	64.7* 40.0* 90.5* 83.3*		febrile UTI, 65% of subjects
Talan, 2000[17]	Placebo-controlled RCT	Median age: 24y (18-58) men: 0%	Ciprofloxacin 500 mg bid po, 7 days (n=128) TMP/SMX 160/800 mg bid po, 14 days (n=127)	96.5 82.9	99.1 89.1	90.6 77.4	84.7 74.1	15 once 400 mg cipro iv 26 once ceftriaxon 1 g iv
Ulleryd, 2003[31]	Open label RCT	Median age: 62y (18-85) men: 100%	Ciprofloxacin 500 mg bid po, 2 weeks (n=38) Ciprofloxacin 500 mg bid po, 4 weeks (n=34)	92.0 97.0	89.0 97.0	83.0 88.0	75.0 85.0	

Clin clinical; Bact: bacteriological; od once daily; bid twice daily; tid three times a day; TMP/SMX trimethoprim-sulfamethoxazole; UTI urinary tract infection; po orally; iv intravenously; NS not stated; * clinical and microbiological cure combined stated; [a]high percentage lost-to-follow-up (≥ 45%); [b] cumulative bacteriological cure

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- •• of major importance

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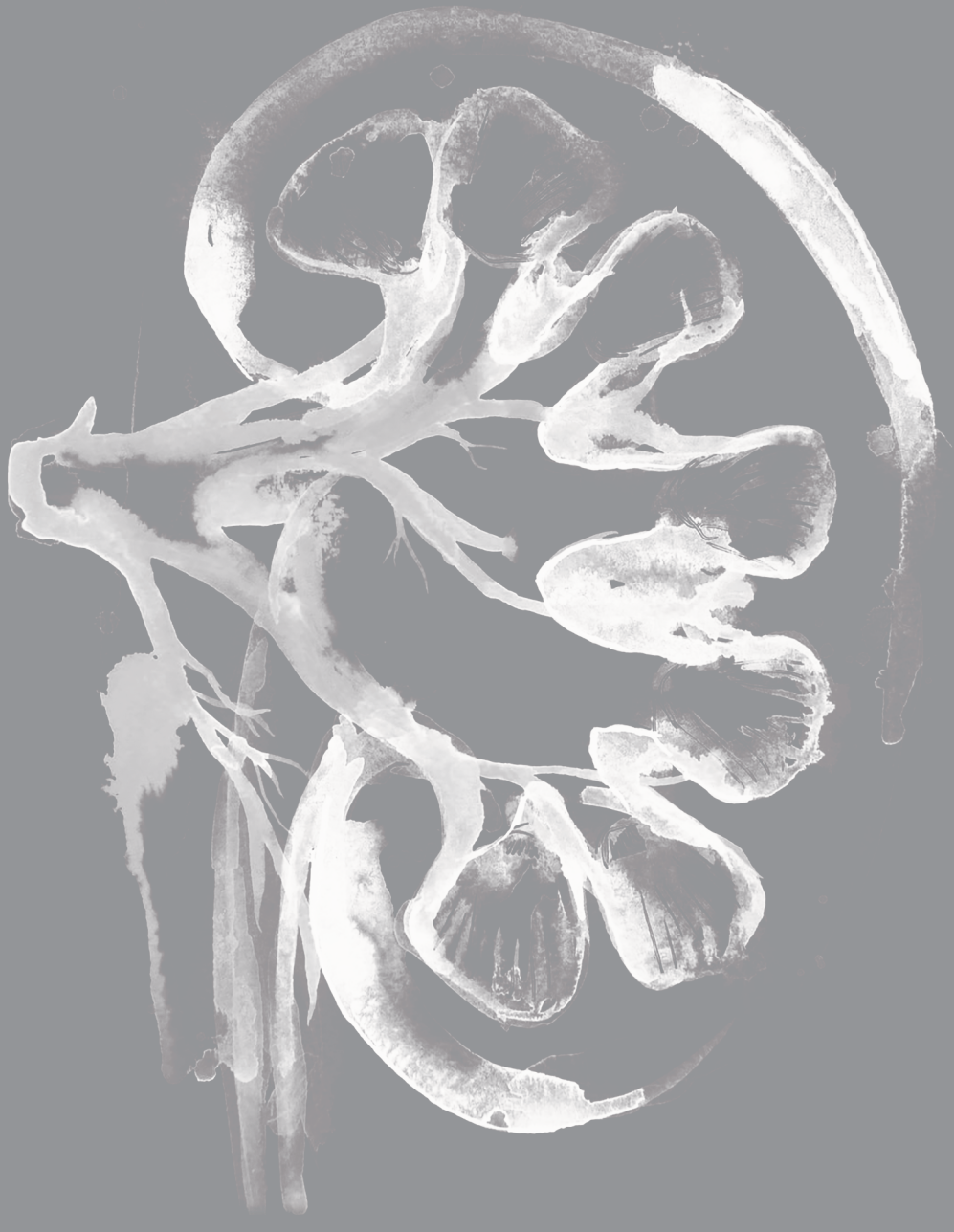

Chapter 3

Ciprofloxacin for 7 days versus 14 days in febrile urinary tract infection: a randomized, double-blind, placebo-controlled non-inferiority trial in men and women

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Abstract

Background

In adults with febrile urinary tract infections (fUTIs), data on optimal treatment duration are limited, especially in men, the elderly and patients with comorbidities.

Methods

A randomized placebo-controlled double-blind multicenter non-inferiority trial among 35 primary care centers and 7 emergency departments of regional hospitals in the Netherlands. Consecutive women and men aged ≥18 years with a presumptive diagnosis of community-acquired fUTI were randomly assigned to receive ciprofloxacin 500 mg orally twice daily for 7 days or for 14 days.

The primary endpoint was the clinical cure rate through the 10- to 18-day post-treatment visit.

Results

Of 357 patients included, 200 were eligible for randomization; 97 patients were randomly assigned to 7 days of ciprofloxacin and 103 patients to 14 days of ciprofloxacin. Overall, in an intention to treat analysis, clinical cure occurred in 85 (90%) patients treated for 7 days and in 94 (95%) of those treated 14 days (difference 4.5%; 90% CI -1.7 to 10.7; p=0.114, non-inferiority confirmed). In women, clinical cure was 94% and 93% in those treated for 7 and 14 days, respectively (47 of 50 vs 54 of 58; p=0.426). In men, however, clinical cure was 86% after 7 days treatment and 98% in those treated 14 days (38 of 44 vs 40 of 41; p=0.031).

Conclusions

In women including postmenopausal women and those with comorbidities, fUTI can be treated successfully with oral ciprofloxacin for 7 days. In men, 7 days of antibiotic treatment for fUTI is inferior to a 14-day course of oral ciprofloxacin.

Introduction

In the last decade, treatment of urinary tract infection (UTI) has become more complicated by rising antimicrobial resistance of Enterobacteriaceae, the most common uropathogens.[1] With a scarcity of new antimicrobial classes in the development pipe-line, it is essential to develop strategies to maintain effectiveness of available antimicrobials.[2] Therefore, among strategies to control resistance, the determination of an optimal duration of treatment is essential in addition to optimization of diagnostics to target treatment and antibiotic stewardship concerning antibiotic choice and dose. Shortening of antimicrobial therapy will lead to less selection pressure on the gut microbiome with benefits to both the individual patient as well as ecological environment including reduction of antibiotic resistance development.[3] Even in a common infection like UTI, there is a scarcity of controlled randomized studies that address minimal yet optimally efficacious duration of UTI treatment. With respect to febrile UTI (fUTI) or acute pyelonephritis, trials have usually focused on young women with uncomplicated UTI and have addressed optimal treatment duration by comparing the same drug for different durations of therapy, or compared various treatment durations of different antimicrobial agents. Recently, a randomized placebo-controlled trial showed that community-acquired acute uncomplicated pyelonephritis in women of all ages can be safely and efficaciously treated with oral ciprofloxacin for 7 days.[4] Clearly, such findings need to be extended to men and patients with significant comorbidities. In the present investigator-initiated randomized trial of treatment duration, we use fUTI as the clinical syndrome of interest because this is a broadly recognized specific clinical presentation of patients. Consecutive patients with fUTI were included, including men and women with comorbidities, and treated with ciprofloxacin for 7 days or 14 days. The aims of the study were to compare clinical and bacteriological cure both at short term and long term.

Methods

Study design and patients

We conducted a randomized placebo-controlled double-blind multicenter non-inferiority trial; the protocol has been published previously.[5]
Consecutive women and men aged 18 years or older with a presumptive diagnosis of community-acquired fUTI established by primary care physician

or on presentation at hospital's emergency department (ED) were recruited. Eligible patients had all of the following criteria: fever of ≥ 38.2°C and/or a history of feeling feverish with shivering or rigors in the past 24 hours, one or more symptoms suggestive of UTI (i.e. dysuria, frequency, urgency, perineal or suprapubic pain, costovertebral tenderness or flank pain) and positive urine nitrate test and/or pyuria (positive leucocyte esterase test or >5 leucocytes per high-power field in a centrifuged sediment). Patients enrolled were competent to provide written informed consent. Exclusion criteria for study entry were: known allergy to fluoroquinolones, pregnancy or lactation, polycystic kidney disease, permanent renal replacement therapy, kidney transplantation, residence outside The Netherlands and inability to speak or read Dutch.

Contra-indications for randomization were: isolation of ciprofloxacin-resistant causal uropathogen, presence of renal abscess, metastatic infectious foci or underlying chronic bacterial prostatitis as defined by recurrent UTI with the same uropathogen. Patients enrolled with fUTI but not randomized to trial medication, remained in the observational part of the study to assess outcome.

The independent medical ethics committees of the participating centers approved the study protocol. The trial was registered at ClinicalTrials.gov [NCT00809913] and trialregister.nl [NTR1583].

Randomization and antimicrobial treatment

Patients were randomized in a 1:1 ratio stratified per center and sex, to receive either a 7-day or a 14-day regimen of antimicrobial treatment. In the second week, treatment was continued double-blinded, with either ciprofloxacin 500 mg or placebo orally twice daily, according to randomization code. In inpatients, the treating physician could administer discretionary empirical intravenous antibiotics at the start of treatment according to local guidelines (in all hospitals participating: a β-lactam antibiotic ± aminoglycoside). These patients were switched as soon as deemed possible to open label oral ciprofloxacin (non-blinded) up to the 7th day after inclusion. The decision whether to treat as outpatient or inpatient, was made by the attending physician based on clinical judgment.

Further details upon randomization, trial medication, microbiological methods and study procedures are previously published.[11]

Main outcome measures

The primary endpoint was the clinical cure rate through the 10- to 18-day post-treatment visit (short-term clinical cure). Clinical cure was defined as being alive with absence of fever and resolution of UTI symptoms (either absence of symptoms or at least 2 points improvement on a 0 through 5 points severity score), without additional antimicrobial therapy (for relapse of UTI). Secondary outcome measures were bacteriological cure through the 10- to 18-day post-treatment visit, clinical cure rate through the 70- tot 84-day post-treatment visit (cumulative clinical cure), all-cause mortality, adverse event rate determined 10-18 days and 70-84 days post-treatment, and rate of UTI relapses. In addition, outcome measures were analyzed as stratified by specific subgroups (i.e., men, patients with complicated UTI, postmenopausal women, patients with any comorbidity and bacteremic UTI). Bacteriologic cure was defined as eradication of the study entry uropathogen with no recurrence of bacteriuria (pathogen growth $<10^4$ cfu/mL in women or $<10^3$ cfu/mL in men of a midstream urine culture combined with disappearance of leucocyturia).[6]

Statistical analysis

The primary endpoint was analyzed on the intention-to-treat (ITT) population, including all randomized patients who received at least one dose of the study drug, and on the per-protocol (PP) population, including all randomized patients who had been given the study drug for a minimum of 24 hours (in case of treatment failure) or who had been taken at least 80% of the study drug (in case of clinical cure).

The study sample size was calculated on the basis of a clinical cure rate of 10 percentage points lower at short-term follow-up in the 7-day treatment arm with the assumption of a 90% clinical cure rate in patients treated for 14 days.[7, 8] We adopted 10% as the margin of non-inferiority as suggested previously.[9] As we are only interested in non-inferiority and not in equivalence, the sample size calculation was based on a one-tailed alpha of 0.05. Assuming a non-inferiority margin of 0.10, 1-tailed alpha of 0.05 and a power of 0.90, the required sample size per group was 200. This implies that the 90% confidence interval of a two-tailed Chi-square test should not cross the predefined risk difference of 10% lower clinical cure rate, or equivalently, the one-sided p-value is less than the 0.05 significance level.[10] Interim analyses were done after randomization of 100 and 200 patients. After the second interim analysis, the principal investigators, who obviously were still blinded with respect to treatment allocation, noted that the overall cure rate

was 92% implying that the trial already had reached sufficient power (e.g. comparable with the power in a recently published similar trial).[4] Besides this, the overall cure rate was remarkably different for women and men (94% versus 91%). For men the principal investigators estimated and concluded the trial would likely end in futility, since the high overall cure rate in men was considered to be unlikely unless very large differences in cure rate between

Table 1. Baseline characteristics of 357 patients with febrile urinary tract infection

	Randomized (n=200)			
	Ciprofloxacin for 7 days (n=97)	Ciprofloxacin for 14 days (n=103)	Not randomized (n=157)	p-value"
Age (years)	60 (48-72)	61 (40-73)	63 (49-75)	0.277
Male sex	44 (45%)	42 (41%)	58 (37%)	0.247
Urologic history				
Indwelling urinary catheter	3 (3%)	2 (2%)	12 (8%)	0.024
Urinary tract disorder*	28 (29%)	28 (27%)	52 (33%)	0.296
Recurrent UTI^	19 (20%)	19/100 (19%)	47/147 (32%)	0.007
Comorbidity				
Diabetes	12 (12%)	17 (17%)	25 (16%)	0.709
Malignancy	3 (3%)	5 (5%)	17 (11%)	0.012
Heart failure	12 (12%)	6 (6%)	19 (12%)	0.340
Cerebrovascular disease	5 (5%)	5 (5%)	13 (8%)	0.210
Chronic renal insufficiency	3 (3%)	2 (2%)	10 (6%)	0.070
COPD	10 (10%)	11 (11%)	23 (15%)	0.236
Immunocompromised	3 (3%)	8 (8%)	14 (9%)	0.209
Presentation				
At emergency department	59 (61%)	68 (66%)	145 (92%)	<0.001
Antibiotic pretreatment	23 (24%)	29 (28%)	56 (36%)	0.048
Fever duration, hours	30 (15-48)	36 (20-60)	48 (19-96)	0.081
Dysuria	82/95 (86%)	78/102 (77%)	102/145 (70%)	0.019
Flank pain	57/96 (59%)	67/102 (66%)	91/144 (63%)	0.914
Suprapubic pain	51/96 (53%)	48/100 (48%)	72/145 (50%)	0.876
Perineal pain	4/96 (4%)	7/98 (7%)	8/140 (6%)	0.986
Outpatient treatment	45 (46%)	45 (44%)	23 (15%)	<0.001
Positive urine culture	69 (71%)	68 (66%)	107 (68%)	0.944
Positive blood culture	20/88 (23%)	15/98 (15%)	45/153 (29%)	0.012
Positive urine and/or blood culture	75 (77%)	70 (68%)	118 (75%)	0.571
Initial intravenous dose(s) of antibiotics	48 (50%)	55 (53%)	133 (85%)	<0.001

Data presented as number (%) or median (IQR)

* any functional or anatomical abnormality of urinary tract except urinary catheter

^ ≥3 UTIs in past 12 months or ≥2 UTIs in past 6 months

" randomized (both 7 and 14 days ciprofloxacin) vs not-randomized patients

the treatment arms were assumed. As this was considered either not-realistic or the justification to halt trial inclusion because then non-inferiority was evidently rejected, we decided to stop the trial at this point.
Descriptive statistics were used to describe the baseline characteristics in each arm with Chi-square tests for binomial and categorical data and Mann-Whitney tests for continuous data. All analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Between November 2008 and May 2013, 357 patients with a diagnosis of fUTI were enrolled into the study. Of these, 200 were randomly assigned to receive ciprofloxacin for 7 days (n=97) or 14 days (n=103). Reasons for exclusion from randomization, ITT and PP analyses are listed in Figure 1.
Of the 157 non-randomized patients, 119 (76%) were evaluable for short-term efficacy and 116 (74%) for cumulative efficacy.
Baseline characteristics of the study population are summarized in Table 1. Randomized, evaluable subjects in the two treatment arms were well matched with respect to demographic characteristics and presentation on study entry. The 157 patients who were not randomized, generally had more comorbidities and were more ill as more were referred to the ED. Additional details are listed in the Supplement. Baseline urine cultures were performed in 341 patients (96%) (Table 2). In 99 (28%) patients, urine culture showed either no significant bacteriuria or a mixed flora; in over half of these cases (58%), patients were pre-treated with antibiotics (in the group randomized to 7 days of ciprofloxacin: 13 (59%), and to 14 days of ciprofloxacin: 20 (63%)); a similar percentage pertained to those not randomized: 23 (51%), Blood cultures were obtained in 339 patients of which 80 (24%) had bacteremia with growth of *E. coli* in the majority of the cases (n=67, 84%).
Both treatment regimens resulted in a high clinical cure rate at short-term follow-up in ITT population: 90% vs 95% in patients treated with ciprofloxacin for 7 or 14 days, respectively (Table 3). The difference in short-term clinical cure rate between both treatment arms was 4.5% (90% CI -1.7 to 10.7, p-value non-inferiority test 0.114), thus the confidence interval surpassed the predefined non-inferiority margin of 10%. The median time to defervescence did not differ between the two groups: 2 (IQR 1-2) days in 7-day ciprofloxacin, 2 (IQR 1-3) days in 14-day ciprofloxacin. Short-term

clinical cure was 85% in non-randomized patients, whereas median time to defervescence amounted to 2 (IQR 1-3) days.
Short-term clinical cure rates were analyzed in preset subgroups of patients. In women, short-term clinical cures for 7- and 14-day arm were 47 of 50 (94%) vs 54 of 58 (93%), respectively. In men, clinical cure rates differed significantly between those treated for 7 or 14 days (38 of 44 [86%] vs 40 of 41 [98%], p=0.031) (Figure 2A and 2B). Clinical cure rates in patients with stepdown treatment were 41 of 47 (87%) and 48 of 52 (92%), in 7- and 14-day arm, respectively. Clinical cure rates were somewhat higher in patients treated with oral ciprofloxacin right from enrollment (44 of 47 [94%] vs 46 of 47 [98%], respectively for 7- and 14-day arm). Patients with positive blood cultures had higher clinical cure rates when treated with ciprofloxacin for 14 days compared to 7 days (15 of 15 [100%] vs 18 of 20 [90%]).
No differences were noted in cure rates between women with a complicated fUTI treated for 7 or for 14 days (33 of 35 [94%] vs 34 of 37 [92%]). Moreover, clinical cure rates did not differ in postmenopausal women treated for 7 or 14 days (28 of 30 [93%] vs 31 of 33 [94%]. Detailed information on subgroup analyses among men and women are listed in Figure 2A and 2B, respectively. Both treatment regimens post-randomization were well tolerated with no differences in side effects.
Post treatment urine cultures (at day 28-32) were obtained in 93 of 94 (99%) patients assigned to 7 days of ciprofloxacin, in 92 of 99 (93%) patients assigned to 14 days of ciprofloxacin, and in 109 of 119 (92%) non-randomized patients, with the short-term follow-up visit. Bacteriologic cure was 91% in the 7-day treatment arm, 97% in patients treated with ciprofloxacin for 14 days, and 86% in non-randomized patients (Table 3). More details upon clinical and microbiological outcomes are listed in the Supplement.

Figure 1. Trial profile
* concurrent medical conditions (n=16), logistic reasons (n=5), abroad during treatment with trial medication (n=3)

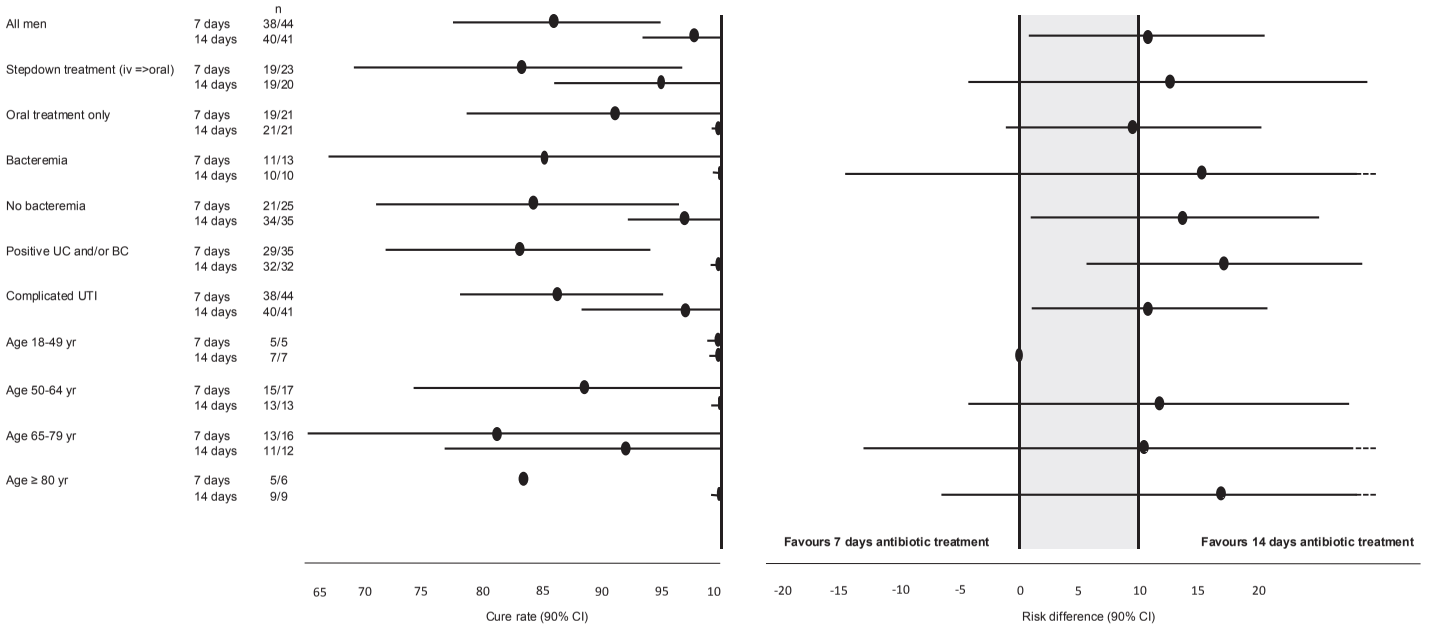

Figure 2a. Cure rate of febrile UTI in men and specific male subgroups
UC: urine culture, BC: blood culture, CI: confidence interval

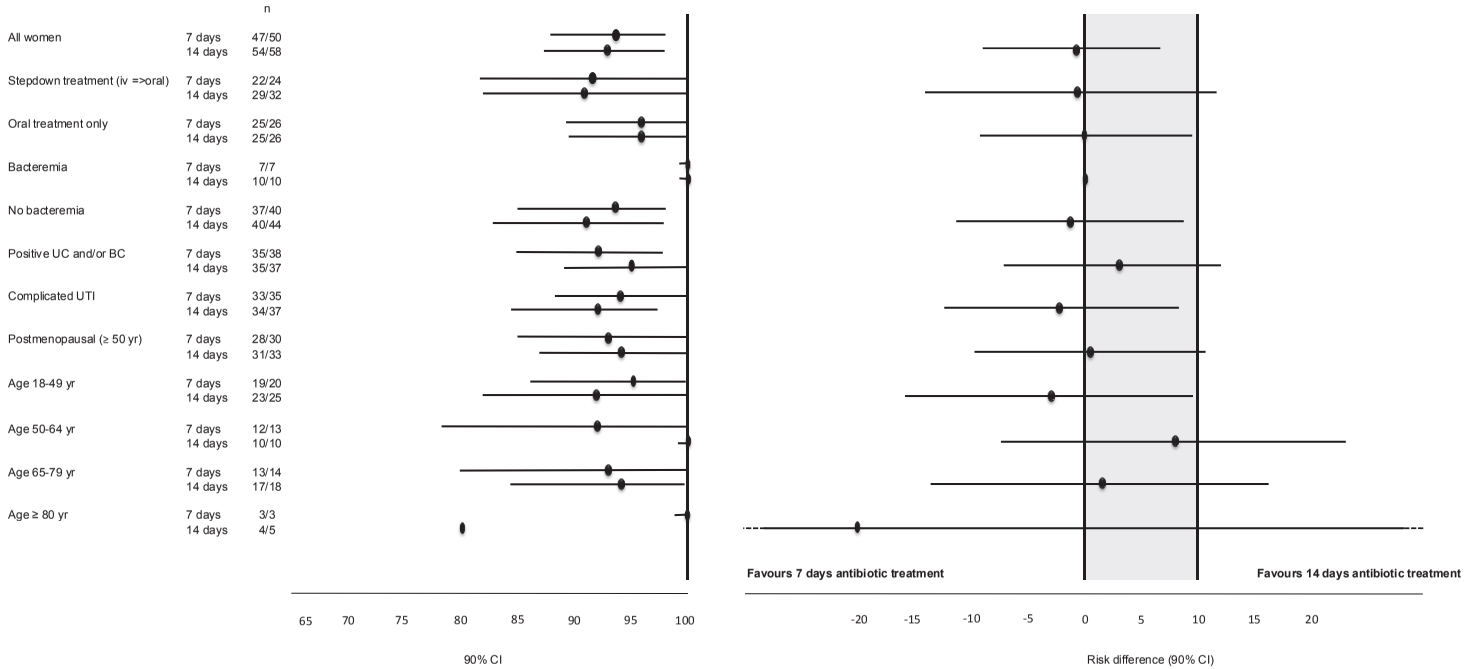

Figure 2b. Cure rate of febrile UTI in women and specific female subgroups
UC: urine culture, BC: blood culture, CI: confidence interval

Table 2. Urine culture results at entry*

	Randomized		Not randomized
	Ciprofloxacin for 7 days	Ciprofloxacin for 14 days	
Escherichia coli	65 (68%)	65 (59%)	85 (51%)
Klebsiella spp.	2 (2%)	4 (4%)	13 (8%)
Proteus spp.	1 (1%)	6 (5%)	6 (4%)
Pseudomonas aeruginosa	–	–	2 (1%)
Enterococcus spp.	1 (1%)	–	8 (5%)
Staphylococcus spp.	–	–	1 (1%)
Other^	3 (3%)	3 (3%)	8 (5%)
None or contaminated culture	22 (23%)	32 (29%)	45 (27%)

Data presented as number (%). Urine culture performed in: 7 days ciprofloxacin: 91 (94%), 14 days ciprofloxacin: 100 (97%), non-randomized: 150 (96%)

* some patients had multiple isolates; ciprofloxacin 7 days: n=6, ciprofloxacin 14 days: n=10, not randomized n=17

^ ciprofloxacin 7 days: *Proteus mirabilis* (1), *Citrobacter sedlakii* (1), *Citrobacter koseri* (1), *Candida* spp. (2)
ciprofloxacin 14 days: *Morganella morganii* (1), β-hemolytische streptokok group B *S. agalactiae* (2)
not randomized: *Serratia marcescens* (1), β-hemolytic streptococci group B (1), *Enterobacter cloacae* (1), *Streptococcus bovis* (1), *Citrobacter koseri (1), Morganella morganii (1), Proteus mirabilis (1), β-hemolytische streptokok group A (1)*

Table 3. Clinical and bacteriologic outcomes in the intention-to-treat and per-protocol population

	Randomized				
	Ciprofloxacin for 7 days	Ciprofloxacin for 14 days	Difference (90% CI)	Non-inferiority test p-value	Not randomized population
Intention-to-treat population	(n=94)	(n=100)			
Short-term efficacy	(n=94)	(n=99)			(n=119)
Clinical cure	85 (90%)	94 (95%)	4.5% (-1.7 to 10.7)	0.114	101 (85%)
Bacteriologic cure	86/93 (91%)	89/92 (97%)	4.3% (-1.2 to 9.8)	0.101	94/109 (86%)
Cumulative efficacy	(n=94)	(n=94)			(n=116)
Clinical cure	87 (93%)	86 (91%)	-1.1% (-7.6 to 5.5)	0.394	88 (76%)
Per-protocol population	(n=92)	(n=93)			
Short-term efficacy	(n=92)	(n=92)			NA
Clinical cure	83 (90%)	87 (95%)	4.3% (-2.1 to 10.8)	0.135	
Bacteriologic cure	84/91 (92%)	83/86 (97%)	4.2% (-1.5 to 10.0)	0.114	
Cumulative efficacy	(n=92)	(n=87)			
Clinical cure	85 (92%)	79 (91%)	-1.6% (-8.5 to 5.3)	0.352	

Data presented as number (%), unless otherwise indicated. NA: not applicable.
Short-term efficacy: endpoints assessed at 10- to 18-days post-treatment visit.
Clinical cure: being alive with absence of fever and resolution of UTI symptoms through post-treatment visit with no additional antimicrobial therapy for a relapse of UTI prescribed.
Bacteriologic cure: elimination of study entry uropathogen or pathogen growth <10^4 colony forming units/mL (women) or <10^3 colony forming units/mL (men) combined with disappearance of leucocyturia.
Cumulative efficacy: endpoint assessed at 70- to 84-days post-treatment visit.

Discussion

Our findings show that community-acquired febrile urinary tract infection can be safely and efficaciously treated with oral ciprofloxacin for 7 days in women, including the elderly with significant comorbidity, and irrespective of severity of disease at presentation. However in men, the 7-day treatment was significantly inferior to 14 days of treatment.

The main strength of this trial is its pragmatic nature reflecting daily clinical practice with the inclusion of consecutive patients with fUTI, both men and women, irrespective of age and underlying medical conditions, with the notable exception of those with severe kidney disease, antibiotic allergy and pregnancy. Several hospitals were involved, including a referral university hospital, and general practitioners who enrolled about one fourth of our patients. Therefore, patients recruited into the study are considered representative of individuals with acute community-acquired fUTI, encompassing acute pyelonephritis and prostatitis. Of note, the findings hold for both the intention-to-treat and the per protocol analysis, underlying the high compliance by patients randomized with respect to the treatment protocol and precluding that poor study procedures may have concealed differences in patient management.

In contrast, the group of patients who were not randomized (because of the isolation of ciprofloxacin-resistant causal uropathogen, renal abscess or underlying chronic bacterial prostatitis) had a significantly higher treatment failure rate.

Our study lacks statistical power to draw confident conclusions on the various subgroups because of the limited number of patients enrolled. However, further enrollment was precluded by the already significant difference in outcome between 7 and 14 days of treatment in the male subgroup.

Our findings extend recent findings of a highly similar controlled randomized study done in women with acute pyelonephritis in Sweden, showing non-inferiority of 7 and 14 days of antimicrobial treatment.[4] Although that study did not exclude the elderly or those severely ill, their patient group was younger with less comorbidity and complicated UTI than that enrolled in our study.

In men, our results indicate an increase in rate of clinical and bacteriological treatment failure after the 7-day treatment as compared to 14 days. Of note, this lack of efficacy could not be attributed upfront to a propensity of prostatitis in men, as the difference was especially clear in those men presenting with clinically evident costovertebral tenderness, although the number of

cases constrained a purposeful exploration of subgroups. Given the identical efficacy of the 7-day and 14 -day treatment in women, and the absence of a relation with manifest or possible prostatitis as reason for the inferiority of 7-day treatment in men, our findings cannot reliably explain this outcome. There is a lack of studies on optimal treatment duration of fUTI in men. One study directly compared different treatment duration in an open, prospective and randomized trial in 72 men with community-acquired fUTI showing similar bacteriological cure rates with ciprofloxacin 500 mg orally twice daily for either 2 or 4 weeks.[11] Similarly, a randomized, double-blind trial in Sweden lent support for the efficacy of 14 day treatment with fluoroquinolones in men.[12] Taken together, the studies confirm that at present, a 14-day treatment regimen of fluoroquinolones is the minimum period necessary for optimal therapy of fUTI in men. Recently however, a retrospective analysis of a large database of male veterans indicated that more than 7 days of antibiotic treatment (the vast majority being treated with ciprofloxacin) was not associated with a reduction of UTI recurrence.[13] In addition, this study showed that treatment with β-lactams was associated with a higher risk of recurrence as compared to fluoroquinolone treatment. Furthermore, they showed that UTI recurrence was independently associated with comorbidities and age. As in our study about half of the patients were initially treated with a β-lactam intravenously, implying less penetration into the prostrate,[14] this may have influenced our results and possibly this may explain the larger difference in cure rates within the subgroup of men with stepdown treatment. Interestingly, in line with this we found no significant difference in men who were solely treated with ciprofloxacin whereas in men aged less than 50 years, there was a similar cure rate with antibiotic treatment for 7 or 14 days. Future studies should address whether in men less than 50 years, fUTI can be efficaciously treated with 7 days of ciprofloxacin.

Given the consistency of our findings and those of the recent study in Sweden[4], we conclude that women can be treated orally with 7 days of adequately dosed fluoroquinolones, unless the urinary isolate is proven not susceptible to this antibiotic. Ciprofloxacin was chosen as treatment because of its reliable intestinal resorption and bioavailability, and excellent antimicrobial activity against a broad spectrum of susceptible gram-negative microorganisms, the most common etiologic microbiological agents in UTI, making it a drug of choice in both outpatient as well as hospital setting. As a surplus, activity against perineum and vagina colonizing Enterobacteriaceae may help prevent early recurrences.[15] Current results obtained with ciprofloxacin may likely be extrapolated to the other fluoroquinolones with

gram-negative activity but not to other antibiotic classes. An important concern has been the rise of ciprofloxacin resistance in the community, i.e., up to 15% of Enterobacteriaceae currently being resistant in The Netherlands, that, if it continues at the current rate, may prelude the use of fluoroquinolones as first-choice empiric oral treatment of fUTI. Of great concern, in other countries this figure has been reported as high as 40 to 50%.[16, 17] In countries with concurrent high rates of co-trimoxazole resistance in Enterobacteriaceae, there may be no oral antibiotic option left for general practitioners to treat fUTI at home, raising health care costs. These findings underscore the importance of controlling antimicrobial resistance, through antibiotic stewardship including the administration of antibiotics with optimal duration.

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Supplement

Baseline characteristics

In the 7-day treatment arm, 23 (24%) patients had been pretreated for presumptive UTI with: norfloxacin (n=1, 4%), none with ciprofloxacin, nitrofurantoin (n=5, 22%), trimethoprim ± sulfamethoxazole (n=3, 13%), amoxicillin ± clavulanic acid (n=12, 52%), phosphomycin (n=1, 4%) and others (n=1, 4%). Of those randomized to 14 days ciprofloxacin, 29 (28%) had been pretreated with ciprofloxacin (n=3, 10%), norfloxacin (n=1, 3%), nitrofurantoin (n=6, 21%), trimethoprim ± sulfamethoxazole (n=7, 24%), amoxicillin ± clavulanic acid (n=8, 28%), others (n=3, 10%) and unknown (n=1, 3%). In the non-randomized group, 56 (36%) had been pretreated with ciprofloxacin (n=8, 14%), norfloxacin (n=2, 6%), nitrofurantoin (n=8, 14%), trimethoprim ± sulfamethoxazole (n=7, 13%), amoxicillin ± clavulanic acid (n=21, 38%), phosphomycin (n=1, 2%), others (n=4, 7%) and unknown (n=5, 9%).

About half of the patients were initially treated with intravenous antibiotics, and this did not differ between treatment arms: in the 7 days of ciprofloxacin, 48 (50%) patients (cefuroxime n=21, 44%; cefuroxime + gentamicin n=22, 46%; other n=5, 10%) and in the 14 day, 55 (53%) patients (cefuroxime (n=32, 58%), cefuroxime ± gentamicin (n=20, 36%), ciprofloxacin i.v. (n=1, 2%) and other antibiotics (n=2, 4%)). In the non-randomized group, 133 (85%) patients had initial dose(s) of intravenous antibiotics, i.e., cefuroxime (n=61, 46%), cefuroxime ± gentamicin (n=49, 37%), ciprofloxacin (n=4, 3%) and other (n=18, 14%). Of note, the median time till switch from intravenous to oral antibiotics was 3 days (IQR 2-4), and did not differ between the groups.

Clinical outcome

During short-term follow-up, nine patients assigned to ciprofloxacin for 7 days had a clinical recurrence. Three patients had an episode of (afebrile) acute cystitis at day 17, 18 and 20, whereas six patients had an additional episode of fUTI at day 9, 14, 15, 17, 20 and 26 after treatment. Among patients assigned to 14 days of ciprofloxacin, one patient had an acute cystitis at day 30 and four patients had recurrent fUTI at day 8, 9, 19 and 20.

For cumulative clinical cure rate, 94 patients were evaluable in each treatment arms. Clinical cure rates were high: 93% vs 91% in patients treated with ciprofloxacin for 7 of 14 days (Table 3). During late follow-up, seven patients assigned to 7 days had a clinical recurrence. Six patients had an

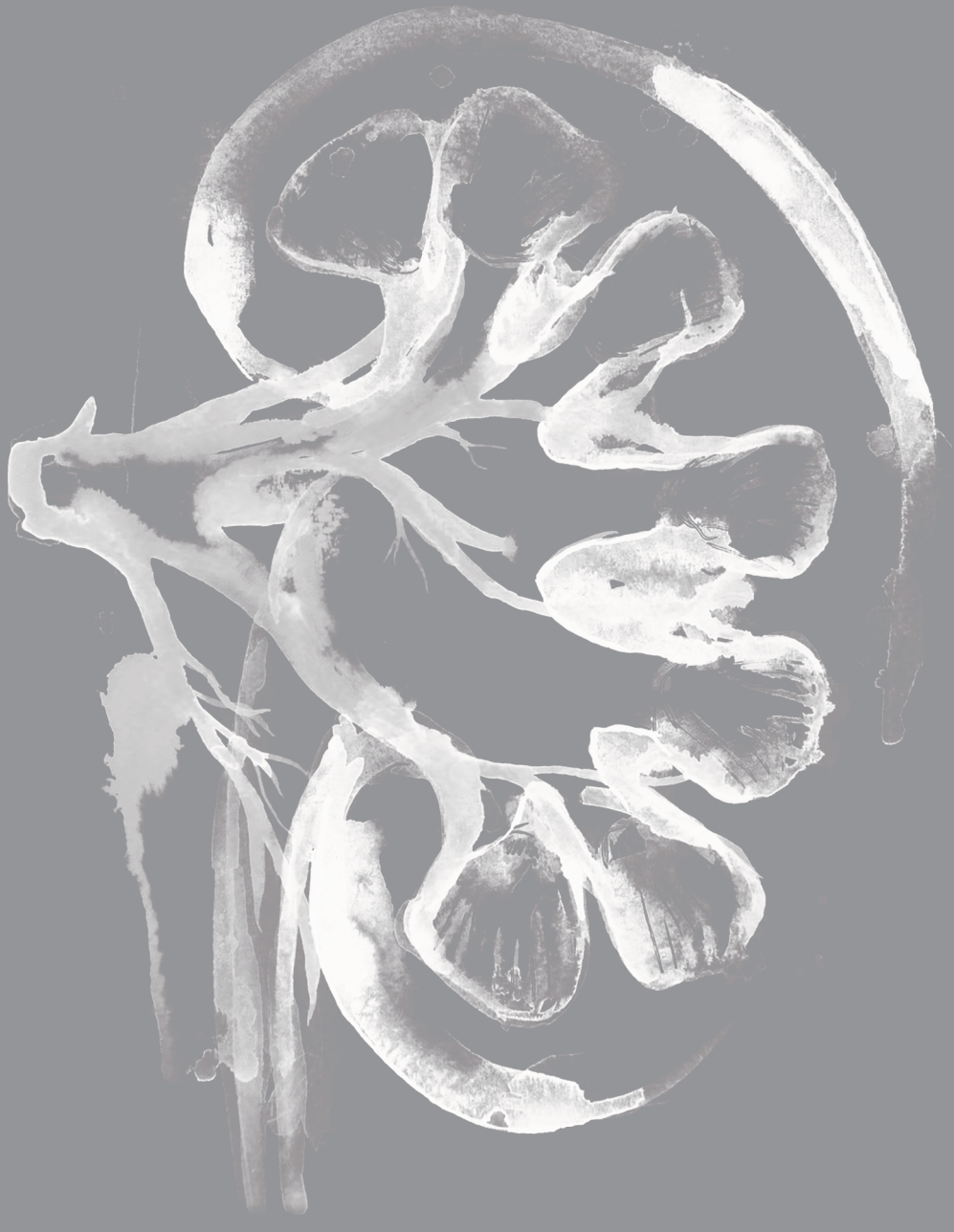
episode of (afebrile) acute cystitis at day 38, 40, 56, 63, 64 and 83 and one patient had an additional episode of fUTI. Among patients assigned to 14 days, seven patients had an (afebrile) acute cystitis at day 40, 44, 71 and 77 (n=3 day unknown) and one patients had recurrent signs of fUTI at day 90. One patient assigned to ciprofloxacin for 7 days was readmitted at day 9 because of treatment failure, and was treated intravenously with cefuroxime followed by oral ciprofloxacin for 14 days, now with good clinical response. None of the patients assigned to the 14-days treatment arm were readmitted because of treatment failure.

During the study period, no patients given 7 days of ciprofloxacin died. One patient, an 84-year old man assigned to 14 days of ciprofloxacin, died on day 92 due to pneumonia and sepsis. Five non-randomized patients died during follow-up due to concurrent medical problems.

With respect to side effects, one patient who received placebo, discontinued trial drug because of mucosal candida infection (day 2 after start placebo). Five patients on ciprofloxacin discontinued trial drug because of itching exanthema (n=2, both on day 3, i.e., day 10 of treatment) or feeling tired (n=3; day 1,3 and 5). During trial drug period, patients reported the following adverse events in 7- and 14 days treatment arm: nausea (7% vs 4%), vomiting (2% vs 1%), diarrhea (3% vs 2%), headache (16% vs 4%), dizziness (10% vs 9%), itching exanthema or rash (4% vs 4%) and myalgia (10% vs 12%).

Microbiological outcome

In the group assigned to 7 days of ciprofloxacin, seven patients had asymptomatic bacteriuria at short-term follow-up (five with *E. coli*, one with *Klebsiella oxytoca* and one with *Enterococcus faecalis*). Three patients treated with ciprofloxacin for 14 days had asymptomatic bacteriuria at short-term follow-up (one with *E. coli*, one with *E. faecalis* and one with coculture of *E. faecalis* and *S. aureus*). Fifteen non-randomized patients had asymptomatic bacteriuria at short-term visit: seven with *E. coli*, one with *E. coli* and *E. faecalis*, one with *Klebsiella* spp and *S. saprophyticus*, one with *Proteus* spp, two with *E. faecalis*, one with *E. faecalis* and *P. aeruginosa*, one with *P. aeruginosa* and one with *Enterobacter cloacae*.



Chapter 4

Diabetes mellitus and the course of febrile urinary tract infection

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Abstract

Objective

Evaluate the impact of diabetes mellitus on the clinical presentation and microbiological and clinical outcomes of febrile urinary tract infection (UTI).

Research design and methods

A prospective observational multicenter cohort study including 858 adults with community-onset febrile UTI presenting at seven emergency departments and 35 primary care units. The effect of pre-existing diabetes on presentation, microbiological and clinical outcome was assessed and multivariable logistic regression performed to establish whether diabetes was an independent risk factor for a complicated course.

Results

Of 858 patients, 140 had diabetes (93% type 2 diabetes). Patients with diabetes were older, more frequently male, and had a higher rate of cardiovascular and urinary tract comorbidities. Diabetes was not associated with longer fever duration or prolonged hospital admission. Patients with diabetes more often had bacteremia at presentation, ICU admission, recurrent UTI, asymptomatic bacteriuria and mortality during 30 days of follow-up. However, when adjusted for possible confounders, diabetes was not an independent risk factor for any of these complications, although women with diabetes were at increased risk of asymptomatic bacteriuria after one month. The higher prevalence of complications in diabetics was mainly explained by an increased prevalence of cardiovascular comorbidity and higher age.

Conclusions

Although it is widely held that patients with diabetes have a more complicated course of infections, our data show that diabetes is not independently associated with adverse outcomes in an unselected population of patients with febrile UTI. Cardiovascular comorbidity and increased age are the main risk factors for a complicated course.

Introduction

Diabetes mellitus affects about 6% of the population worldwide, and this number is expected to grow in the coming years.(1,2) Asymptomatic bacteriuria in diabetes - a common finding in up to one quarter of patients - predisposes to urinary tract infections(UTIs).(3,4) This increased risk of acquiring UTIs in diabetic patients is clear.(5,6) It is less clear whether UTIs in diabetic patients compared to non-diabetic patients follow a more complicated course, although various treatment guidelines reflect this perception.(7,8) In previous studies on diabetes and the outcome of febrile UTI, diabetes was associated with a higher rate of bacteremia and a longer duration of hospitalization and fever.(9-11) Two studies reported a higher mortality(10,12) and a recent meta-analysis demonstrated an increased hazard ratio for death from infection other than pneumonia (HR 2.39).(13) On the contrary, increased mortality was not associated with diabetes in a large review of hospitalizations due to pyelonephritis.(14)

However, these studies specifically focusing on urinary tract infections in diabetes are limited by small sample size, inadequate adjustment for confounding and varying inclusion criteria.

Therefore, this prospective observational cohort study was performed to evaluate the impact of diabetes on the clinical presentation of febrile UTI and its microbiological and clinical outcomes.

Research design and methods

We conducted a prospective observational multicenter cohort study including consecutive patients with febrile UTI from January 2004 until January 2011. Participating centers were 35 primary health centers and the emergency departments of 7 hospitals, all clustered in one area of the Netherlands. The local ethics committees approved the study and all participants provided written informed consent.

Inclusion criteria were age ≥ 18 years, fever (≥ 38.0°C) and/or a history of fever or shaking chills within 24 hours before presentation, at least one symptom of UTI (dysuria, perineal pain or flank pain) and a positive nitrite dipstick test or leucocyturia as defined by a positive leucocyte esterase dipstick test or the presence of more than five leucocytes per high-power field in a centrifuged sediment. Exclusion criteria were current treatment for urolithiasis or hydronephrosis, pregnancy, known allergy to fluoroquinolones,

receipt of hemo- or peritoneal dialysis, a history of kidney transplantation, or known presence of polycystic kidney disease. Patients were only included once in the study.

Procedures

Demographic and clinical data were collected at baseline, at 3-4 days and 28-32 days after enrollment. Microbiological data were collected at baseline and since August 2006 a second urine culture was obtained after 28-32 days. The presence of diabetes was included in the standard questionnaire at baseline, which was filled out by qualified research nurses or the clinical investigator by reviewing the medical record completed with a patient interview. When diabetes was newly diagnosed during admission, the patient was regarded as diabetic patient. In case the patient was lost to follow-up, mortality was assessed using interviews from patient's GP and/or hospital chart review and/or local governmental mortality registries. Blood and urine cultures were performed using standard microbiological methods. MIC breakpoints for resistance were based on EUCAST criteria (www.eucast.org).

Definitions

A urinary tract disorder was defined as the presence of any anatomical or functional abnormality of the urinary tract excluding the presence of a urinary catheter or history of nephrolithiasis. These two variables were analyzed separately. Recurrent UTI was defined as two or more episodes of UTI in the last six months, or three or more episodes of UTI in the last year. The isolation of coagulase-negative staphylococci from the blood culture was considered to indicate contamination and thus absence of bacteremia. Adequate treatment was defined as appropriate antibiotic treatment, taking into account the causal uropathogen(s) in urine- and blood culture and its resistance pattern. Asymptomatic bacteriuria was defined as the growth of $\geq 10^5$ CFU/mL of a uropathogen in a midstream urine sample collected 28-32 days after enrolment without symptoms suggesting UTI (dysuria/flank pain/fever).(15) Recurrent UTI was defined as the growth of $\geq 10^3$ CFU/mL of a uropathogen in a midstream urine sample collected 28-32 days after enrolment plus ≥1 symptom suggesting UTI.(16,17) Due to logistic reasons, in 500 patients (58%) a urine culture at 28-32 days after inclusion could be obtained; outcomes of asymptomatic bacteriuria and recurrent UTI could only be assessed in these patients.

Data on bacteremia were missing in 51 patients because no blood cultures were performed at presentation. Survival data were lacking in four patients

due to loss to follow-up; those patients were excluded from analysis of 30-day mortality. All patients were relatively young (22, 24, 26 and 67 years), and three had no underlying illnesses except for a stroke in the medical history of a 67-year old male, so risk of mortality is low.

Statistical analysis

Descriptive analysis included means with standard deviation (SD) or medians with interquartile range (IQR), as appropriate. Comparison of groups was performed using the Mann-Whitney U-test or unpaired t-test for continuous variables and the Chi-squared test for categorical variables. Odds ratios and 95% confidence intervals were calculated. Factors included in the logistic regression models for adverse outcomes (bacteremia, asymptomatic bacteriuria, recurrent UTI and 30-day mortality) using Enter selection method were age, sex, comorbidities, bacteremia at presentation (for 30-day mortality), and receiving an adequate antibiotic treatment (for recurrent UTI) if the *p* value was <0.2. Interactions between paired variables were tested. A two-tailed *p*-value <0.05 was considered to indicate statistical significance. All analyses were performed using SPSS 20.0 (SPSS Inc, Chicago, IL, USA).

Results

Of 858 consecutive patients, 140 had diabetes (130 (93%) type 2 diabetes), of which 41 (30%) used insulin, and 19 (14%) were managed by diet only. Diabetic patients were older (median age 73 [IQR 60-81] vs 64 [IQR 42-77] years), more frequently male (48% vs 35%, *p* 0.006), more often presented at the emergency department (86% vs 76%, *p* 0.008) and had a higher rate of cardiovascular and urinary tract comorbidities compared to 718 patients without diabetes. Further baseline characteristics of the study population are summarized in Table 1.

Signs and symptoms

Patients with diabetes had comparable signs and symptoms at presentation compared to non-diabetic patients, except for systolic blood pressure (138 ± 25 mmHg vs 129 ± 22 mmHg, respectively) and flank pain (50% versus 65%, respectively). After correction for age (both continuous and in quartiles), diabetes no longer had a significant influence on the absence of flank pain in the diabetic patients.

Table 1. Baseline characteristics of 858 patients presenting with febrile UTI

Characteristic	All (n=858)	Diabetes (n=140)	No diabetes (n=718)	p-value
Age, median [IQR] years	66 [46-78]	73 [60-81]	64 [42-77]	<.001
Male sex	320 (37)	67 (48)	253 (35)	.006
Antibiotic pre-treatment	254 (30)	48 (34)	206 (29)	.189
Urologic history				
Urinary tract disorder*	210 (24)	48† (34)	162 (23)	.005
Indwelling urinary catheter	58 (7)	16 (11)	42 (6)	.016
Recurrent UTI‡	269 (31)	54 (39)	215 (30)	.044
Comorbidity				
Malignancy	91 (11)	19 (14)	72 (10)	.230
Heart failure	128 (15)	39 (28)	89 (12)	<.001
Cerebrovascular disease	112 (13)	25 (18)	87 (12)	.074
Chronic renal insufficiency	78 (9)	26 (19)	52 (7)	<.001
Chronic obstructive pulmonary disease	118 (14)	28 (20)	90 (13)	.023
Presentation				
At emergency department	662 (77)	120 (86)	542 (76)	.008
Shaking chills	489/783 (63)	79/125 (63)	410/658 (62)	.851
Dysuria§	613 (76)	102 (83)	511 (75)	.065
Flank pain	526/837 (63)	66/132 (50)	460/705 (65)	<.001
Fever duration at presentation, median hours [IQR]‖	30 [12-60]	36 [15-72]	29 [12-60]	.397
Heart rate >90 beats/minute	448/850 (53)	73/139 (53)	375/711 (53)	1.000
Systolic blood pressure, mean mmHg ± SD	130 ± 23	138 ± 25	129 ± 22	<.001
Diastolic blood pressure, mean mmHg ± SD	72 ± 14	72 ± 16	72 ± 14	.940

Data are presented in n (%) unless otherwise stated. UTI = urinary tract infection, IQR = interquartile range, SD = standard deviation.

* defined as any functional or anatomical abnormality of the urinary tract except urinary catheter and history of nephrolithiasis.

† prostatic hypertrophy (20), malignancy of the urinary tract (6), neurogenic bladder (5), status after nephrectomy (3), and other anatomical or functional disorders of the urinary tract (14)

‡ defined as ≥ 3 UTIs in the past 12 months or ≥ 2 UTIs in the past 6 months

§ not recorded in patients with an indwelling urinary catheter

‖ recorded in 660 patients

Microbiological outcome

In 809 patients (94%), a urine culture was performed at baseline. *Escherichia coli* was the most common isolated uropathogen in both diabetic and non-diabetic patients (Table 2). *Klebsiella* spp. (9% vs 4%), *Enterococcus* spp. (11% vs 3%) and *Staphylococcus* spp. (5% vs 2%) were isolated more frequently in diabetic patients. Also urine culture of diabetic patients more frequently revealed more than one uropathogen (11% vs 4%). A comparable distribution

Table 2. Uropathogens isolated from urine culture at inclusion

Uropathogen	Diabetes (n=133)	No diabetes (n=676)
Escherichia coli	69 (52)	393 (58)
Proteus spp.	5 (4)	23 (3)
Klebsiella spp.	12 (9)	28 (4)
Pseudomonas aeruginosa	4 (3)	18 (3)
Enterococcus spp.	14 (11)	19 (3)
Staphylococcus spp.	6 (5)	10 (2)
Candida spp.	0 (0)	2 (0,3)
Other	14 (11)	89 (13)
None/ contaminated	23 (17)	121 (18)

Data are presented as n (%). Urine culture was not performed in 49 patients.

of uropathogens among diabetic and non-diabetic patients was seen in an analysis excluding patients with an indwelling urinary catheter (n =753).
Resistance patterns of *E. coli* urinary isolates were comparable between diabetic and non-diabetic patients: amoxicillin-clavulanic acid (17% vs 12%), trimethoprim-sulfamethoxazole (25% vs 30%) and ciprofloxacin (19% vs 12%), even when only patients without indwelling catheter and without antibiotic pre-treatment were analyzed (data not shown).
Baseline blood cultures were performed in 807 patients (94%). Diabetic patients more often had bacteremia (29% vs 20%). Also in patients with low bacterial counts in their urine culture ($< 10^5$ CFU/mL), bacteremia was more prevalent in diabetic patients versus non-diabetic patients (22% vs 16%, respectively). In the latter comparison patients with a urinary catheter or patients who had received antibiotic pre-treatment were excluded (Table 4).

Clinical outcome

Upon presentation, diabetic patients were more frequently admitted to the hospital (81% vs 64%). Moreover, they were more frequently admitted to the intensive care unit during hospitalization (6% vs 3%). However, when admitted, fever duration and length of hospital stay were comparable. Diabetic patients were more often bacteremic at presentation (30% vs 22%). After one month, diabetic patients had a higher rate of asymptomatic bacteriuria (13% vs 9%; OR 1.5, 95% CI 0.8-3.0), recurrent UTI (9% vs 3%; OR 2.9, 95% CI 1.2-7.2) and mortality (6% vs 2%; OR 3.3, 95% CI 1.3-8.0), although absolute numbers were low (Table 3). In a subgroup analysis of diabetic patients, use of insulin was not associated with any of the adverse outcomes.

Table 3. Relation between diabetes mellitus and clinical and microbiological outcome of 858 patients presenting with febrile UTI

Outcomes	All (n=858)	Diabetes (n=140)	No diabetes (n=718)	Univariate OR[5] [95% CI]	Multivariate OR[5] [95% CI]
Clinical					
Hospital admission	575 (67)	114 (81)	461 (64)	2.4 [1.6-3.8]	-
Hospitalization duration (days), median [IQR]	6 [4-9]	6 [4-11]	6 [4-8]	-	-
ICU admission	32 (4)	9 (6)	23 (3)	2.1 [0.9-4.6]	-
Bacteremia at presentation	185/807 (23)	40/134 (30)	145/673 (22)	1.6 [1.0-2.3]	1.2 [0.8-1.8]
Defervescence[1] (days), median [IQR]	2 [1-3]	2 [1-3]	2 [1-3]	-	-
30-day mortality	21/854 (2)	8 (6)	13/714 (2)	3.3 [1.3-8.0]	2.0 [0.7-5.8]
Microbiological[2]					
30-day asymptomatic bacteriuria[3]	49/500 (10)	12/92 (13)	37/408 (9)	1.5 [0.8-3.0]	1.1 [0.5-2.5]
30-day recurrent UTI[4]	21/500 (4)	8/92 (9)	13/408 (3)	2.9 [1.2-7.2]	2.2 [0.7-6.8]

Data are presented in n (%) unless otherwise stated. UTI = urinary tract infection, OR= odds ratio, CI = confidence interval, IQR = interquartile range, ICU = intensive care unit

[1] recorded in 667 patients: 105 (75%) diabetic patients and 562 (78%) non-diabetic patients

[2] of 500 patients with a urine culture performed after 30 days

[3] defined as the growth of a uropathogen $\geq 10^5$ in a midstream urine sample without symptoms of UTI

[4] defined as the growth of a uropathogen $\geq 10^3$ in a midstream urine sample plus ≥1 symptom(s) of UTI

[5] univariate and multivariate OR of risk factor diabetes mellitus for the corresponding outcomes

Multivariate analysis

After adjusting for possible confounders, independent risk factors for bacteremia were age (OR 1.0, 95% CI 1.0-1.0) and chronic renal insufficiency (OR 1.9, 95% CI 1.1-3.1) but diabetes was not (OR 1.2, 95% CI 0.8-1.8). Risk factors for 30-day mortality were age (OR 1.1, 95% CI 1.1-1.2) and heart failure (OR 3.2, 95% CI 1.2-8.9). Diabetes was not a significant risk factor for mortality (OR 2.0, 95% CI 0.7-5.8). Similarly, bacteremia was not associated with mortality (OR 1.6, 95% CI 0.6-4.4). None of the variables we studied were independently associated with recurrence of UTI during 30 days of follow-up (Table 3).

Also, diabetes was not independently associated with asymptomatic bacteriuria after one month (OR 1.1, 95% CI 0.5-2.5). Only having an indwelling catheter was significantly associated (OR 8.0, 95% CI 3.6-17.7). Potential interactions between variables (e.g diabetes mellitus and sex) were additionally tested, but they did not significantly change the models, except for asymptomatic bacteriuria. Of female diabetic patients, 15% had asymptomatic bacteriuria after one month compared to 4% in female non-diabetic patients (OR 4.3, 95% CI 1.5-11.9). In male, 11% of diabetic patients had asymptomatic bacteriuria compared to 17% in non-diabetic (OR 0.6, 95% CI

Table 4. Relation between urine bacterial load and bacteremia in adults with febrile UTI

CFU/mL urine	% bacteremia diabetic patients	% bacteremia non-diabetic patients
$<10^3$	1/1 (100%)	1/9 (11%)
10^3-10^4	1/2 (50%)	2/17 (12%)
10^4-10^5	0/7 (0%)	7/36 (19%)
$>10^5$	19/59 (32%)	65/277 (23%)
Total	21/69 (30%)	76/343 (22%)

Analysis performed in all patients with positive urine culture; patients pretreated with antibiotics or patients with indwelling urinary catheter were excluded from analysis.

0.2-1.7). So, there is an important interaction between diabetes and sex for the risk of asymptomatic bacteriuria (adjusted OR 0.1, 95% CI 0.02-0.7 for male diabetic patients).

Conclusions

In this prospective observational multicenter cohort study, diabetes mellitus was not independently associated with increased mortality or a complicated outcome of febrile urinary infection compared to non-diabetic patients. The prevalence of adverse outcomes was higher in diabetic patients, but mainly attributable to concurrent illnesses, especially cardiovascular comorbidities, and a higher age of the diabetic population. This is in line with the fact that most of the diabetic patients had type 2 diabetes.

Clinical symptoms of febrile UTI were comparable between diabetic and non-diabetic patients, apart from the observation that diabetic patients experience less flank pain, as was reported previously.(9) This might possibly be explained by diabetic neuropathy, although diabetes had no significant influence on the absence of flank pain in the diabetic patients after correction for age.

The lack of flank pain in a substantial part of the study population shows once more that flank pain has a low predictive value in the identification of complicated UTI, whereas presence of fever effectively excludes the presence of a non-complicated UTI. Therefore, the determination of fever in patients with suspected UTI should be the starting point in further diagnostic and therapeutic steps, because that is the most reliable distinction between cystitis/urethritis and UTIs associated with tissue invasion.

Although diabetic patients had a nearly similar clinical presentation except for flank pain, they more often had bacteremia. Interestingly, bacteremia did not affect duration of fever and hospitalization. The higher rate of bacteremia might be partly due to presentation later in the course of UTI, as suggested by a longer fever duration before inclusion. Another explanation could be that their immune response is less efficient. Various in vitro, animal and a few patient studies investigated the host response in diabetes,(18,19) which seems to be altered and could predispose diabetic patients to e.g. recurrent infection. Of these, several mechanisms could explain our clinical findings. Firstly, although the systemic cytokine response to infection seems not to be altered,(18,20) a lower pro-inflammatory cytokine amount was found in urine from diabetic patients with asymptomatic bacteriuria,(18) possibly reflecting an attenuated local immune response. This could be the explanation why in this study, like another study(21), less virulent uropathogens, such as *Enterococcus* spp., more often were cultured in diabetic patients. However, these differences also could be due to more frequent usage of antibiotics or more hospital admissions of diabetic patients, as *Enterococcus* spp. are frequent causative micro-organisms of nosocomial infections.(22,23) Secondly, even when the same uropathogen is involved, there could be a better adhesion of bacteria on the uroepithelium by their type 1 fimbriae, as was shown in *E. coli*, especially in poorly controlled diabetic patients.(24) In our study, we did not systematically collect the level of control of diabetes at presentation. As a consequence, this findings could not be confirmed within our group of diabetic patients. However, considering the similar clinical presentation in both groups, this phenomenon might be more responsible for the acquisition of UTI and not associated with different presentation or outcome of UTI. Lastly, more bladder voiding problems are encountered in diabetic patients due to autonomic dysfunction,(25) which leads to stasis of urine and possibly higher risk of bacterial growth and lack of bacteriologic cure.(26,27)

Our findings support previous reports on the lack of an association between diabetes per se and an increased mortality in urinary tract infection. Age and comorbidity, especially heart failure in our study, were more predictive of mortality and should be accounted for, as was also demonstrated in a study of elderly patients with urosepsis.(28) Another study of 206 elderly patients with febrile UTI did show a higher mortality in diabetic patients, but here no correction was made for comorbidity.(10) A retrospective cohort study comparing diabetic persons with age and sex matched controls found a higher risk ratio for death due to infectious diseases, but did not report

febrile UTI specific mortality and did not take into account possible differences in comorbidities between diabetic persons and controls.(29) Similarly in other infections, such as community-acquired pneumonia, a higher risk of death in diabetic patients was assumed to be associated with a higher incidence of acute kidney injury and acceleration of underlying cardiovascular disease.(20) Therefore, when diabetes patients have cardiovascular or renal complications of disease, these conditions will be the main determinants of outcome in infections.

Diabetes mellitus was not an independent risk factor for asymptomatic bacteriuria after one month in our study population. However, there was an significant interaction between diabetes and sex, showing an increased risk of asymptomatic bacteriuria in women with diabetes compared to men. This is supported by previous research, which showed that women with diabetes have a higher risk of asymptomatic bacteriuria compared to healthy women(19,30-32). The results in men however are not supported by a recent meta-analysis, which demonstrated also a higher prevalence of asymptomatic bacteriuria in men with diabetes compared to healthy men.(32) Our study do not find support for an increased duration of antimicrobial treatment of febrile UTI in diabetic compared to non-diabetic patients, since clinical and microbiological outcomes after one month did not differ significantly between both groups, and diabetic and non-diabetic patients were treated alike.

In conclusion, our data show that diabetes is not independently associated with adverse outcomes in an unselected population of patients with febrile UTI, although it is widely held that patients with diabetes have a more complicated course of infections. Cardiovascular comorbidity and increased age are the main risk factors for a complicated course of febrile UTI.

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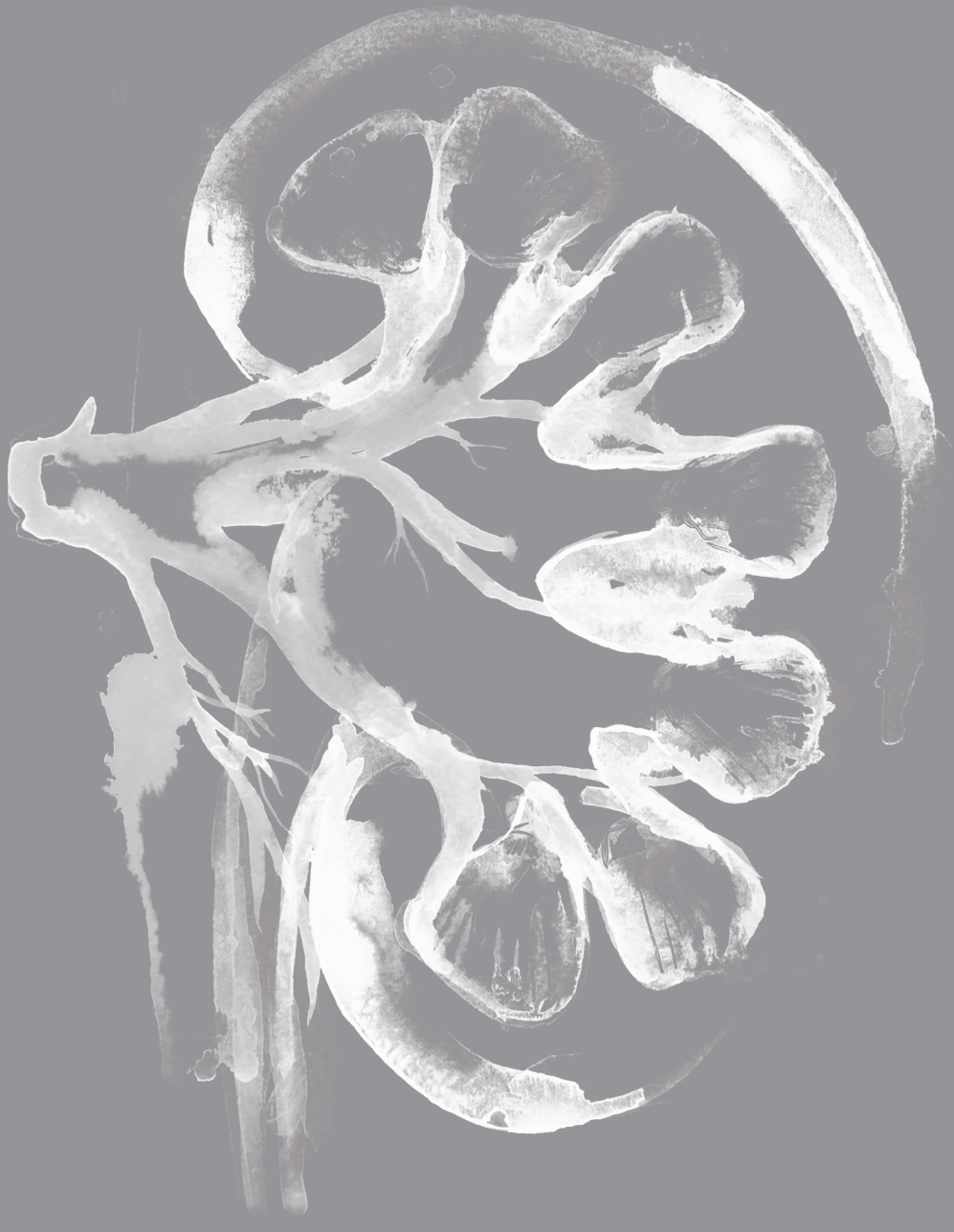

Chapter 5

Prognostic value of pro-adrenomedullin, procalcitonin and C-reactive protein in predicting outcome of febrile urinary tract infection

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Abstract

Bacterial infections such as febrile urinary tract infection (*f*UTI) may run a complicated course which is difficult to foretell on clinical evaluation only. Because the conventional biomarkers erythrocyte sedimentation rate (ESR), leukocyte count, C-reactive protein (CRP) and procalcitonin (PCT) have a limited role in the prediction of a complicated course of disease, a new biomarker - plasma midregional pro-adrenomedullin (MR-proADM) - was evaluated in patients with *f*UTI. We conducted a prospective multicentre cohort study including consecutive patients with *f*UTI at 35 primary care centres and 8 emergency departments. Clinical and microbiological data were collected and plasma biomarker levels were measured at presentation to the physician. Survival was assessed after 30 days. Of 494 *f*UTI patients, median age was 67 [IQR 49-78] years, 40% were male; two third of them had significant co-existing medical conditions. Median MR-proADM level was 1.42 [IQR 0.67-1.57] nmol/L; significantly elevated MR-proADM levels were measured in patients with bacteraemia, those admitted to the ICU, and in 30- and 90-day non-survivors, as compared to patients without these characteristics. The diagnostic accuracy for predicting 30-day mortality in *f*UTI, reflected by the area-under-the-curve of receiver operating characteristics were: MR-proADM 0.83 (95%CI: 0.71-0.94), PCT 0.71 (95%CI: 0.56-0.85); whereas CRP, ESR and leukocyte count lacked diagnostic value in this respect. This study shows that MR-proADM assessed on first contact predicts a complicated course of disease and 30-day mortality in patients with *f*UTI and in this respect has a higher discriminating accuracy than currently available biomarkers ESR, CRP, PCT and leukocyte count.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections. Febrile UTI (*f*UTI), reflecting acute pyelonephritis, prostatitis or urosepsis, is a potentially serious infection with a mortality rate of about 0.3%, but in bacteremic fUTI the mortality may be as high as 7.5-30% (1;2). Moreover, bacteraemia in *f*UTI is associated with prolonged hospitalization and a complicated course (3-5), and occurs in up to 30% of those admitted to hospital and in 15% of patients treated at home (6). Evaluation of clinical symptoms fails to provide accurate guidance to the clinician which patients have bacteraemia or who may run a complicated course, and which patients may be safely treated at home. At present, there is a lack of robust inflammatory biomarkers that may help determine severity of disease in *f*UTI (7;8). A promising new biomarker is midregional pro-adrenomedullin (MR-proADM). Adrenomedullin (ADM) has been detected in a variety of tissues including kidneys. It has immune modulating, metabolic and bactericidal activity, and is involved in regulation of complement activity (9-12). Reliable plasma measurement of ADM is challenging due to half-life time of 22 minutes (13). MR-proADM, the more stable mid-regional fragment of adrenomedullin, has been identified in plasma of patients with septic shock (13-15).
The aim of the present study is to assess the prognostic value of plasma MR-proADM in adult patients with *f*UTI with respect to bacteraemia, need for hospital admission and a complicated course, as compared to current available biomarkers like blood leukocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and procalcitonin (PCT).

Patients and methods

We conducted a prospective observational multicentre cohort study of patients presenting with a presumptive diagnosis of *f*UTI from January 2004 until March 2011. Participating centres were 35 primary care centres and 8 emergency departments (ED) in the Netherlands as described previously (6,8). The local ethics committees approved the study and all participants provided written informed consent. Over 7 years, we established a cohort of 869 patients. From this database, we randomly selected (every other patient, e.g. the 1st, 3rd,5th, etc.) frozen plasma samples of 494 patients for measurement of MR-proADM.

Inclusion criteria were age ≥18 years, fever (≥38.0 °C) and/or a history of fever or shaking chills within 24 hours before presentation, at least one symptom of UTI (dysuria, perineal pain or flank pain) and a positive nitrite dipstick test or leucocyturia. Exclusion criteria were current treatment for urolithiasis or hydronephrosis, pregnancy, hemo- or peritoneal dialysis, history of kidney transplantation or presence of polycystic kidney disease. Patients were only included once.

Procedures and definitions

Clinical data and laboratory values were collected within 24 hours of enrolment by standardized questionnaires and reviewing the medical record. All patients were empirically treated with antibiotics according to local and national policy. Blood cultures and clean midstream-catch urine cultures were obtained before starting antimicrobial therapy and analysed using standard microbiological methods. Bacteraemia was defined as growth of any pathogen in the blood culture, except coagulase-negative *staphylococci.*

Plasma ethylenediaminetetraacetic acid (EDTA) blood samples were collected, centrifuged and stored at -80 °C within two hours of patient enrolment. MR-proADM and PCT levels were measured after completion of all study enrolments, using a Time Resolved Amplified Cryptate Emission technology assay (TRACE®, Kryptor Compact, MR-proADM sensitive and PCT sensitive; Thermofisher - Brahms AG; Henningsdorf, Germany). The median concentration of MR-proADM in a cohort of healthy individuals was 0.39 nmol/L (97.5^{th} percentile: 0.55 nmol/L) (16). According to the manufacturer recommendation we tested MR-proADM levels for different cut-off values (16;17). Results of PCT measurement to predict bacteraemia have been described previously (8). Measurements of CRP, ESR and leukocyte count were only done at enrolment when indicated by the attending physician. All eight participating EDs applied similar techniques. CRP was measured using immunoturbidimetric assay, cut-off values varied from 6-10 mg/L. ESR was measured using Westergren method, cut-off values: <20mm/hour for males and females ≤50 years, 30mm/hour for females > 50 years and 15mm/hour for males >50 years. Leukocyte count was measured using flow cytometry, cut-off value: 10.0 $x10^9$/L. Data on biomarkers available in our study population were: CRP (n=319), ESR (n=158), leukocyte count (n=372) and PCT (n=321).

End points

MR-proADM values were evaluated for their predictive acumen of primary and secondary endpoints, in comparison to that of the other biomarkers. The primary endpoint was 30-day mortality. Secondary endpoints were presence of bacteraemia at admission, need for hospital admission as estimated by the Acute Pyelonephritis Severity Index score (APSI score), and need for ICU admission. The APSI score is a prediction rule allocating points to age, sex, nursing home residency, comorbidities, and vital signs at presentation (18).

Statistical analysis

The histogram of biomarker values were skewed and log-normalized before analysis. Descriptive analysis included means with confidence intervals (CIs) or medians and ranges, as appropriate. Univariate analysis was performed using ANOVA, student's *t*-test or where appropriate Mann-Whitney *U*-test for continuous variables and Chi-square tests for categorical variables. Continuous variables were added into the models as continuous variables (except for the APSI-score) and log-normalized if data were not normal distributed. The APSI-score was analysed as a binary variable, using a cut-off value of 100 points, based on previous data (18).

To assess the prognostic ability of MR-proADM compared to PCT and other conventional biomarkers in predicting the primary and secondary endpoints, AUC of ROC-curves were calculated. The main conclusion regarding the predictive ability of MR-proADM was based on this analysis. For each biomarker corresponding positive and negative predictive values and likelihood ratios were calculated for standardized cut-off values in predicting the primary endpoint. Kaplan-Meier survival curves were generated to illustrate survival probability and clinical outcome for different levels of MR-proADM. The log rank test was used to test the difference between survival curves. Survival analysis was performed on the whole cohort, ROC-analysis and sensitivity analysis on the subset of patients with data on the concerning biomarkers available. A p-value <0.05 was considered to indicate statistical significance. SPSS software (SPSS Inc., Chicago, Ill, version 20.0) was used for statistical analysis.

Results

In total 494 patients were randomly selected from our existing database resource. There were no significant differences between our study population

and the remainder of the database population, except for having a significantly older population (p=0.027) with significantly more diabetics (25%; p<0.001) in the selected study group (data not shown). Median age of our study population was 67 [IQR 49-78] years, 40% were male and 66% had co-existing medical conditions. Of 376 patients included at the Emergency Department, 329 (88%) were hospitalized. None of the patients recruited in primary care were hospitalized. (Table 1).

Table 1. Baseline characteristics of 494 patients presenting with febrile UTI

Characteristic	Febrile UTI patients n = 494
Age, median years [IQR]	67 [49-78]
Male sex	198 (40)
Antibiotic pre-treatment	171 (35)
Comorbidity	
Any	325 (66)
Diabetes mellitus	121 (25)
Malignancy	56 (11)
Heart failure	76 (15)
Cerebrovascular disease	73 (15)
Chronic obstructive pulmonary disease	77 (16)
Chronic renal insufficiency	54 (11)
Urologic history	
Urinary tract disorder[a]	126 (26)
Indwelling urinary catheter	40 (8)
Recurrent UTIs[b]	158 (32)
Presentation	
At emergency department	376 (76)
Shaking chills	290 (59)
Dysuria[c]	366 (74)
Flank pain	283 (57)
Fever duration at presentation, median hours [IQR]	32 [16-66]
Heart rate >90 beats/minute	258 (52)
Systolic blood pressure, mean mmHg ± SD	130 ± 23
Diastolic blood pressure, mean mmHg ± SD	72 ± 14

Data presented as n (%) unless otherwise stated. UTI=urinary tract infection, IQR=interquartile range, SD=standard deviation.

[a] any anatomical or functional abnormality of the urinary tract except urinary catheter and history of nephrolithiasis.

[b] defined as ≥ 3 UTIs in the past 12 months or ≥ 2 UTIs in the past 6 months.

[c] not recorded in patients with indwelling urinary catheter.

In the ED group, 30-day mortality was 3% (n=12) versus an absence of 30-day mortality in the primary care group. A total of 101 (22%) patients with blood cultures taken at presentation (n=463) presented with bacteraemia, with significantly more patients in the ED group (n=90/347, 26%, p<0.001). Nineteen (5%) of ED patients were admitted to the ICU, of these two died. (Table 2).

Table 2. Overview of primary and secondary endpoints in 494 patients with febrile UTI

Endpoint	Febrile UTI patients (n= 494)
Bacteraemia at presentation[a]	101/463 (22)
Hospitalization duration (days), median [IQR]	4 [0-7]
ICU admission	19 (4)
APSI score >100[b]	77 (16)
Mortality	
day 3	2/492 (0.4)
day 30	12/485 (3)
day 90	19/474 (4)

Data are presented in n (%) unless otherwise stated. IQR = interquartile range, ICU = Intensive Care Unit, APSI = Acute Pyelonephritis Severity Index.

[a] no blood culture performed in 31 patients.

[b] prediction rule allocating points to age, sex, nursing home residency, comorbidities and vital signs at presentation; patients with APSI score <100 can be safely treated at home without risk of readmission and mortality.

MR-proADM versus other biomarkers in predicting 30-day mortality

To define the prognostic accuracy of different biomarkers for predicting 30-day mortality, ROC analyses were performed. The AUC for MR-proADM (n=494) was 0.83 (95%CI 0.71-0.94), leukocyte count (n=372): 0.44 (95%CI 0.26-0.62), ESR (n=158): 0.60 (95%CI 0.43-0.78), CRP (n=319): 0.59 (95%CI 0.37-0.81) and PCT (n=321): 0.71 (95%CI 0.56-0.85). Based on the constructed AUCs, MR-proADM has a higher discriminating accuracy for predicting 30-day mortality as compared to the other conventional biomarkers.

The 97.5th percentile cut-off value of normal provided by the manufacturer is 0.55 nmol/L but in our target group this cut-off lacks specificity. Thus, the PPV, NPV and likelihood ratios for different MR-proADM cut-off values were calculated (Table 3). Our data indicates a plasma MR-proADM level of 1.00 nmol/L was the optimal cut-off value to stratify 30-day mortality in patients with *f*UTI. Using this cut-off, we calculated a sensitivity of 91.7% with a specificity of 48.0%; NPV 99.6%; PPV 4.3%; LR+ 1.8; LR- 0.2 (Table 3).

Table 3. Sensitivity, specificity, PPV, NPV, LR+ and LR- of different infectious biomarkers for predicting 30-day mortality

	Cut-off value	No. cases under cut-off	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	LR + (95% CI)	LR – (95% CI)
MR-proADM (nmol/L)	0.55[a]	79 (16%)	100.0 (73.4-100.0)	15.6 (12.5-19.2)	2.9 (1.5-5.1)	100.0 (95.1-100.0)	1.2 (1.1-1.2)	0.0 (0.0-0.0)
	1.00	234 (47%)	91.7 (61.5-98.6)	48.0 (43.4-52.6)	4.3 (2.2-7.5)	99.6 (97.6-99.9)	1.8 (1.5-2.1)	0.2 (0.0-1.1)
	1.50	362 (73%)	83.3 (51.6-97.4)	74.6 (70.5-78.5)	7.7 (3.8-13.7)	99.4 (98.0-99.9)	3.3 (2.4-4.4)	0.2 (0.1-0.8)
	1.88	406 (82%)	66.7 (35.0-89.9)	83.1 (79.4-86.4)	9.1 (4.0-17.1)	99.0 (97.4-99.7)	3.9 (2.5-6.2)	0.4 (0.2-0.9)
Procalcitonin (µg/mL)	0.25	129 (26%)	72.7 (39.1-93.7)	40.7 (35.1-46.3)	4.2 (1.8-8.1)	97.7 (93.3-99.5)	1.2 (0.8-1.8)	0.7 (0.3-1.8)
C-reactive protein	6	6 (1%)	100.0 (73.4-100.0)	1.3 (0.5-2.7)	2.5 (1.3-4.3)	100.0 (54.1-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
(mg/L)	8	10 (2%)	100.0 (73.4-100.0)	2.1 (1.0-3.9)	2.5 (1.3-4.3)	100.0 (69.0-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
	10	13 (3%)	100.0 (73.4-100.0)	2.8 (1.5-4.7)	2.5 (1.3-4.4)	100.0 (75.1-100.0)	1.0 (1.0-1.0)	0.0 (0.0-0.0)
ESR (mm)	20	74 (15%)	91.7 (61.5-98.6)	15.0 (11.9-18.6)	2.7 (1.3-4.7)	98.6 (92.5-99.8)	1.1 (0.9-1.3)	0.6 (0.1-3.7)
Leukocyte count (x10^9/L)	10	106 (22%)	75.0 (42.8-94.2)	21.8 (18.1-25.8)	2.4 (1.1-4.5)	97.2 (91.9-99.4)	1.0 (0.7-1.3)	1.2 (0.4-3.1)

PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio; ESR: erythrocyte sedimentation rate. Data on biomarkers available: MR-proADM (n=494), procalcitonin (n=321), C-reactive protein (n=319), ESR (n=158), leucocyte count (n=372).

[a] 97.5th percentile Brahms MR-pro-ADM Kryptor cut-off value in 144 healthy individuals.

Need for hospital admission

In the prediction of need for hospitalization, as based on an APSI score >100 points, MR-proADM outperformed PCT (n=321 patients with both data available) given the AUC for MR-proADM of 0.82 (95% CI 0.77-0.88) compared to 0.69 (95%CI 0.62-0.77) for PCT. For prediction of bacteraemia, MR-proADM and PCT performed about equally (MR-proADM: AUC 0.78 (95%CI 0.72-0.85) and PCT 0.81 (95%CI 0.75-0.87)). As the predictive values might have been influenced by antibiotic (pre)treatment (in 35% of the patients), analysis was also done separately in those with and without antibiotics on study enrolment (n=113 *vs* n=208). Corresponding AUCs for MR-proADM were 0.75 (95%CI 0.65-0.85) and 0.79 (95%CI 0.70-0.88), and for PCT 0.80 (95%CI 0.71-0.89) and 0.80 (95%CI 0.72-0.88) respectively, indicating that antibiotic pretreatment did not alter the predictive value of MR-proADM with respect to bacteraemia. In the prediction of either bacteraemia or need for hospital admission, CRP, ESR and blood leukocytes lacked predictive power (all AUC <0.60). For prediction of the need for ICU admission, pro-ADM and PCT performed almost identical (i.e., AUC of 0.77 and 0.75, respectively, n=321).

MR-proADM and clinical parameters

In addition to 30-day mortality, median MR-proADM level was significantly correlated with bacteraemia (bacteremic versus non-bacteremic patients: 1.60 [IQR 1.01-3.28] versus 0.96 [IQR 0.61-1.37] nmol/L), need for ICU-admission (ICU versus non-ICU patients: 2.01 [IQR 1.37-4.61] versus 1.04 [IQR 0.65-1.50] nmol/L) and APSI score (1.95 [IQR 1.27-2.90] nmol/L in patients with a score >100 points versus 0.94 [IQR 0.62-1.36] nmol/L in patients with a score ≤100 points). Furthermore, MR-proADM levels increased with age and were significantly higher in patients with heart failure and chronic renal insufficiency.

The Kaplan-Meier curves showed no 30- or 90-day mortality in patients with MR-proADM levels in the 1st quartile (n=123) of the whole group. In the 2nd quartile (n=124) three events occurred, four in the 3rd quartile (n=124), and eleven in the 4th quartile (n=123). All three events in the 2nd quartile occurred late in current disease episode: day 16, 27 and 33. One event in the 3rd quartile occurred on day 3, the other three occurred late after disease onset (day 41, 42 and 66). Events in the 4th quartile occurred primarily in the early stage of current disease episode. This suggests that events in the 2nd and 3rd quartile are likely due to pre-existing co-morbidity, while events

in the 4th quartile occur as result of the current active disease (Figure 1). The 30-day cumulative survival rate was 1.00 in the 1st quartile, 0.98 in the 2nd quartile (log rank *p* =0.157), 1.00 in the 3rd quartile (log rank *p* 1.00) and 0.92 in the 4th quartile (log rank *p* =0.001). The 90-day cumulative survival rate was 1.00 in the 1st quartile, 0.98 in the 2nd quartile (log rank *p* =0.083), 0.97 in the 3rd quartile (log rank *p* =0.046) and 0.91 in the 4th quartile (log rank *p* =0.001).

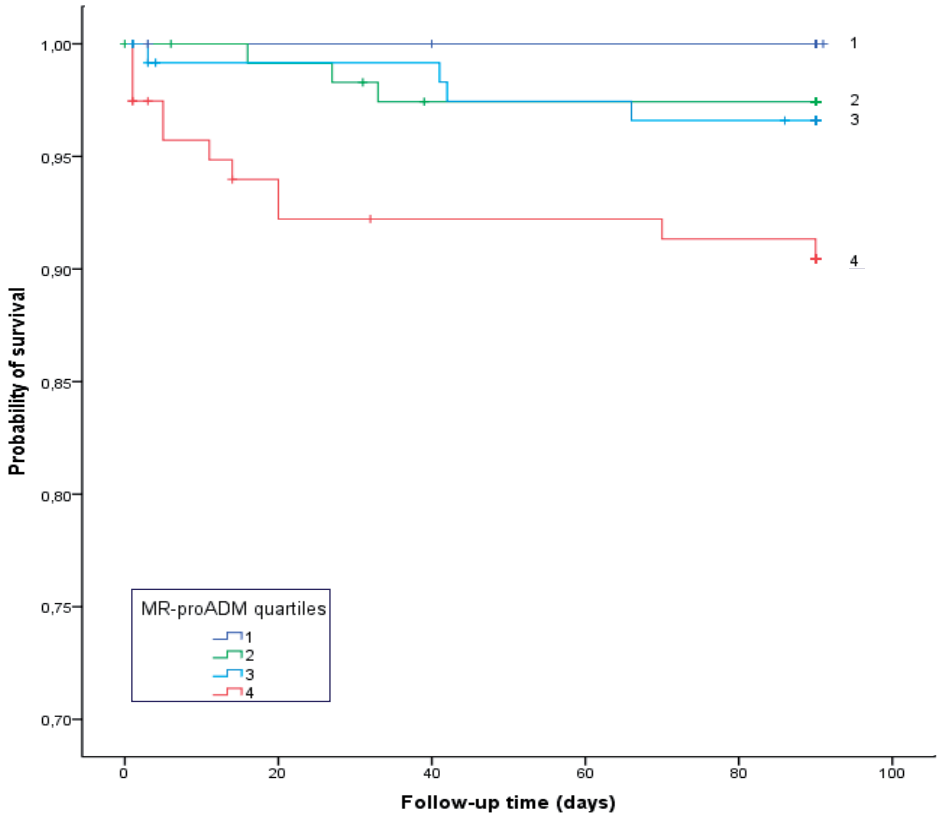

Figure 1. Kaplan-Meier curves of 90-day mortality according to quartiles of mid-regional pro-adrenomedullin (MR-proADM)

Discussion

The main finding of the present study is that MR-proADM, determined on first contact in a patient with presumptive *f*UTI, predicts a complicated course of disease necessitating hospital admission and admission to the ICU, and a worse outcome of infection as reflected by 30-day mortality. MR-proADM

more accurately predicts outcome than currently used biomarkers. Furthermore, we found significantly higher plasma MR-proADM levels in patients presenting with bacteraemia. Given these characteristics, measurement of MR-proADM in patients with a presumptive diagnosis of *f*UTI may provide the clinician more accurate guidance than currently applied biomarkers, e.g., with respect to admission of high-risk patients, and thus help focus resources to the patients that need them most.

Strengths of this study are its prospective design in which *f*UTI patients were included in both primary care and hospital ED setting, reflecting a real-life, full spectrum of invasive UTI recognizable to every clinician. Also, the large sample size of a clinically and microbiologically well characterized disease group is a strength. To our knowledge, this is the first large prospective study focusing on the predictive value of MR-proADM in adult patients with *f*UTI, and making a comparison to currently available biomarkers of inflammation like PCT and CRP. Travaglino et al. showed that in febrile ED patients, MR-proADM and PCT levels correlated with APACHE-II score and the combined use of both biomarkers might be helpful in predicting hospitaliztation (19).
There are also some limitations. We determined MR-proADM levels once, at first contact with the physician. This precludes the analysis whether a rise or decline in MR-proADM levels correlates to changes in the clinical course of disease, as has been determined for e.g. PCT (20). However, our findings show that having a single baseline value can provide clinicians guidance in predicting a complicated clinical course at the ED or primary care. This is where patients initially present and decisions have to be made regarding treatment and hospital admission. When interpreting MR-proADM, it should be taken into account that certain patient characteristics like age and heart failure may affect the plasma level of MR-proADM, as well as disease duration before presentation. A technical limitation might be that the measurement of MR-proADM and other biomarkers was done afterwards, and not immediately 'at the bedside'. However, it has been shown that frozen storage and consequent freeze-thaw cycles of blood samples has no influence on the analyte and measured concentration (15;21). Finally, it should be realized that our findings pertain to *f*UTI and need be confirmed in other infectious conditions. Of note, similar findings have been made in other infectious states albeit usually in much smaller groups of patients and rarely prospectively (14;22-25).

We hypothesize that at least two mechanisms might be responsible for the marked increase of MR-proADM in *f*UTI. *In vitro* and *in vivo* studies have shown that the onset of inflammation is accompanied by changes in both ADM and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Plasma ADM levels are markedly increased in patients with septic shock, supporting the hypothesis that pro-inflammatory cytokines augment ADM production and may increase plasma levels of ADM (11;26-28). However, ADM is also capable of upregulation of interleukine-6 in non-stimulated and LPS-stimulated macrophages, thereby suppressing LPS-induced TNF-α production (29). This suggests that ADM acts as part of a regulatory loop balancing pro-inflammatory cytokines with its anti-inflammatory actions. Since LPS and cytokine levels were not measured in our study, we cannot refute or confirm this hypothesis. Secondly, a decreased clearance of ADM by the kidneys may be – at least in part – responsible for increased proADM levels in *f*UTI. This is supported by our data with a higher median MR-proADM in patients with chronic renal insufficiency and studies that have shown a significant correlation between MR-proADM and creatinine levels (14;30).

In conclusion, we show that MR-proADM has a strong predictive value for 30-day mortality in patients with *f*UTI compared to more conventional biomarkers. Next, studies may wish to confirm the selected cut-off value as a predictor of complicated course and evaluate in daily follow up measurements of MR-proADM the relationship to treatment and clinical recovery. Such studies will establish whether MR-proADM could function as a new prognostic tool for guidance in risk stratification and clinical outcome in patients with *f*UTI.

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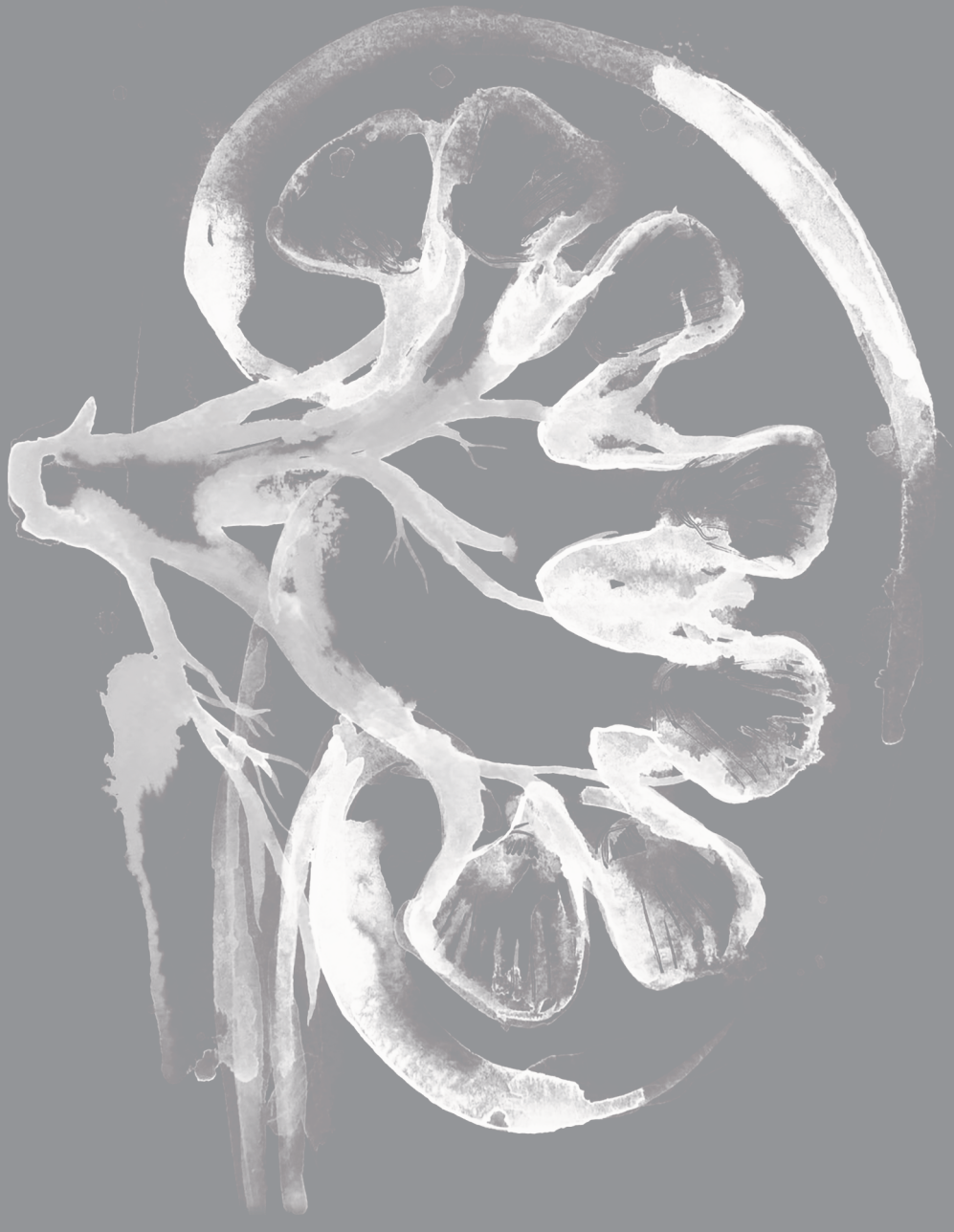

Chapter 6

Urinary proteins, vitamin D and genetic polymorphisms as risk factors for febrile urinary tract infection and relation with bacteremia: a case control study

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Abstract

Objective

Febrile urinary tract infection (UTI) is a common bacterial disease that may lead to substantial morbidity and mortality especially among the elderly. Little is known about biomarkers that predict a complicated course. Our aim was to determine the role of certain urinary cytokines or antimicrobial proteins, plasma vitamin D level, and genetic variation in host defense of febrile UTI and its relation with bacteremia.

Methods

A case-control study. Out of a cohort of consecutive adults with febrile UTI (n=787) included in a multi-center observational cohort study, 46 cases with bacteremic *E.coli* UTI and 45 cases with non-bacteremic *E. coli* UTI were randomly selected and compared to 46 controls. Urinary IL-6, IL-8, LL37, β-defensin 2 and uromodulin as well as plasma 25-hydroxyvitamin D were measured. In 440 controls and 707 UTI patients polymorphisms were genotyped in the genes *CXCR1*, *DEFA4*, *DEFB1*, *IL6*, *IL8*, *MYD88*, *UMOD*, *TIRAP*, *TLR1*, *TLR2*, *TLR5* and *TNF*.

Results

IL-6, IL-8, and LL37 are different between controls and UTI patients, although these proteins do not distinguish between patients with and without bacteremia. While uromodulin did not differ between groups, inability to produce uromodulin is more common in patients with bacteremia. Most participants in the study, including the controls, had insufficient vitamin D and, at least in winter, UTI patients have lower vitamin D than controls. Associations were found between the CC genotype of *IL6* SNP rs1800795 and occurrence of bacteremia and between *TLR5* SNP rs5744168 and protection from UTI. The rare GG genotype of *IL6* SNP rs1800795 was associated with higher β-defensin 2 production.

Conclusion

Although no biomarker was able to distinguish between UTI with or without bacteremia, two risk factors for bacteremia were identified. These were inability to produce uromodulin and an *IL6* rs1800795 genotype.

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections and has a high risk of recurrence. The predominant causal pathogen is uropathogenic *Escherichia coli* and it is thought that innate immune responses to this pathogen can both control and predispose to subsequent recurrence of UTI [1]. Although UTI by itself rarely causes significant complications, fever indicates the presence of tissue inflammation that can be accompanied with bacteremia and the urosepsis syndrome that may eventually lead to septic shock and death [2].

We have set up a large prospective multicenter cohort study to investigate various aspects of febrile UTI [3–5]. In this study we focus on urinary markers of host defense, plasma vitamin D and the role of genetic variation.

The cytokines IL-6 and IL-8 are mediators of inflammation in response to bacterial infection, and when measured in plasma or serum these may be used as early biomarkers of infection. IL-6 and IL-8 levels are also known to be elevated in the urine of patients with UTI, whereas reportedly none are measurable in the urine of healthy controls [6–10].

The human body produces several antimicrobial proteins, amongst others cathelicidin LL37 and β-defensins, that are part of our first line defense against bacterial infections. These antimicrobial proteins are produced by epithelial cells, including those of the urinary tract [11,12]. Urinary cathelicidin is elevated in children with pyelonephritis [11] and was recently reported to be also elevated in adult women during UTI [13]. Whether the amounts of cathelicidin or β-defensin produced in the urine of UTI patients is associated with disease severity such as bacteremia is unknown.

Uromodulin (also known as Tamm Horsfall protein) is the most abundant urinary protein in mammals. It adheres to fimbriae of uropathogenic *E. coli*, thus preventing its attachment to the epithelium. Uromodulin also has an immunomodulatory function [14]. In young women with recurrent UTI urinary uromodulin concentrations were similar to those in healthy controls [15]. Whether uromodulin lowers the risk for developing febrile UTI or associated bacteremia is unknown.

Vitamin D is known to play an important role in the first defense against bacterial infections; e.g. by induction of the antimicrobial proteins cathelicidin and β-defensin. High prevalence of vitamin D deficiency and insufficiency have been reported in elderly people (≥ 65 years) in the Netherlands [16,17] just as in many other regions around the world [18]. It has been shown in *in vitro* experiments that bladder epithelium from women taking vitamin D

supplements are capable of producing larger amounts of cathelicidin upon infection [19]. Thus vitamin D deficiency is believed to be an attributable factor in host susceptibility to UTI as has also been suggested in a recent case-control study [20]. Whether vitamin D status of UTI patients affects cathelicidin and β-defensin production in urine and the occurrence of bacteremia is unknown.
Many studies have found that host genetic factors influence susceptibility to human infectious diseases [21,22]. Also in UTI genetic factors likely play a role, as illustrated by the finding that positive family history is a risk factor for recurrent UTI [23] and reviewed in [1]. Studies evaluating the role of genetic factors in UTI were mainly performed in children [24–29]. No studies have investigated the contribution of host genetic factors to the development of UTI complicated by bacteremia.

The aims of the current study were: first, to investigate the production of cytokines and antimicrobial proteins in the urine of UTI patients in order to determine whether any of these proteins are risk factors for febrile UTI or predictive biomarkers for bacteremia; second, to determine whether plasma vitamin D is correlated with urinary protein production or clinical outcome; and third, to determine whether there is a correlation between genetic variants and the production of any of the proteins or occurrence of bacteremia.

Materials and Methods

Patients and controls

We conducted a prospective observational multi-center cohort study. Patients were recruited from 35 family practices and emergency departments of eight hospitals in the region. Consecutive patients (n=787) with a presumptive diagnosis of febrile UTI were considered for enrollment and those who met the entry criteria and provided written informed consent were included. Inclusion criteria for the patients were: age ≥18 years, fever (≥38.0°C) and/or a history of fever and chills within 24 hours before presentation, at least one symptom of UTI (dysuria, increased urination frequency, urgency, perineal pain, flank pain or costovertebral tenderness) and a positive nitrite dipstick test or leukocyturia as defined by a positive leukocyte esterase dipstick test or the presence of more than five leukocytes per high-power field (pyuria) in a centrifuged sediment. Exclusion criteria were: pregnancy, hemodialysis or peritoneal dialysis, a history of kidney transplantation, known presence

of polycystic kidney disease or current treatment for urolithiasis or hydronephrosis. From the patients with *E. coli* as the causal uropathogen 45 patients without bacteremia and 46 with bacteremia were randomly selected for the plasma and urine measurements (Figure 1). Controls (n=369) were recruited from the same primary healthcare centers and emergency departments if they were aged ≥18 years, had no symptoms of UTI, and provided written informed consent. Of these controls included from the primary health care centers, 46 non-febrile individuals were randomly selected for the plasma and urine measurements (Figure 1). The study was approved by the Medical Ethics Committee of the Leiden University Medical Center (approval number P08.065). Blood and urine samples were taken before the start of antimicrobial treatment. Plasma and urine were stored at -80°C within two hours after enrollment. Blood and urine cultures were analyzed using standard microbiological methods. DNA was isolated from white blood cells by a salting out method essentially as described [30] and stored at -20°C.

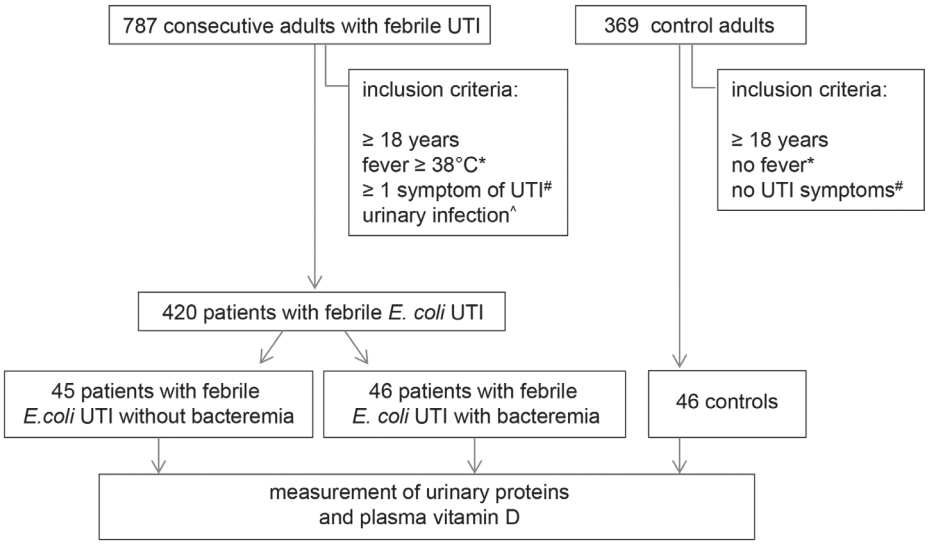

Figure 1. Selection of UTI patients and controls for the urine and plasma measurements

* and/or a history of fever and chills within 24 hours before presentation.

symptoms of UTI: dysuria, increased urination frequency, urgency, perineal pain, flank pain or costovertebral tenderness.

^ urinary infection as determined by a positive nitrite dipstick test or leukocyturia as defined by a positive leukocyte esterase dipstick test or the presence of more than five leukocytes per high-power field (pyuria) in a centrifuged sediment.

Measuring proteins in urine

Protein specific enzyme-linked immunosorbent assays (ELISA) were used to determine concentrations in urine essentially according the manufacturer's protocol with minor modifications. Urine samples were diluted two and ten times for the IL-6 ELISA, eight and 64 times for the IL-8 ELISA (Biosource). The detection limit of both ELISAs was 30 pg/ml. Urine samples were diluted four and 32 times for the LL37 ELISA (Hycult Biotechnology), the detection limit was 2 ng/ml. Urine samples were diluted four and 32 times for the β-defensin 2 ELISA (Antigenix America Inc), the detection limit was 10 pg/ml. Urine samples were diluted 200 and 400 times for the Uromodulin ELISA (MD Bioproducts), the detection limit was 20 ng/ml. Optical densities were determined at 450 nm in an iMark microplate reader (Bio-Rad).
In order to account for the gravity of the urine, protein concentrations were divided by the urine creatinine concentration. Urinary concentrations of creatinine were measured with the CREA plus (Cobas) enzymatic assay in an automated clinical chemistry analyzer.

Vitamin D assay

The concentration of 25-hydroxyvitamin D (25(OH)D) was determined in plasma by a competitive electrochemiluminescence immunoassay (ECLIA), essentially according to the manufacturer's protocol (Elecsys Vitamin D Total assay, Roche Diagnostics). Based on consensus in the literature [31] we defined vitamin D deficiency as a plasma 25(OH)D concentration ≤20 ng/ml, relative vitamin D insufficiency at plasma 25(OH)D concentrations between 20 and 30 ng/ml, and all concentrations ≥30 ng/ml as sufficient.

Genotyping

Of the 787 enrolled patients, 707 were included in the genotyping assays (of 80 samples DNA was not isolated or of low quality), as well as 440 controls (369 controls from the cohort study as well as anonymous controls). Genotyping of polymorphisms was performed by use of a Sequenom MassArray platform according to the manufacturer's protocols (Sequenom, San Diego, USA). Multiplex assays were designed with Assay designer software (Sequenom). In brief, after a multiplex PCR on 5 ng of DNA, a primer extension reaction was performed to introduce mass-differences between alleles and, after removing salts by adding a resin, 15 nl of the product was spotted onto a target chip with 384 patches containing matrix. Mass differences were detected using a Bruker Autoflex MALDI-TOF mass spectrometer and genotypes were assigned real-time using Typer 3.1 software (Sequenom). Several samples representing

the various genotypes were sequenced to confirm the genotyping results. As quality control, 10% of samples were genotyped in duplo; no inconsistencies were observed. Primer and probe sequences can be found in Table 1.

Statistical analyses

All data were entered in an SPSS database (SPSS Inc., Chicago, IL, USA; version 20.0) for statistical analysis. Graphs were generated using GraphPad Prism (GraphPad Software Inc., San Diego, California, USA; version 5.01). Descriptive analysis included medians and ranges, or means or percentages with standard deviation (sd), as appropriate. Univariate analysis was performed using the Student's t–test, the Kruskal-Wallis test or the Mann–Whitney U-test for continuous variables and the Pearson Chi-Square test for categorical variables. Measures for association were expressed as odds ratios (ORs) with their 95% confidence intervals (CI) for categorical variables.

Results

Description of patients and controls for urine and plasma measurements

During the study period, 787 patients with febrile UTI were enrolled. *E. coli* was the most frequent causal uropathogen, present in 420 (53%) of the patients. From these, 45 patients without bacteremia and 46 patients with bacteremia were selected and compared to 46 controls (Figure 1). Controls had a median age (58 years, IQR 49-75) comparable to UTI patients without bacteremia (63 years, IQR 46-75), but significant lower than UTI patients with bacteremia (73 years, IQR 60-82). The groups were comparable with respect to sex, BMI, use of immunosuppressants and vitamin D supplements. Further baseline characteristics of the study population are summarized in Table 2.

Cytokines in urine do not distinguish between patients with our without bacteremia

The production of the cytokines IL-6 and IL-8 was determined in urine samples from 44 UTI patients without bacteremia, 45 UTI patients with bacteremia and 46 controls. IL-6 was detected in 30 of all 89 (34%) UTI patients, in 12 of 44 (27%) without bacteremia and in 18 of 45 (40%) with bacteremia, and in none of the controls. Due to the large number of samples with undetectable IL-6 in each group, the median IL-6 concentrations were the same in the three groups. Both patient groups are significantly different from the controls (each $p<0.001$) but not significantly different from each other ($p= 0.21$) (Figure 2A).

Table 1. Primer and probe sequences for the multiplex genotyping assay

gene	SNPid	F primer sequence	R primer sequence	Probe sequence
TNF	rs1800629	ACGTTGGATGGGAGGCAATAGGTTTTGAGG	ACGTTGGATGTTCTGGGCCACTGACTGATT	GGCTGAACCCCGTCC
DEFB1	rs1800972	ACGTTGGATGCTGTCAGCTCAGCCTCCAAA	ACGTTGGATGTCATGGCGACTGGCAGGCAA	CAGGAACTGGGGAGA
UMOD	rs4293393	ACGTTGGATGGCTGAGAATGGCTGAAAGTC	ACGTTGGATGTGTTGTACAGAGTGGGTCAG	CAGGTCCAGTGATGTC
TIRAP	rs8177374	ACGTTGGATGGTACATGAATCGGAGCTCAG	ACGTTGGATGGCCGAGGGCTGCACCATCC	CACCATCCCCCTGCTGT
IL6	rs1800795	ACGTTGGATGAGCCTCAATGACGACCTAAG	ACGTTGGATGGATTGTGCAATGTGACGTCC	GTGACGTCCTTTAGCAT
TLR1	rs5743618	ACGTTGGATGTAACTCTGCTGATCGTCACC	ACGTTGGATGTGAGATACCAGGGCAGATCC	CAGGGCAGATCCAAGTAG
CXCR1	rs2234671	ACGTTGGATGGAATCTCAGTGGCATCCAGG	ACGTTGGATGTGAGGACCCAGGTGATCC	ggCAGGTGATCCAGGAGA
DEFA4	rs28661751	ACGTTGGATGATCACCTCTTGCCTGGAGTG	ACGTTGGATGCAGCCATGAGGATTATCGCC	tAGGATTATCGCCCTCCTC
TLR2	rs5743708	ACGTTGGATGAAAAAAGCCATTCCCCAGCG	ACGTTGGATGCAGGTAGGTCTTGGTGTTCA	TCTTGGTGTTCATTATCTTC
TLR5	rs5744168	ACGTTGGATGTCCTGGAAAAATTACAGACC	ACGTTGGATGAGATATCGGGTATGCTTGG	GGTTGTAAGAGCATTGTCTC
MYD88	rs6853	ACGTTGGATGGCGTACAAAACATGTAGAAG	ACGTTGGATGCACCTGTCCCCCTTTAATAC	ccGGCATTTTAAAGCCATCTC
DEFA4	rs736227	ACGTTGGATGGTTCCCAGCATGACATTCTC	ACGTTGGATGGTTTCACATACTGCTGCACG	ggtgaACGCGTGTCGATTAAC
CXCR1	rs3138060	ACGTTGGATGTGTGGGAGCTGAGGATTTCT	ACGTTGGATGTCCTCTTCACCTGCTAACTC	CTGCTAACTCCATGTATGAGTG
IL8	rs4073	ACGTTGGATGGGTACTATGATAAAGTTATCT	ACGTTGGATGCTGAAGCTCCACAATTTGGT	CTCCACAATTTGGTGAATTATCAA
IL6	rs10499563	ACGTTGGATGAAGCCTGGTCTGGCCTGTAT	ACGTTGGATGACCTGAAAGGAGGTAGCAGA	GATTTCTTAATTATTATACAAGCACA

IL-8 was detected in 77 of all 89 (87%) UTI patients, in 34 of 44 (77%) without bacteremia and in 43 of 45 (96%) with bacteremia, and in only four of the 46 controls (9%). The median value of the controls is 0.1 pg IL-8 / µmol creatinine, of the UTI patients without bacteremia 91 pg/µmol and of those with bacteremia 232 pg/µmol. Both patient groups are significantly different from the controls (each $p<0.0001$) but not significantly different from each other ($p= 0.06$) (Figure 2B).
A positive correlation was demonstrated between IL-8 production and age ($r=0.302$, $p<0.001$), IL-6 production ($r=0.534$, $p<0.001$), and temperature at onset ($r=0.516$, $p<0.001$). IL-8 was not correlated with presence of urinary tract disorders, recurrent UTIs or antibiotic pre-treatment.

Antimicrobial proteins in urine do not correlate with bacteremia

The antimicrobial protein β-defensin 2 was detected in 23 of all 89 (26%) UTI patients, in 10 of 44 (23%) with bacteremia and in 13 of 45 (29%) without bacteremia, and in five of the 46 controls (11%). Due to the large number of samples with undetectable β-defensin 2 in each group, the median concentrations were similar in the three groups. Only the UTI patient group with bacteremia was significantly different from the controls ($p<0.05$) (Figure 3A). Urinary β-defensin 2 showed no correlation with age, IL-8 production and temperature at onset. The β-defensin 2 was also not correlated with presence of urinary tract disorders, recurrent UTIs or antibiotic pre-treatment.
Cathelicidin LL37 was detectable in 53 of all 89 (60%) UTI patients, in 23 of 44 (52%) without bacteremia and in 30 of 45 (67%) with bacteremia, and in two of the 46 controls (4%). The median value of the controls is below the detection limit, of the UTI patients without bacteremia it is 1.2 ng LL37/µmol creatinine and of those with bacteremia 2.6 ng/µmol. Both patient groups are significantly different from the controls (each $p<0.0001$) but not significantly different from each other ($p= 0.23$) (Figure 3B). LL37 showed no difference in the presence of urinary tract disorders, recurrent UTIs and antibiotic pretreatment.

Lack of uromodulin in urine increases risk of bacteremia

Uromodulin was detectable in 78 of all 89 (88%) UTI patients, in 42 of 44 (95%) without bacteremia and in 35 of 45 (78%) with bacteremia, and in 42 of the 46 (91%) controls. The median value of the controls is 2366 ng uromodulin /µmol creatinine, of the UTI patients without bacteremia it is 1941 ng/µmol and of those with bacteremia 1851 ng/µmol. The uromodulin production is not significantly different between the groups (Figure 3C).

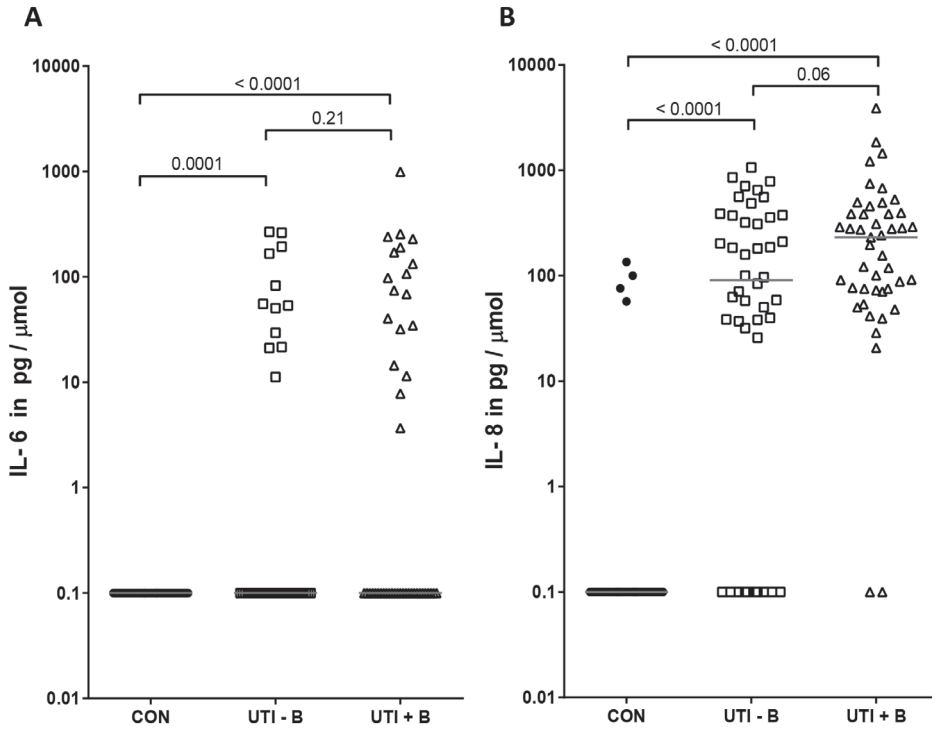

Figure 2. IL-6 or IL-8 concentrations do not distinguish UTI patients with bacteremia from those without bacteremia

IL-6 (Figure 2A) and IL-8 (Figure 2B) concentrations were determined in the urine of 46 controls (CON), 44 cases with febrile UTI without bacteremia (UTI–B), and 45 cases with febrile UTI with bacteremia (UTI+B). Cytokine concentrations depicted were corrected for urine gravity, for samples below the detection limit the corrected cytokine concentration was set to 0.1. Solid bars represent medians, for statistical analysis a Mann-Whitney test was used.

Most people produce uromodulin in the urine regardless of infection and it appears that inability to produce any uromodulin is more common in the patients with bacteremia than in patients without bacteremia (p=0.015). The odds ratio for developing bacteremia for UTI patients who do not produce uromodulin is 6.0 (95% CI: 1.2-29.2).

Uromodulin showed no correlation with blood leukocyte count, age, IL-8 or temperature at onset. Also, uromodulin did not differ in the presence of leukocyturia, urinary tract disorders, recurrent UTIs or antibiotic pre-treatment.

Most participants in the study had insufficient vitamin D levels

Plasma 25(OH)D was measured in 46 controls, 43 UTI patients without bacteremia and 44 UTI patients with bacteremia. All groups had comparable median plasma 25(OH)D concentrations (Figure 4A and Table 3).

Table 2. Baseline characteristics of the study population

	controls	febrile UTI without bacteremia	febrile UTI with bacteremia
	n = 46	n = 45	n = 46
Age in years, median [IQR]	58 [49 - 75]	63 [46 - 75]	73 [60 - 82]
Male sex	18 (39)	16 (36)	18 (39)
Comorbidity			
Any	24 (52)	33 (73)	36 (78)
Urinary tract disorder [a]	5 (11)	10 (22)	9 (20)
Urinary catheter	0 (0)	2 (4)	3 (7)
History of nephrolithiasis	5 (11)	4 (9)	5 (11)
Recurrent UTIs [b]	1 (2)	16 (36)	6 (13)
Diabetes mellitus	3 (7)	5 (11)	12 (26)
Malignancy	2 (4)	2 (4)	4 (9)
Hypertension	17 (37)	24 (53)	26 (57)
Heart failure	0 (0)	5 (11)	8 (17)
Cerebrovascular disease	1 (2)	5 (11)	12 (26)
Chronic renal insufficiency	0 (0)	3 (7)	6 (13)
COPD	2 (4)	7 (16)	3 (7)
Medication			
Immunosuppressants	1 (2)	1 (2)	4 (9)
Vitamin D supplements	2 (4)	4 (9)	4 (9)
Season of inclusion			
Winter (Dec - Feb)	46 (100)	8 (18)	5 (11)
Spring (Mar - May)	-	6 (13)	10 (22)
Summer (June - Aug)	-	11 (24)	17 (37)
Fall (Sept - Nov)	-	20 (44)	14 (30)
BMI [c]			
Mean BMI in kg/m^2 (sd)	26.5 (4.6)	26.6 (4.0)	27.3 (7.2)

Data are presented as n (%) unless otherwise stated. UTI = urinary tract infection, IQR = interquartile range, sd = standard deviation, COPD = chronic obstructive pulmonary disease, BMI = body mass index. [a] Defined as any functional or anatomical abnormality of the urinary tract except urinary catheter and history of nephrolithiasis. [b] Defined as ≥2 UTIs in the last 6 months or ≥3 UTIs in the last 12 months. [c] Eight missing BMI data: 2 in controls, 1 in UTI without bacteremia and 5 in UTI with bacteremia.

Table 3. Plasma 25(OH)D concentrations and vitamin D status

	controls n = 46	febrile UTI without bacteremia, n = 43	febrile UTI with bacteremia, n = 44
Median 25(OH)D in ng/ml [IQR]	17.6 [13.9 - 26.4]	19.5 [14.1 - 26.1]	17.9 [9.4 – 24.7]
vitamin D sufficient	6 (13%)	6 (14%)	8 (18%)
vitamin D insufficient	14 (30%)	15 (35%)	11 (25%)
vitamin D deficient	26 (57%)	22 (51%)	25 (57%)

UTI= urinary tract infection, IQR = interquartile range. Vitamin D sufficiency is defined as 25(OH)D of ≥30 ng/ml, vitamin D insufficiency is defined as 25(OH)D >20 and <30 ng/ml, vitamin D deficiency is defined as 25(OH)D of ≤ 20 ng/ml.

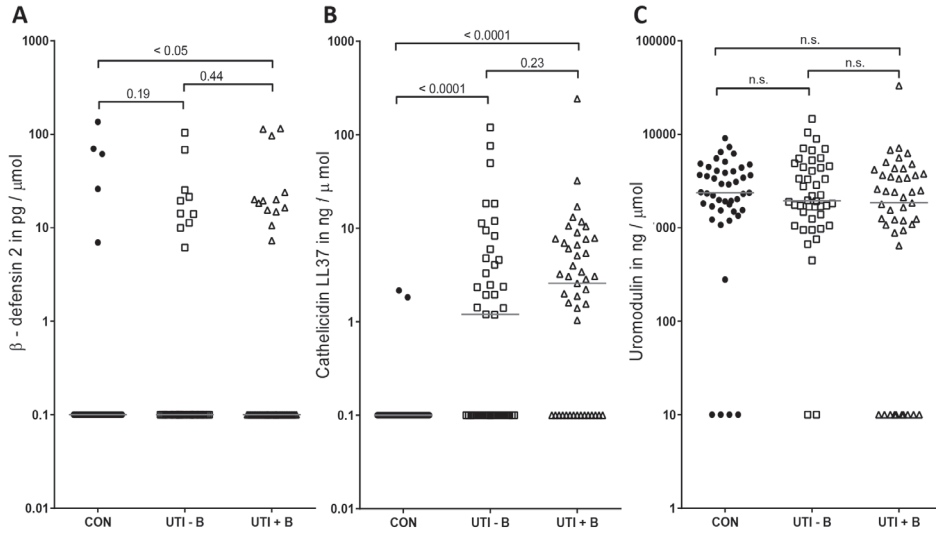

Figure 3. Antimicrobial protein concentrations do not distinguish UTI patients with bacteremia from those without bacteremia

Concentrations of the antimicrobial proteins β-defensin 2 (A) Cathelicidin LL37 (B) and Uromodulin (C) were determined in the urine of 46 controls (CON), 44 febrile UTI cases without bacteremia (UTI–B), and 45 febrile UTI cases with bacteremia (UTI+B). Protein concentrations depicted were corrected for urine gravity, for samples below the detection limit the corrected protein concentration was set to 0.1 (β-defensin 2, Cathelicidin LL37) or 10 (Uromodulin). Solid bars represent medians, for statistical analysis the Mann-Whitney U test was used.

Of the 133 individuals analysed, only 22 (17%) had a sufficient vitamin D level. More than half (n=73, 55%) had a vitamin D deficiency, while severe vitamin D deficiency (with 25(OH)D ≤10 ng/ml) was found in 25 participants (19%): 5 controls (11%), 8 UTI patients without bacteremia (19%) and 12 UTI patients with bacteremia (27%). Because vitamin D concentrations vary by season, we examined the effect of seasonal variation on our findings. All controls were recruited in winter. Patients with UTI were included in all seasons: 58 (44%) in winter, 16 (12%) in spring, 27 (20%) in summer and 32 (24%) in fall. The vitamin D concentration was significantly lower in UTI patients recruited in winter than in other seasons. More importantly, the controls that were all recruited in winter had a higher vitamin D concentration compared to cases recruited in winter (17.6 [IQR 13.9-26.4] ng/ml vs. 13.0 [IQR 6.9-17.3] ng/ml, p=0.015) (Figure 4B). Cathelicidin LL37 (r= -0.69, p=0.436) and β-defensin 2 (r= -0.003, p=0.969) in the urine were not correlated with plasma vitamin D.

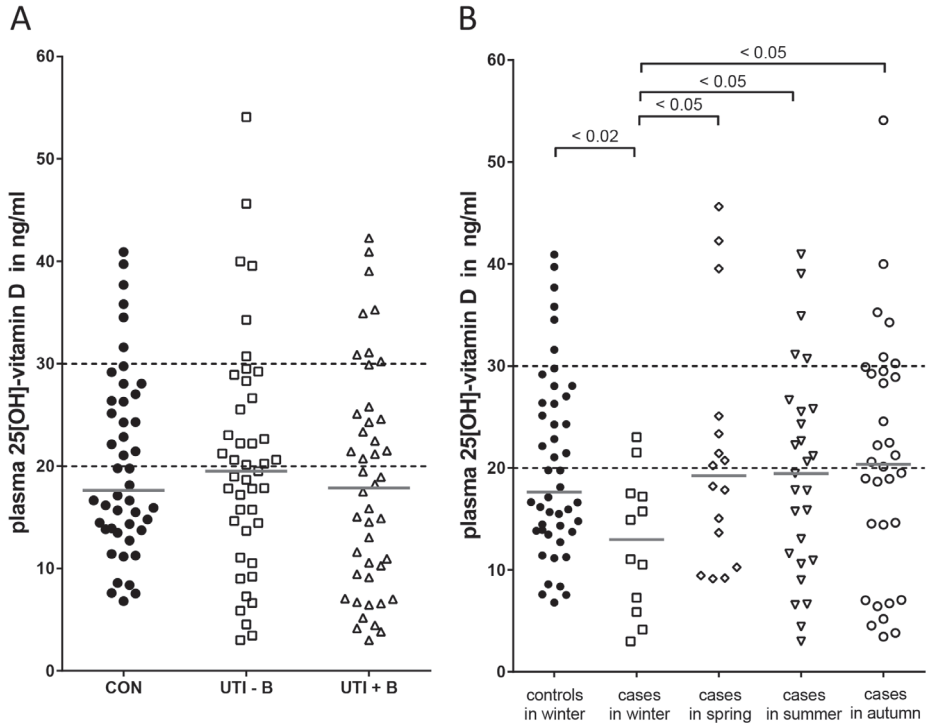

Figure 4. Plasma vitamin D concentrations in controls and UTI patients

Plasma 25(OH) vitamin D concentrations were determined in 46 controls (CON) and 43 UTI patients without bacteremia (UTI-B) and 44 UTI patients with bacteremia (UTI+B) (A). Analysis of plasma 25(OH)D vitamin D concentrations according to season of sampling (B). All controls were recruited in winter. 12 UTI patients were recruited in winter, 16 in spring, 27 in summer and 32 in autumn. Solid bars represent median concentrations; dotted lines represent upper limit of vitamin D deficiency (≤20 ng/ml) and lower limit of vitamin D sufficiency (≥30 ng/ml). The Mann-Whitney U test was used to determine whether groups were statistically different.

Distribution of genetic variation in cases and controls

In most infectious diseases, in addition to exposure, strain virulence and environmental factors, genetic host variations also play a role in susceptibility to disease. To determine whether genetic variations attribute to susceptibility to febrile UTI, we genotyped 15 single nucleotide polymorphisms (SNPs) in 12 genes of 440 controls and 707 febrile UTI patients (Table 4). All SNPs were in Hardy Weinberg equilibrium in the controls.

None of the SNP alleles (data not shown) or genotypes were found to be associated with susceptibility to febrile UTI when analyzing controls versus patients (Table 4). Because the presence of urinary tract disorders or chronic renal insufficiency may mask genetic effects we re-analyzed the data after exclusion of individuals with known urinary tract disorders or chronic renal insufficiency. This revealed that the *TLR5* SNP rs5744168 was significantly

Table 4. Genotypes of genetic variations in controls and UTI patients

Gene	SNPid	common name or location	genotypes	controls genotypes, n (%)	UTI patients genotypes, n (%)	p-value
CXCR1	rs3138060	217C>G	CC	380 (87.6%)	623 (88.3%)	0.581
		in intron	CG	52 (12.0%)	82 (11.6%)	
			GG	2 (0.4%)	1 (0.1%)	
CXCR1	rs2234671	2608 G/C*	GG	379 (87.1%)	620 (88.0%)	0.574
		Ser276Thr	CG	54 (12.4%)	84 (11.9%)	
			CC	2 (0.5%)	1 (0.1%)	
DEFA4	rs28661751	A8P	CC	427 (98.4%)	700 (99.3%)	0.373
			CG	7 (1.6%)	6 (0.7%)	
			GG	0	0	
DEFA4	rs736227	in 3'UTR	TT	196 (45.4%)	324 (46.1%)	0.962
			CT	196 (45.4%)	313 (44.5%)	
			CC	40 (9.2%)	66 (9.4%)	
DEFB1	rs1800972	-44C>G*	CC	264 (60.8%)	426 (60.4%)	0.450
		-668C>G*	CG	147 (33.9%)	252 (35.7%)	
			GG	23 (5.3%)	27 (3.8%)	
IL6	rs10499563	-6331T>C*	TT	277 (63.8%)	403 (57.4%)	0.100
			CT	141 (32.5%)	269 (38.3%)	
			CC	16 (3.7%)	30 (4.3%)	
IL6	rs1800795	-174G>C*	GG	147 (33.7%)	249 (35.2%)	0.711
			CG	209 (47.9%)	340 (48.2%)	
			CC	80 (18.4)	117 (16.6%)	
IL8	rs4073	-251A/T*	TT	130 (29.9%)	207 (29.4%)	0.985
			AT	212 (48.7%)	346 (49.1%)	
			AA	93 (21.4%)	151 (21.5%)	
MYD88	rs6853	in 3'UTR	AA	331 (76.6%)	551 (78.6%)	0.120
			AG	92 (21.3%)	145 (20.7%)	
			GG	9 (2.1%)	5 (0.7%)	
UMOD	rs4293393	5' near gene	TT	286 (65.9%)	460 (65.2%)	0.354
			CT	127 (29.3%)	220 (31.1%)	
			CC	21 (4.8%)	26 (3.7%)	
TIRAP	rs8177374	S180L*	CC	310 (71.1%)	498 (70.0%)	0.995
		C539T*	CT	116 (26.6%)	189 (26.7%)	
			TT	10 (2.3%)	16 (2.3%)	
TLR1	rs5743618	1805 G/T*	GG	179 (42.0%)	315 (45.4%)	0.379
		S602I*	GT	193 (45.3%)	285 (41.1%)	
			TT	54 (12.7%)	94 (13.5%)	
TLR2	rs5743708	Arg753Gln*	GG	408 (93.2%)	662 (93.8%)	0.773
		R753Q*	AG	30 (6.8%)	44 (6.2%)	
			AA	0	0	
TLR5	rs5744168	R392X*	CC	363 (84.2%)	626 (89.0%)	0.072
		Arg392Stop*	CT	63 (14.6%)	72 (10.2%)	
		1174C>T*	TT	5 (1.2%)	6 (0.8%)	
TNF	rs1800629	-308 G/A*	GG	282 (64.6%)	486 (68.8%)	0.170
		in promoter	AG	144 (33.0%)	197 (27.9%)	
			AA	11 (2.5%)	23 (3.3%)	

* name used in the literature

different between patients and controls (p=0.011). In addition we analyzed the distribution of genotypes between UTI patients with and those without bacteremia. The distribution of one SNP, rs1800795 in the gene *IL6*, was significantly different as the genotype CC is more common among UTI patients with bacteremia (p= 0.009) (Table 5).

The production of proteins can often be correlated to genetic variations in for instance the promoter region or translation start site. Most individuals in which urinary protein concentrations were measured in this study were also genotyped, therefore we were able to investigate whether any of the genotypes were correlated to urinary protein production. A significant difference in β-defensin 2 production was found between the *DEFB1* rs1800972 genotypes, as most individuals with an CC or CG genotype did not produce β-defensin 2 while the three individuals with an GG genotype did (p=0.0002) (Figure 5). No significant correlations were found between other genotypes and protein production (data not shown).

Table 5. Genotypes of *IL6* variation in UTI patients with and without bacteremia

Gene	SNPid	genotypes	UTI without bacteremia, n (%)	UTI with bacteremia, n (%)	p-value
IL6	rs1800795	CC	76 (15.0%)	37 (23.9%)	0.009
		CG	257 (50.9%)	57 (36.8%)	
		GG	172 (34.1%)	61 (39.3%)	

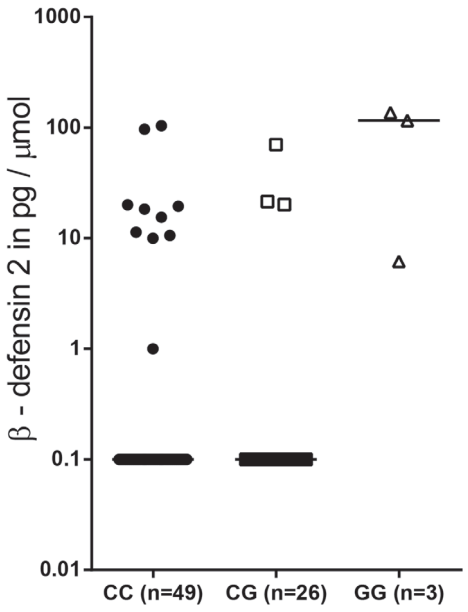

Figure 5. β-defensin 2 production is associated with a DEFB1 genotype

Concentration of the antimicrobial protein β-defensin 2 grouped per DEFB1 rs1800972 genotype, the groups are significantly different (p=0.0002). Protein concentrations were corrected for urine gravity, for samples below the detection limit the corrected cytokine concentration was set to 0.1. Solid bars represent medians, for statistical analysis the Kruskal-Wallis test was used.

Discussion

We analyzed various proteins in urine of UTI patients and controls from a prospective cohort study and found that while IL-6, IL-8 and cathelicidin LL37 are different between UTI patients and controls, these proteins, as well as β-defensin 2, cannot be used as biomarkers for bacteremia. We did find that while uromodulin production is in general not different between patients and controls, complete absence of uromodulin production in the urine is a strong risk factor for bacteremia. In addition we found that plasma vitamin D may have a protective effect against UTI as, at least in winter, vitamin D is lower in UTI patients compared to controls.

The strength of this study is that we have collected the largest prospective UTI cohort thus far with a clinically and microbiologically well-characterized disease group. Our emphasis in the analyses was on the identification of host defense risk factors for febrile UTI and predictive markers for bacteremia. Therefore we determined urinary proteins and plasma vitamin D in 91 patients (of which 46 had bacteremia) and 46 controls, and genotyped polymorphisms in genes thought to play a role in UTI in a large number of patients (n=707) and controls (n=440). The three groups of individuals in which urinary proteins and plasma vitamin D were determined were in general well matched, except for age which was higher in patients with UTI and bacteremia.
In general, IL-6 and IL-8 levels are elevated in the urine of patients with UTI and children with acute pyelonephritis, whereas none are measurable in the urine of controls [6–10]. This study confirms that IL-6 and IL-8 are elevated in UTI patients; we did however also find four controls that produced IL-8. One of the controls had asymptomatic bacteriuria; for the other three it is unclear why they produce IL-8. Production of IL-8 has also occasionally been observed in healthy children [8,9]. While IL-6 and IL-8 are good biomarkers for infection, we found that they cannot distinguish between patients with or without bacteremia. In a Swedish study a significant difference in IL-6 production was found between bacteremic and non-bacteremic patients, 24 hours after inclusion [32]. In that study, UTI patients were all hospitalized, suggesting they had far more severe clinical symptoms than our patients who were largely recruited at family practices. In addition, similar to the samples in our cohort, at inclusion IL-6 production in the Swedish patients was not significantly different between patients with or without bacteremia [32].

As previously reported in children and adults with UTI [11,13], we also found that urinary cathelicidin LL37 is elevated in adults with UTI and appears to be a biomarker for infection. Cathelicidin LL37 was however not different between patients with and without bacteremia, making it unsuitable as a biomarker for bacteremia.
β-defensin 2 has been previously shown by RT-PCR and immunohistochemistry to be produced in kidneys from patients with chronic upper UTI undergoing nephrectomy but not in normal kidneys [12]. In addition it was reported that β-defensin 2 mRNA transcription by renal tubular cells *in vitro* could be upregulated by *E. coli* [33]. We determined the β-defensin 2 protein production by ELISA and found that β-defensin 2 was detectable in the urine of a minority of both controls and patients and did not differ between them. There was also no difference in β-defensin 2 production between patients with or without bacteremia, making it unsuitable as a biomarker for bacteremia.
Nitschke *et al* observed that stimulation with IL-6 did not affect β-defensin 2 production in renal tubular cells [33].We determined whether there was a relation between IL-6 and β-defensin 2 in urine. 30 out of 89 patients produce IL-6 and 23 out of 89 patients produce β-defensin 2 while only six produce both, which is even slightly less than the expected number (eight) if they are not correlated. This suggests that these two proteins are not induced by the same stimuli.
Uromodulin is known to be produced constitutively in urine and was previously reported not to be correlated with recurrent UTI in young women [15]. We found that the production also did not differ between the elderly patients and controls in our cohort or between patients with or without bacteremia. We did however observe that the inability to produce uromodulin in the urine greatly increased the risk of developing bacteremia (OR 6.0, 95% CI: 1.2-29.2). Uromodulin is produced in the thick ascending loop of Henle and secreted into the urine via proteolytic cleavage. It is a known biomarker for kidney disease [34]. Of the 16 individuals who did not produce detectable uromodulin only a few had a condition that affected the kidneys: two patients with bacteremia had chronic kidney insufficiency and one other had urothelial cell carcinoma. One control had only one kidney but that in itself does not explain the lack of uromodulin production. Lack of uromodulin production in the urine appears to be a valuable biomarker for bacteremia in vulnerable UTI patients.

The majority of the individuals in our study had, according to commonly used standards [31], insufficient vitamin D in their blood, while severe vitamin D

deficiency was found in many as well. However, there is still debate about the definition of vitamin deficiency [35] but regardless of the criteria used we found comparable median plasma vitamin D concentrations between the three groups. Unfortunately, all samples for vitamin D measurement in controls were recruited in winter. That may mask a difference between UTI patients and controls. When comparing controls with patients recruited in the same season, controls had significantly higher plasma vitamin D. Previously, low vitamin D levels were shown to be associated with bacterial and viral infections [36]. A recent study also showed that vitamin D levels in patients with recurrent UTI were found to be lower than in controls [20]. However, in that study it was unclear whether recruitment of cases and controls were equally distributed upon the different seasons. Based on *in vitro* experiments with bladder biopsies from women before and after vitamin D supplementation it has been suggested that the protective effect of vitamin D against UTI may be through the regulation of cathelicidin LL37 production in the bladder [19]. In this respect, one might expect that higher vitamin D levels might to some extent have a protective effect against invasive UTI and bacteremia via cathelicidin. We however did not find an association of plasma vitamin D with urinary cathelicidin LL37. Furthermore, there was no association between either vitamin D level or urinary cathelicidin LL37 with the presence of bacteremia.

We selected fifteen polymorphisms in genes with a known role in the recognition, the defense, or immune response to uropathogens (*TLR1, TLR2, TLR5, TIRAP, MYD88*, *IL6, IL8, DEFA4, DEFB1, UMOD, CXCR1, TNF*). None of the alleles or genotypes was associated with febrile UTI when analyzing all controls versus all patients. Because the presence of urinary tract disorders or chronic renal insufficiency may mask genetic effects we re-analysed these data after exclusion of individuals with known urinary tract disorders or chronic renal insufficiency. This revealed an association between *TLR5* SNP rs5744168 and *protection from* UTI. The same SNP was previously found to be associated with *increased susceptibilit*y to recurrent UTI in Caucasian American women (339 recurrent UTI, 321 pyelonephritis, 317 controls) [26]. The discrepancy in the allele associated with protection from UTI suggests that the *TLR5* SNP is not itself the functional variant causing the protective effect but rather linked to a functional variation in the vicinity. The different genetic backgrounds in the two studies may cause the functional variant to co-segregate with different alleles. The *TLR5* polymorphism is also a lot more common in our population than in the Caucasian American population (CT

and TT 15.8% in our controls vs 7.4% in the Caucasian American controls) thus revealing a difference in distribution. More SNPs in and around *TLR5* need to be analyzed to pinpoint the causal variation.
Various other genetic variations have also been reported to be associated with protection from, or the risk of developing UTI [27]. We analyzed several of these but none were associated with febrile UTI in our cohort: the *TLR2* polymorphism Arg753Gln that was associated with UTI in Turkish children (124 patients, 116 controls) [24], the *TLR1* 1805T allele that was associated with pyelonephritis in Caucasian American women (339 recurrent UTI, 321 pyelonephritis, 317 controls) [26] and five *CXCR1* polymorphisms (of which we tested two) that were associated with acute pyelonephritis in a Swedish cohort (60 patients, 226 controls) [37]. These associations have thus far not been confirmed in other independent cohorts either. The lack of association in our cohort may be due to a difference in genetic background or due to the selection of elderly patients in our cohort, as at an elderly age comorbidity and an aging immune system may have more effect on susceptibility to UTI than subtle genetic variations. We analyzed the largest cohort so far (440 controls and 707 patients) therefore the power to detect an association (if present) is greater than in the other cohorts.

We found one polymorphism, the *IL6* SNP rs1800795, specifically the CC genotype, to be associated with development of bacteremia. This SNP is located in the promoter of *IL6* and is known to affect the IL-6 concentration in blood plasma, with the GG genotype producing more IL-6 [38,39]. Unfortunately, because not enough individuals produce IL-6 in their urine we were unable to determine whether the GG genotype is also correlated with IL-6 production in urine. It is however likely that the CC genotype increases susceptibility to UTI due to low IL-6 production. In one study, a significantly higher urinary IL-6 production was found in bacteremic than in non-bacteremic patients, at 24 hr after inclusion [32]. In that study it was also found that IL-6 response kinetics were different in bacteremic and non-bacteremic patients, with IL-6 in non-bacteremic patients peaking early and declining afterwards while IL-6 in bacteremic patients peaks late and stays high [32]. It would be interesting to determine whether these response kinetics are under genetic control. In mouse models of bladder infection with uropathogenic *E. coli*, transcription of the *il6* gene was found to be very highly upregulated at 2 hr and moderately upregulated at 24 hr after infection, with response kinetics varying between mouse strains, suggesting genetic control [40]. In addition, the causative uropathogen was also found

to influence the response kinetics, with group B streptococcus inducing a far less pronounced response than *E. coli* [41].

In conclusion, we did not identify a convenient biomarker that will allow us to distinguish between patients with or without bacteremia. We did find a risk factor, lack of uromodulin, that is present in few patients but greatly increases their risk of developing bacteremia. In addition we identified several modest genetic associations, especially between *TLR5* SNP rs5744168 and UTI and between *IL6* SNP rs1800795 and occurrence of bacteremia.

Acknowledgement

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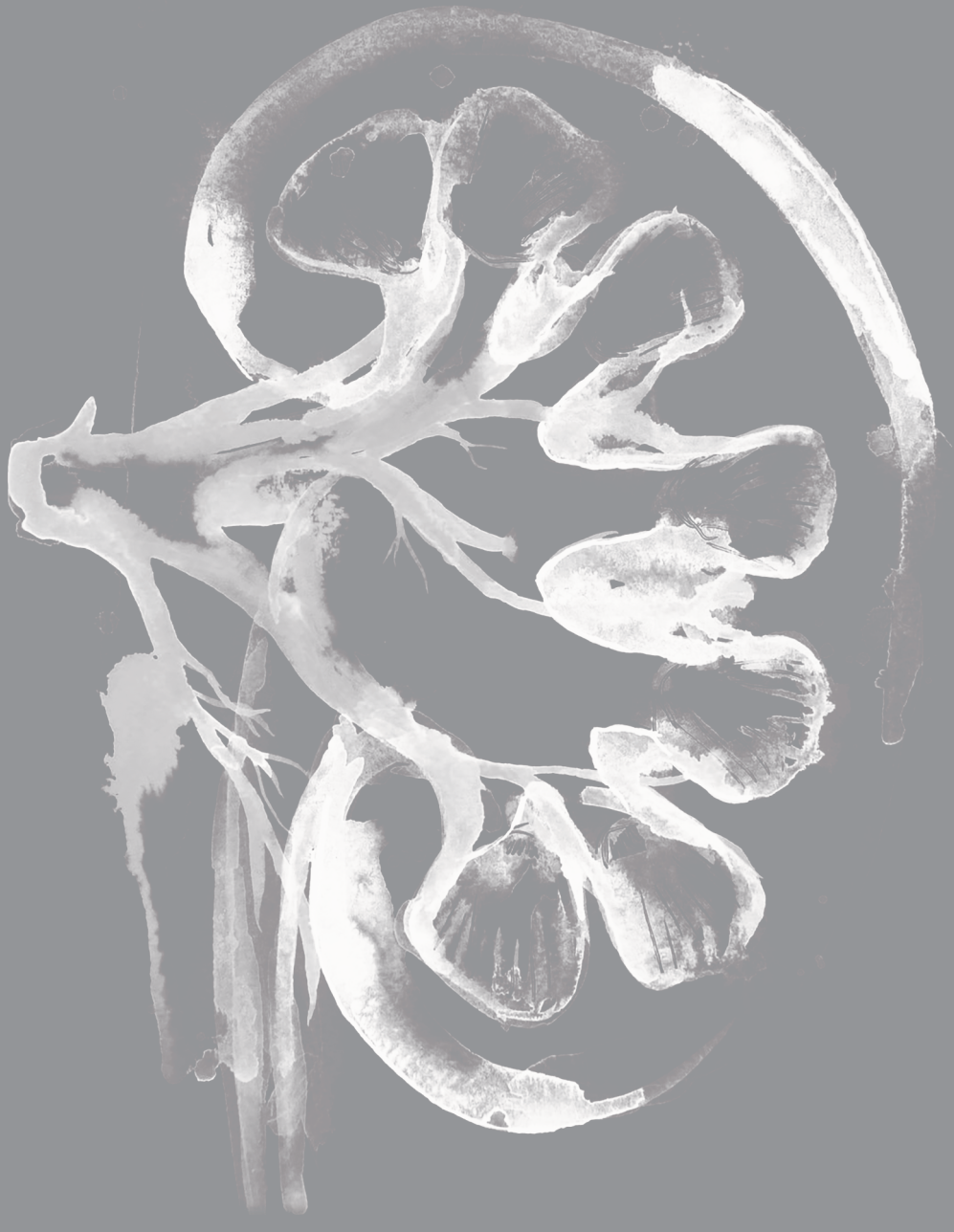

Chapter 7

Procoagulant tissue factor activity on microparticles is associated with disease severity and bacteremia in febrile urinary tract infections

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Abstract

Introduction

Inhibition of tissue factor, the primary initiator of coagulation in sepsis, attenuates morbidity in primates infused with *Escherichia coli*. In a human endotoxemia model, microparticles expressing procoagulant TF (MP-TF) are released in blood concurrently with markers of inflammation and coagulation. We investigated whether the release of MP-TF into blood is accompanied by procoagulant and inflammatory changes in patients with *E. coli* urinary tract infection.

Materials and methods

In a multicenter cohort study, we determined clinical disease severity using APACHE II scores and measured plasma MP-TF activity, TAT, sE-selectin, sVCAM-1, procalcitonin and monocyte count in blood of 215 patients with community-acquired febrile *E. coli* urinary tract infections.

Results

Plasma MP-TF activity on admission corresponded with clinical disease severity (APACHE II score; P=0.006) and correlated significantly but weakly with plasma markers of disease severity (sE-selectin, sVCAM-1, procalcitonin). Additionally, median plasma MP-TF activity was higher in patients than in healthy controls (197 vs. 79 fM Xa/min; P<0.0001), and highest in bacteraemic patients (325 fM Xa/min). MP-TF activity showed a weak inverse correlation with monocyte count (rs -0.22; P=0.016) and a weak correlation with TAT (rs 0.23, P=0.017). After 3 days of antibiotic treatment, upon resolution of the infection, plasma MP-TF activity and TAT concentrations declined.

Conclusions

Microparticle-associated procoagulant tissue factor activity is related to disease severity and bacteremia in febrile *E. coli* UTI patients and may contribute to the prothrombotic state in gram-negative sepsis.

Introduction

Sepsis is associated with activation of the coagulation cascade, which may range from subclinical to widespread microvascular thrombosis and disseminated intravascular coagulopathy. Blood microparticles are highly mobile carriers of pro-inflammatory mediators and procoagulant proteins and could play a major role in the onset of sepsis-related morbidities and mortality.
Tissue factor (TF), the primary initiator of coagulation *in vivo*, is thought to play an important role in sepsis. In primates, inhibition of the tissue factor pathway with anti-TF monoclonal antibodies, tissue factor pathway inhibitor (TFPI) or active site-inactivated FVIIa attenuated coagulopathy and prevented acute lung injury, renal failure and mortality in septic shock caused by *Escherichia coli* [1-3]. Furthermore, in human endotoxemia models, coagulopathy improved following infusion of TFPI or recombinant human IL-10 (known to inhibit LPS-induced TF activity and monocyte TF-expression, respectively) [4, 5].
In a kinetic study of healthy volunteers challenged intravenously with purified *E. coli* lipopolysaccharide, we demonstrated that microparticles bearing functional procoagulant TF (MP-TF activity) are concurrently released with markers of inflammation and coagulation [6]. Interestingly, the subject with the most prominent clinical response to endotoxin also had the highest MP-TF activity. The role of microparticles expressing tissue factor (MP-TF) in the pathogenesis of sepsis has not yet been elucidated.
As inflammatory conditions caused by an intact pathogen may similarly induce shedding of MP-TF, we investigated the relation between MP-TF activity, bacteremia and clinical disease severity using the APACHE II score in a large cohort of patients with febrile *E. coli* urinary tract infection (UTI). In addition, we assessed whether MP-TF activity decreased upon resolution of the infection and explored the association between MP-TF activity, markers of disease severity (sE-selectin, sVCAM-1, procalcitonin) and blood monocyte count. Finally, we examined levels of thrombin-antithrombin complex (TAT) as a marker of coagulation in a subset of patients.

Materials and methods

Study design

From January 2004 to December 2009, we enrolled 787 consecutive patients presenting with community-onset febrile urinary tract infections at the emer-

gency departments of seven hospitals and 35 affiliated primary healthcare centers in the western part of The Netherlands [7]. This multicenter cohort study was approved by the Medical Ethical Committees of the participating centers and all patients gave written informed consent.

Inclusion criteria were age ≥18 years, ear temperature ≥38.0°C or a history of fever and rigors within 24 hours prior to presentation, at least one symptom of UTI (dysuria, frequent or urgent urination, perineal pain, flank pain or costovertebral tenderness), and positive nitrite dipstick test or leukocyturia. Leukocyturia was defined as a positive leukocyte esterase dipstick test or the presence of >5 leucocytes per high-power field in the urine sediment. Exclusion criteria were current treatment for urolithiasis or hydronephrosis, hemodialysis, kidney transplantation or polycystic kidney disease.

From the initial cohort of 787 patients, we selected all 420 patients in whom *E. coli* was cultured from the urine sample obtained upon admission. We excluded 156 patients with pre-existing disorders or severe co-morbidity, i.e. cancer, autoimmune disease, diabetes, cerebrovascular accident or heart failure, because elevated plasma levels of microparticles and MP-TF activity have been reported in patients with these disorders [8-12]. This exclusion criterion resulted in a cohort of 264 fairly healthy patients at the onset of their urinary tract infection, since abovementioned disorders and comorbidities were more prevalent amongst the majority of the critically-ill patients with the highest APACHE II scores. Another 49 patients were excluded because blood cultures or frozen plasma samples could not be retrieved, rendering a final study cohort of 215 patients.

As required by the study protocol, all patients received empirical intravenous or oral antibiotic treatment upon admission according to local hospital policy. Antimicrobial therapy consisted of intravenously administered cefuroxime (n=89; of which 39 in combination with gentamicin) or oral treatment with ciprofloxacin (n=106). A small number of patients (n=20) was treated with other oral or intravenous antibiotic agents directed against gram negative micro-organisms.

Healthy volunteers without history of fever or infectious disease were recruited in the participating primary healthcare centers and amongst laboratory staff. Age and gender were similar to that of the study patients (63% female, median age 59 years [range 24-76]), as well as the protocol for blood collection and processing.

Procedures and definitions

Clinical data and routine laboratory measurements were collected by the clinical investigators and qualified research nurses. Baseline data of the patients were obtained within 24 hours using a standardized questionnaire and by reviewing medical records. Double data entry was performed by two independent data managers and both entries were compared for discrepancies.

We calculated the most commonly used clinical disease severity score for septic patients, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score. Patients were allocated to previously reported score categories, allowing assessment of the severity of the disease and providing an estimate of in-hospital mortality risk (an APACHE II score of 1-4 corresponded with a 4% mortality rate, whereas APACHE II scores of 5-9, 10-14, and 15-19 translated into an observed mortality rate of 8%, 15% and 25%, respectively) [13]. Clean midstream-catch or catheter-port urine were collected upon admission and cultured using local standard microbiological methods. Urine samples were considered infected in case of bacterial growth $>10^3$ CFU per ml urine or a bacterial monoculture $>10^2$ CFU per ml urine in the presence of leukocyturia.

Disease burden was systematically quantified in all 215 patients by taking blood cultures on admission. Patients were considered to have bacteremia when *E. coli* was cultured.

At baseline (day 0) and three days thereafter (day 3), venous blood samples were collected into EDTA [ethylenediaminetetraacetic acid] BD Vacutainer tubes (Franklin Lakes, NJ, USA) applying minimal venostasis and discarding the first tube. Plasma was prepared by removing cells through a single centrifugation step at 3500 x *g* (5 minutes at room temperature). Aliquots were transferred immediately to polypropylene tubes and frozen at -80°C to enable future simultaneous analysis of day 0 and 3 samples. Sample processing time from venepuncture to storage at -80°C was less than an hour for the majority of study patients and well within two hours for all patients as required by the study protocol. Plasma samples remained deep-frozen until analysis.

Isolation of microparticles and MP-TF activity assay

After thawing of deep-frozen EDTA-anticoagulated plasma samples, microparticles were pelleted and repeatedly washed with pH 7.45 filtered 0.32% citrate/PBS buffer (30 minutes at 18,890 x *g* with minimum brake, 20°C) thus diluting plasma constituents and EDTA more than 200-fold. The mic-

roparticle suspension was subsequently recalcified and incubated in a 1:5 ratio (v/v) with 10 mM pH 7.45 Hepes, 137 mM NaCl, 4 mM KCl, 5 mg/ml ovalbumin, 50 nM hirudin, 6 mM $CaCl^2$ and 25 μM negatively charged phospholipid vesicles (dioleoylphophatidylserine/dioleoylphosphatidylcholine 1/9). TF/FVII complex formation was initiated by the addition of FVII (Kordia, The Netherlands). The reaction was started by the addition of S2765 (Chromogenix, Italy) and FX (Kordia, The Netherlands). Subsequently, cleavage of the chromogenic substrate S2765 by the generated FXa was recorded during 90 minutes (absorbance at 405 nm). Parallel experiments were performed in the absence of FVII and in the presence of excess polyclonal sheep anti-human TF-IgG (Affinity Biologicals Inc., Canada) to demonstrate FVII and TF-dependency, respectively. MP-TF activity, defined as FVII- and TF-dependent FXa formation, was calculated as previously described [8] and expressed as fM Xa/min in plasma assuming a 100% microparticle recovery. None of the plasma samples used for isolation of microparticles had been thawed before and all samples were analysed within one year after completion of the study. In previous experiments on plasma samples stored for more than 15 months, we did not observe degradation of active TF on microparticles after prolonged frozen storage; the same MP-TF activity was found in aliquotted samples from 16 patients after prolonged frozen storage.
Nineteen healthy volunteers were recruited to establish reference values for MP-TF activity, although power calculations indicated that a sample size of merely 9-10 for each group would suffice to detect a statistical significant difference. We defined elevated plasma MP-TF activity as levels >172 fM Xa/min, indicating the 99^{th} percentile of MP-TF activity of these 19 healthy controls (e.g. mean MP-TF activity + 2 SD).

Other assays

Endothelial activation and leukocyte recruitment play a key role in inflammation and are characterized by ectodomain shedding of adhesion molecules. As levels of soluble adhesion molecules have been related to disease severity [14], soluble E-selectin and VCAM-1 levels were measured by ELISA according to the manufacturer's protocol (Diaclone, Besançon, France). Procalcitonin, a sensitive biomarker differentiating bacterial infection from non-infectious inflammation [15], was routinely measured using a Time Resolved Amplified Cryptate Emission technology assay (TRACE®, Kryptor compact, PCTsensitive; Brahms AG; Hennigsdorf, Germany). C-reactive protein (CRP) levels were not measured considering the higher diagnostic accuracy of procalcitonin, which is already peaking at 8 hours following an infectious stimulus, in

contrast to 36 hours for CRP. To detect whether (subclinical) coagulation had occurred *in vivo*, thrombin-antithrombin complex, was quantified using an enzyme immunoassay according to the manufacturer's protocol (Enzygnost, Malburg, Germany). TAT levels of > 4.2 µg/L were considered to be elevated.

Statistical analysis

As the distributions of biomarkers were skewed, univariate analysis was performed using the Mann-Whitney *U*-test. Additionally, (M)ANOVA was used to compare between APACHE II disease severity groups after correction for the skewed distribution by log-transformation of data. When appropriate the paired-samples T-test was performed on log-transformed data. Correlations were assessed using the Spearman's rho (*rs*) test. *P*-values <0.05 were considered significant. For all analyses, SPSS Statistics software (version 20.0; IBM Corporation, Armonk, NY, USA) was used.

Results

Patient characteristics

The median age in the study population of 215 patients with febrile *E. coli* UTI was 51 years (range 18-96 years) and 73% were female. Almost all patients were capable of daily self-care activities. The majority of patients (70.1%) had signs of pyelonephritis, 64.6% presented with rigors, while only 22.3% had bacteremia confirmed by blood culture testing, and 37.1% reported vomiting. Relevant comedication consisted of antihypertensive agents (23.3%) and agents to reduce the heart rate (10.2%). A large proportion of patients (63.3%) took antipyretics at the time of admission. Antibiotic treatment was already started in 34 of 215 (15.8%) patients for a median period of 2 days (range 1-10 days) prior to inclusion, and switched upon inclusion in 32 of 34 patients.

As classified by the APACHE II disease severity score, most patients had a low mortality risk. Only thirty-four patients (15.8%) had an APACHE II score of 10-14, whereas 10 patients (4.7%) had an APACHE II score of 15-17 corresponding with a mortality risk of 15% and 25%, respectively. None of the patients had an APACHE II score ≥ 18. Approximately half of the patients (n=107) was admitted to the hospital, from whom only one patient was directly admitted to the intensive care unit (ICU). None of the 215 patients died from urosepsis. Furthermore, none of the 215 patients was diagnosed with clinically manifest thrombosis.

Disease severity and plasma MP-TF activity at baseline

Median plasma MP-TF activity was higher in patients with febrile *E. coli* UTI than in healthy subjects (197 fM Xa/min [IQR 113-398] vs. 79 fM Xa/min [IQR 57-126]; Fig. 1A). Patients with proven bacteremia had higher median plasma MP-TF activities (325 fM Xa/min [IQR 166-641 fM Xa/min] than patients with localized infection and negative blood cultures (186 fM Xa/min; IQR 110-324; Fig. 1B). Plasma MP-TF activity also corresponded with clinical disease severity as determined by APACHE II scores (Fig. 1C). Patients with APACHE II scores of 0-4 had a median plasma MP-TF activity of 152 fM Xa/min [IQR 98-280], whereas patients in higher APACHE II score categories had higher median MP-TF activities: 227 fM Xa/min [IQR 123-501] for patients with an APACHE II score of 5-9, 259 fM Xa/min [IQR 144-599] for patients with an APACHE II score of 10-14, and 471 fM Xa/min [IQR 180-848] for patients with an APACHE II score of 15-17. The differences between the four APACHE groups as analyzed by ANOVA after log-transformation of mean MP-TF activity revealed a significant increase in differences between the four categories (P=0.006). Within each of the APACHE II score categories, the mean MP-TF activity was higher in patients with bacteremia than in those without. Overall, this effect of bacteremia on MP-TF activity was significant (P=0.031 in MANOVA).

Plasma disease severity markers sE-selectin and sVCAM-1 correlated moderately with APACHE II score (rs 0.35 and 0.39, respectively; P<0.0001), but only weakly with MP-TF activity (rs 0.28 and 0.30, respectively; P<0.0001; n=213). Plasma procalcitonin showed a slightly better correlation with MP-TF activity (rs 0.35, P<0.0001).

MP-TF activity, TAT levels and monocyte count at baseline

As monocytes have been shown to be the sole hematopoietic source of TF and activated monocytes shed microparticles expressing procoagulant tissue factor [16, 17], we examined the correlation between MP-TF activity and blood monocyte count. From 117 of 134 patients (87.3%) presenting with febrile *E. coli* UTI at one of the emergency departments, monocyte counts were available. Monocyte counts showed a weak inverse correlation with plasma MP-TF activity (rs -0.22; P=0.016). Of 106 patients, plasma samples were available for determination of TAT levels. MP-TF activity correlated weakly with TAT (rs 0.23, P=0.017; n=106). Interestingly, monocyte count showed a moderate inverse correlation with TAT (rs -0.40, P=0.003; n=55). In contrast, MP-TF activity did not correlate with total leukocyte count (which in septic patients mainly consists of neutrophils).

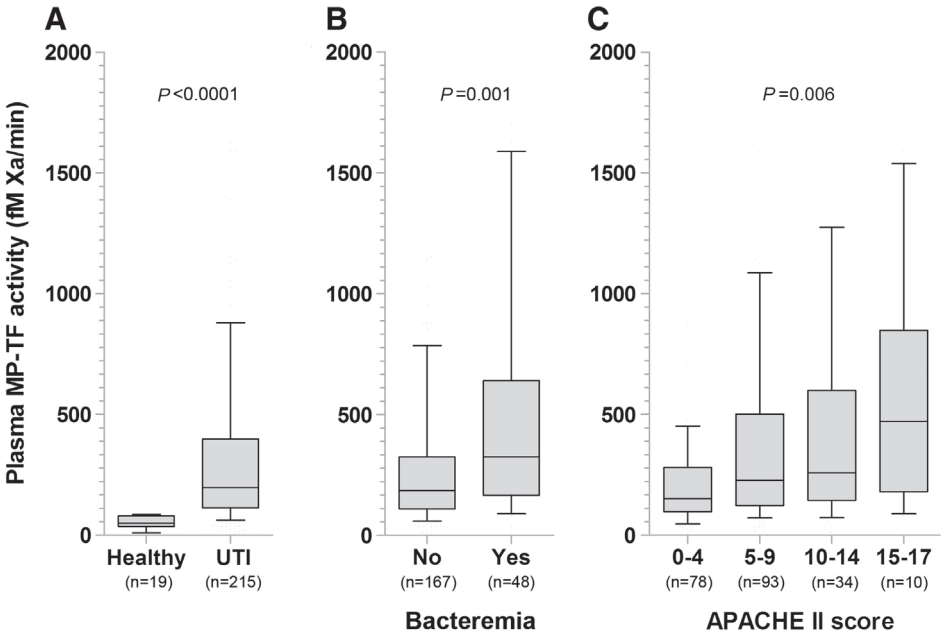

Figure 1. Boxplot showing plasma MP-TF activity in (A) 19 healthy volunteers and 215 patients with febrile *E. coli* UTI, (B) patients with localized disease and no bacteremia (n=167) vs. patients with bacteremia (n=48), and (C) patients with different APACHE II scores. Whiskers indicate the 10^{th} and 90^{th} percentile. Depicted *P*-values were calculated using the Mann-Whitney *U*-test for panels A and B, and ANOVA after log-transformation of data for panel C.

MP-TF activity and TAT on days 0 and 3

As all patients received antibiotic treatment upon admission, we examined MP-TF activity on day 3. In 98 patients, EDTA-anticoagulated plasma samples collected on day 3 were available allowing paired analysis of MP-TF activity on days 0 and 3 (Fig 2A). Overall, mean MP-TF activity declined with 95 fM Xa/min over 3 days of antibiotic treatment (P=0.0003; Paired-Samples T-test after log-transformation). Although the overall MP-TF activity decreased, the MP-TF activity on day 3 showed a 1.2 to 22-fold increase in ten patients. Six of these patients remained febrile or showed signs of inflammation on day 3. Interestingly, the patient who experienced the highest increase in blood MP-TF activity from 183 to 3928 fM Xa/min was an elderly patient with proven bacteremia, who deteriorated clinically despite adequate oral antibiotic treatment. His urine contained $>10^5$ CFU's and the time to positive blood culture was 12 hours. Although vomiting and diarrhea were not reported, this patient had prolonged fever and malaise. As for the eleven patients with the highest MP-TF activities (i.e. > 1500 fM Xa/min), symptoms improved

promptly upon antibiotic treatment. Nine of the eleven patients had to be hospitalized for a median period of 7 days (range 2-28). From four of these eleven patients with MP-TF activities > 1500 fM Xa/min, day 3 samples were available showing a marked decrease in MP-TF activity to 98-265 fM Xa/min. In 106 patients, TAT levels were determined. TAT ranged from < 2.0-145 µg/l and was elevated in approximately 50% of patients. From 11 patients with MP-TF activities > 1500 fM Xa/min, TAT levels were available for 5 patients, all being elevated, the patient with the highest MP-TF activity (3597 fM Xa/min) having one of the highest TAT levels (133 µg/ml). Of 40 patients, plasma was available for paired analysis of TAT on day 3 (Fig 2B). Mean TAT declined with 7.6 µg/l (*P*=0.0096; paired-samples T-test after log-transformation). Interestingly, the patient with prolonged fever who showed the highest increase in MP-TF activity also showed one of the highest increases in TAT from 5.0 to 24.5 µg/ml. The second patient with an increase in TAT on

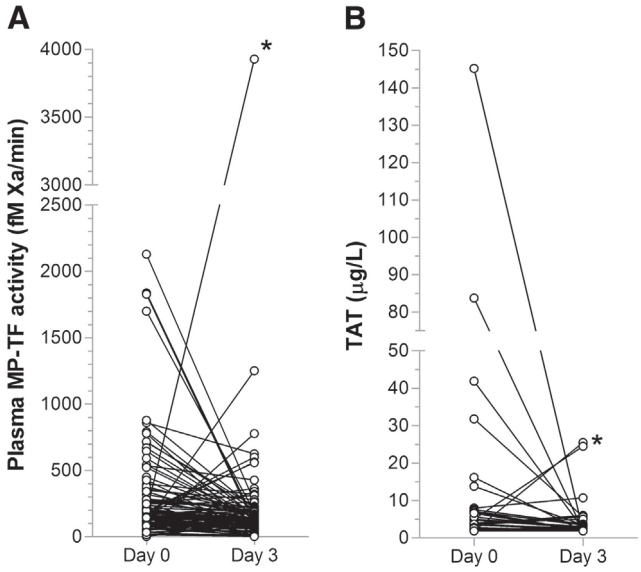

Figure 2. MP-TF activity and TAT at baseline and on day 3 following antibiotic treatment. (A) Median MP-TF activity at baseline (183 fM Xa/min) was higher than on day 3 (129 fM Xa/min; *P*=0.002; n=98). Ten patients showed an increase in MP-TF activity on day 3. In the remaining 88 patients, MP-TF activity decreased or varied within the normal range (< 172 fM Xa/min). Interestingly, the patient with the highest increase in MP-TF activity on day 3 had deteriorated clinically despite antibiotic treatment (*). (B) Median TAT levels at baseline (3.7 µg/l) were higher than on day 3 (2.3 µg/l; *P*=0.003; n=40). The majority of patients showed a decline in TAT following resolution of the infection. Interestingly, the patient with the highest increase in MP-TF activity also exhibited one of the highest increases in TAT (*). Finally, paired-samples T-test after log-transformation of data showed significant decrease in mean MP-TF activity and TAT levels (*P*=0.0003 and *P*=0.0096, respectively).

day 3 (1.9 to 25.5 µg/ml) similarly showed a concurrent increase in plasma MP-TF activity from 261 to 365 fM Xa/min. This elderly lady had been treated with oral antibiotics for recurrent urinary tract infections in a nursing home. She presented with an APACHE II score of 17 and a deteriorating localized urinary tract infection despite antibiotic treatment.

Discussion

In this large cohort study of 215 patients with febrile *E. coli* UTI, which reflects daily clinical practice and entails the most commonly cultured pathogen from urine (i.e. over 70%), we demonstrate that functional procoagulant tissue factor on blood microparticles (MP-TF activity) is higher in patients than in healthy individuals and is associated with disease severity and the presence of bacteremia. After three days of antibiotic treatment, MP-TF activity declined coinciding with defeverescence and resolution of the infection. In a minority of patients, mostly with prolonged fever and inflammatory symptoms, an increase in MP-TF activity was observed on day 3. Interestingly, one bacteraemic patient with delayed resolution of his illness and another patient who deteriorated despite antibiotic treatment which was started prior to admission showed a marked increase in MP-TF activity on day 3 and a concurrent increase in TAT levels. These observations illustrate that height and kinetics of MP-TF activity reflect disease severity and clinical deterioration in sepsis and underscores an association of plasma MP-TF activity with coagulation *in vivo.*

There was a large interindividual variation in MP-TF response to *E. coli* and a considerable number of patients (42%) did not have elevated MP-TF activities on day 0. This may reflect differences in the genetic ability of patients to respond to the pathogen (responders and non-responders) [6, 18], differences in plasma endotoxin concentrations [19] and differences in the time interval between the onset of *E. coli* infection and the time of inclusion into the study. Also, the exclusion of patients with co-morbidity may have resulted in the inclusion of a subgroup of patients who were less prone to bacteremia and severe inflammatory disease.

Our findings are in accordance with the observation by Aras *et al.* as well as our own previous observation that intravenous administration of *E. coli* LPS to healthy volunteers results in a marked but transient increase in procoagulant tissue factor activity of blood microparticles isolated from EDTA and citrated plasma, respectively [6, 20]. Monocytes are thought to be the

sole hematopoietic source of TF and show significantly elevated levels of TF mRNA *in vitro* upon stimulation with *E. coli* LPS [16, 21]. It is highly likely, that the inflammatory response induced by invasion of an intact pathogen into urinary tract tissues similarly results in early activation of monocytes and the release of microparticles bearing TF [6], even though we only found a weak correlation between MP-TF activity and monocyte count. In our study, 70% of the patients had symptoms of pyelonephritis and since glomeruli exhibit strong TF expression [22], apoptotic glomerular cells may hypothetically form an alternative source of MP-TF activity. Provided the availability of a monoclonal antibody able to reliably assess tissue factor on microparticles, multicolor flowcytometry experiments could aid in establishing the cellular source of MP-TF. However, only limited amounts of plasma were available from patients participating in this multicenter study. Since MP-MP fusion events are likely to occur *in vivo*, thus complicating the correct identification of the cellular source of MP-TF, and flowcytometry does not detect the smallest microparticles due to limitations of the 488 nm laser light wavelength used, we did not further explore this.

Plasma MP-TF activity correlated weakly with TAT levels. This is not surprising as TAT concentrations *in vivo* are subject to the dynamics of TAT formation and degradation. Furthermore, the formation of TAT occurs downstream in the coagulation cascade and is influenced by multiple other factors such as TF on activated monocytes, levels of procoagulant phospholipids on microparticles and activation of the intrinsic route of coagulation [23, 24]. As yet, it is not known whether other forms of TF in plasma increase in infectious disease. Previously, we showed that in a human endotoxemia model the rise and fall of plasma TF antigen formed a mirror image of the monocyte count and that plasma TF antigen peaks earlier than plasma MP-TF activity [6]. Stimulation of monocytes may result in proteolytic cleavage of TF from the cell membrane (increase in soluble truncated TF), an increase in alternatively spliced TF and the shedding of MP-TF. However, truncated TF and alternatively spliced TF have strongly reduced (if at all) procoagulant activity. MP-TF, as measured in our assay, may therefore be the most important procoagulant form of TF in plasma.

Nonetheless, procoagulant microparticles have been shown to contribute to thrombus formation *in vivo* [25, 26]. In agreement herewith, consumption of procoagulant microparticles was suggested in a small study of septic ICU patients with multiple organ dysfunction syndrome and coagulopathy [27]. In addition to their procoagulant properties, microparticles may also function as highly mobile carriers of TF and may transfer TF to other cells through

membrane fusion [16]. MP-TF may furthermore contribute to local containment of the infection through capture and even killing of fibrinogen binding bacteria within microthrombi [28].
In conclusion, we demonstrated that inflammatory conditions caused by an intact pathogen can induce the release of MP-TF into the circulation and that plasma MP-TF activity levels are associated with disease severity and bacteremia. Considering the link between inflammatory and TF-related pathways, future studies of the pathophysiological role of MP-TF in critically ill patients with severe sepsis, septic shock, multiple organ dysfunction syndrome and disseminated intravascular coagulopathy are warranted. Moreover, microparticles carrying functionally active tissue factor may well be desirable targets for therapeutic purposes as, hypothetically, inhibition of procoagulant tissue factor on circulating blood microparticles could abort disease progression and even lethality from *E. coli* septicemias.

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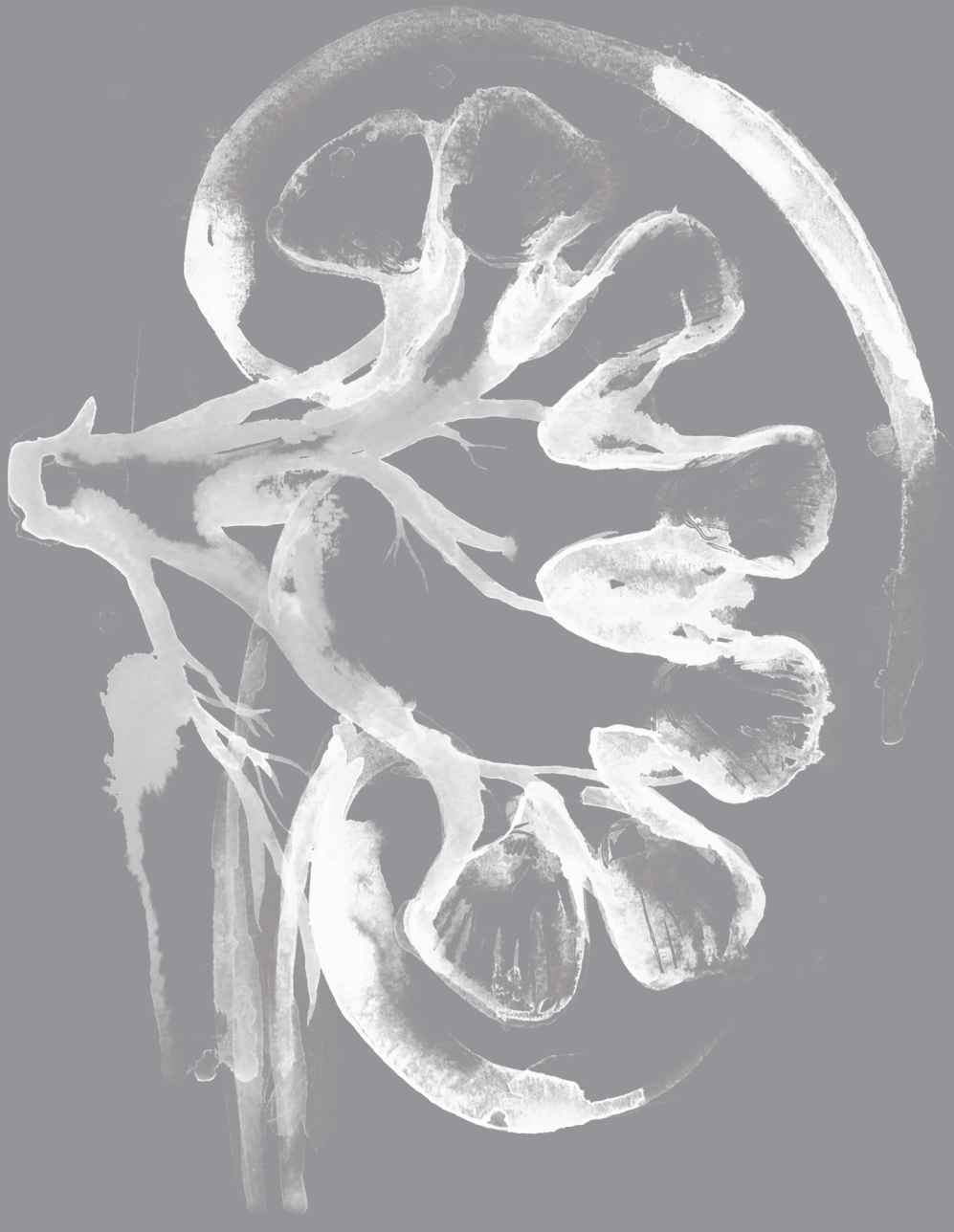

Summary and general discussion

Summary and general discussion

Urinary tract infections (UTIs) are one of the most common infectious diseases. Fever in UTI suggests the presence of tissue inflammation, and points to a diagnosis of acute pyelonephritis, prostatitis or urosepsis. Febrile UTI patients usually present at primary care centres with relatively mild disease. However, its course may be unpredictable as it may rapidly develop into septic shock, a life-threatening condition necessitating hospital emergency care. Evaluation of clinical symptoms often fails to provide accurate guidance to the clinician which patients may run a complicated course.

The overall aim of this thesis was firstly to provide evidence for the clinical implication of biomarkers in blood and urine, as well as genetic markers, for the prediction of the severity and course of febrile UTI. Secondly, this thesis focused on optimization of antimicrobial treatment of febrile UTI. The five main results of this thesis can be summarized as follows:

1. A recent hospitalization, an indwelling urinary catheter and most importantly, individual fluoroquinolone (FQ) use, are independent risk factors for the occurrence of a FQ resistant *Escherichia coli* as cause of febrile UTI (Chapter 1).
2. Women with febrile UTI, including postmenopausal women and those with comorbidities, can be safely and successfully treated with a short, i.e. 7-day course of oral ciprofloxacin. In men, however, treatment duration should be at least 14 days (Chapter 3).
3. Diabetes mellitus per se does not affect the clinical presentation and course of febrile UTI, but concurrent illnesses (e.g. vascular complications of diabetes) and higher age of the diabetic population attribute to a more complicated course (Chapter 4).
4. MR-proADM, a marker of endothelial cell dysfunction, more accurately predicts a complicated course of disease than currently available inflammatory biomarkers (Chapter 5). Importantly, biomarkers derived directly from host defense mechanisms are not suitable to distinguish between febrile UTI patients with and without bacteremia (Chapter 6).
5. Microparticle-associated procoagulant tissue factor activity is related to disease severity and bacteraemia in febrile *E. coli* UTI and may contribute to the prothrombotic state in gram-negative sepsis (Chapter 7).

In this general discussion, the major findings of the studies will be highlighted with a focus on clinical implication. In addition, some methodological issues

and remaining questions will be discussed together with recommendations for future research.

Risk factors for antimicrobial resistance

In patients with febrile UTI, fluoroquinolones (FQs), amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole are the preferred agents for oral antimicrobial treatment, combining a reliable uptake in the gastrointestinal tract with excellent antimicrobial activity. Because of their pharmacokinetic profile (e.g. sufficient tissue level in the prostate)[1] and particularly because of a relatively low rate of antimicrobial resistance compared to β-lactams and trimethoprim-sulfamethoxazole, FQs are the preferred empirical oral agents.[2-5] However, FQ resistance of *Escherichia coli*, the most frequent etiologic bacterial pathogen in UTI, is now emerging in the community, which may limit its use to treat febrile UTI.[6] Worldwide, reported rates of *E. coli* resistance to ciprofloxacin are up to 38%.[7-9] Even in the Netherlands, a country known for its restrictive use of antibiotics and overall low resistance rates, there are signs that the incidence of FQ-resistant *E. coli* is rising, especially among patients at urology services.[10;11] The extensive use of antimicrobials in veterinary and human health care practice may be a potential threat for further emergence and dissemination of resistant pathogens in the community.[6;12]

Antimicrobial resistance is associated with prolonged symptoms and a complicated course in patients with UTI.[13;14] Moreover, FQ resistance in *E. coli* is frequently associated with resistance to other antimicrobial classes.[15] Therefore, when the choice of empirical antimicrobial therapy has to be made, it is important that the attending physician has knowledge about which patients are at a particular risk for a resistant causal uropathogen. However, there is an overall lack of data on risk factors for FQ resistance in community-acquired febrile UTI. **Chapter 1** describes the results of a prospective, multicenter cohort study, in which host-related and environmental risk factors for FQ resistance in febrile *E. coli* UTI were evaluated. In addition, the impact of FQ resistance on clinical outcomes was assessed.

We found that individual use of FQs in the past six months was the most important of several risk factors for FQ resistance that also included a recent hospitalization and the presence of an indwelling urinary catheter. Environmental risk factors, like contact with a household member with UTI or with livestock or pets, were not associated with FQ resistance. Thus, individual FQ use seems to be the driving force for FQ resistance, rather than within-household or animal-human transmission of resistant *E. coli*. These findings

do not lend support for the concern of a human or animal reservoir causing FQ resistance, although it should be emphasized that the current evidence for human-human and animal-human transmission of FQ-resistant *E. coli* seems to be limited to specific strains.[16-19] Moreover, FQ-resistant *E. coli* strains in e.g. a gut reservoir do need to have specific virulence factors, like P-fimbriae, to be able to adhere to the renal epithelium to cause a febrile UTI.[20]

Besides that, our study does not exclude a possible two-hit mechanism for FQ resistance with an initial input of FQ-resistant strains from e.g. food supply of colonized animals into the population followed by selection at the individual level by personal FQ use. Therefore, further studies are urgently warranted to explore this hypothesis, particularly as current data on the relationship between animal-derived food and FQ-resistant *E. coli* in humans reveal conflicting results, but at least indicate that this might be or become a major threat for the human community.[21-24]

FQ resistance was associated with high rates of cross resistance to amoxicillin-clavulanic acid (33%) and trimethoprim-sulfamethoxazole (65%). Presence of extended-spectrum β-lactamase (ESBL) was almost excluded in case of FQ susceptibility. That highlights the importance of detection of risk factors for FQ resistance as these may also be risk factors for ESBL-production.

Interestingly, we found no differences in clinical outcome of patients with a FQ-resistant strain who were empirically treated with ciprofloxacin compared to those treated with 'appropriate' (i.e. based on susceptibility testing results) antibiotics. The majority of patients recovered on ciprofloxacin as their fever resolved before the outcome of the urine culture and susceptibility testing of the etiologic bacterial pathogen became available and antimicrobial treatment was subsequently switched. There are several explanations for this finding. Firstly, this may indicate that febrile UTI is to some extent a self-limiting disease, dealt with by host defense mechanisms that may be assisted by antibiotics but not totally depend on their action. Secondly, ciprofloxacin may be possibly effective in vivo even in ranges above in vitro resistance level used in our study (ciprofloxacin MICs > 1 mg/L according to EUCAST-criteria), as was also suggested in recent literature.[25;26] The absence of a relation between FQ resistance (already 12% of the isolates in our study) and clinical outcome of febrile UTI in our study do question the clinical relevance of detecting FQ resistance at the individual patient level. First of all, the number of patients in this study was limited and a 'type I error' (wrongfully rejecting the hypothesis that a difference exists) cannot be excluded,

the more so because the expected background mortality is low already. On the other hand, the main risk factor for FQ resistance, individual FQ use, can easily be detected by just taking a thorough recent pharmacological history in febrile UTI patients. In patients with recent FQ use, one might consider not to choose a FQ as empirical antimicrobial therapy or not solely rely on this class of antibiotics, although the clinical relevance of detecting FQ resistance in each individual patient may not hold.
Better still than predict among those with febrile UTI the individuals at risk for FQ resistance would be to preclude the emergence and spread of antimicrobial resistance in the community through strict hygiene and antimicrobial stewardship programs. One such strategy is the optimization of antimicrobial treatment duration.

Optimal duration of antimicrobial therapy

With a lack of new antimicrobial classes in the development pipeline,[27] it is increasingly important to develop strategies to maintain and even increase the effectiveness of available antimicrobial agents. Optimization of treatment duration represents one such important strategy, because the development and spread of antimicrobial resistance is closely related to the total amount of antimicrobials used in countries.[28] The duration of antimicrobial therapy exerts differential selecting pressure on gut flora which leads to selection of resistant strains and reduction of resident commensal bacteria paving the road for e.g. *Clostridium difficile* infection.[29] Moreover, the potential adverse effects of unnecessary extended treatment periods reach beyond the individual treated: the longer antimicrobials are taken and excreted into the environment, the more pressure is exerted on the ecological balance of bacteria outside the human gut.[30] Despite the importance of optimization of treatment duration, there is an overall scarcity of randomized controlled trials to study the minimal yet optimally efficacious duration of treatment, even in a common infection like UTI. Our review, described in **Chapter 2**, discusses the available literature. It showed that studies mainly focused on uncomplicated cystitis and acute pyelonephritis in otherwise healthy women. Young women without comorbidities can be treated for febrile UTI with a 1-week regimen of fluoroquinolones provided a low a priori level of fluoroquinolone resistance or, if proven susceptible, with a 2-week course of trimethoprim-sulfamethoxazole.[7;31;32] Oral β-lactams are probably less effective compared to fluoroquinolones and trimethoprim-sulfamethoxazole.[2;33-35] In contrast to this, the optimal treatment duration for all other patient categories is still

unknown, as (most) randomized trials excluded male patients, the elderly, and those with urinary tract abnormalities or underlying systemic illnesses.
We therefore conducted a randomized placebo-controlled double-blind multicenter non-inferiority trial to determine whether the efficacy and safety of a 7-day course of ciprofloxacin was similar to a 14-day ciprofloxacin course in an unselected population of both men and women. Patients with community-acquired febrile UTI were recruited at regional hospitals and primary care centers and clinical and microbiological cure rates were assessed. The results of this study are discussed in **Chapter 3**.
We found that community-acquired febrile UTI can be safely and efficaciously treated with a 7-day instead of 14-day course of oral ciprofloxacin in women, including the elderly with severe comorbidities, and irrespective of severity of disease at presentation. Both treatment regimens were highly effective in women: 94% vs 93% clinical cure at 2-3 weeks after the end of treatment (for 7 versus 14 days, respectively) and a comparable high bacteriological cure rate. Even in patients with positive blood cultures (~20%), the shorter treatment course was safe and effective, as we reported earlier.[36] These results support and extend the findings from a previous Swedish study performed in women with acute pyelonephritis, showing non-inferiority of 7- and 14-day antimicrobial treatment.[37] However, although that trial did not exclude upfront elderly women or the severely ill ones, their patient group was significantly younger than ours, and moreover less frequently had serious underlying comorbidities.
In contrast herewith, 7-day treatment in men did not reach non-inferiority with a 14-day course of treatment, as shown by an increase in rate of clinical (14% vs 2%) and bacteriological treatment failure after a 7-day compared to a 14-day treatment course, irrespective of comorbidities or complicating factors. Of note, this lack of efficacy could not be attributed upfront to a propensity of prostatitis in men, as the difference was especially evident in men presenting with costovertebral tenderness, generally taken as a sign of pyelonephritis, although the numbers of cases limited a firm exploration of subgroups. Still, the findings suggest that all febrile UTI in men likely involves the prostate, irrespective of the presence of signs of pyelonephritis. Unfortunately, the number of patients included in this study constrained a purposeful exploration of the results in subgroups, e.g. those treated in the hospital compared to those at home. Future studies are needed to address these issues in more detail.
Overall, we can conclude that in women including postmenopausal women and those with significant comorbidities, febrile UTI can be treated success-

fully with a 7-day course of oral ciprofloxacin. In men, however, a short course leads to significantly more clinical failures than a 14-day course of ciprofloxacin, so men should be treated for at least two weeks. Likely, these results also hold for other fluoroquinolones with gram-negative activity, as they have shown being able to effectively eradicate susceptible Enterobacteriaceae from the vaginal and rectal flora, which may help prevent early recurrences[35;38;39] However, the current results should not be one-to-one extrapolated to other antimicrobial classes.

An important concern is the rise of ciprofloxacin resistance in the community, i.e., up to 15% of Enterobacteriaceae currently being resistant in The Netherlands.[11] And more importantly, even higher resistance rates have been reported in other countries.[8;9;40] If it continues at the current rate, this may well prelude the end of use of fluoroquinolones as first-choice empiric oral treatment for febrile UTI. As discussed in **Chapter 1**, clinical outcome is (as of yet) not affected by FQ resistance, although it is not imaginary that MICs will increase with emerging FQ resistance and therewith influence clinical outcome. In countries with high rates of trimethoprim-sulfamethoxazole resistance too, there may eventually be no oral antimicrobial left for primary care physicians to confidently treat febrile UTI at home, raising health care costs due to hospitalization.[41] These findings underscore the importance of controlling antimicrobial resistance, through antibiotic stewardship including the optimal duration of antimicrobial treatment and restricted use of ciprofloxacin: only in febrile UTI.

In the future, an alternative strategy could possibly be alteration of the gut microbiome, e.g. by fecal transplant, as is currently being practiced in *C. difficile* colitis,[42;43] therewith decreasing the chance of periurethral colonization with resistant pathogens from the rectum. In women, there are promising results with vaginally applied lactobacilli,[44] which maybe could also be used to alter the perineal flora. Another approach could be the application of intravesical instillation of antibiotics like gentamicin, as is currently investigated in patients with recurrent UTI; such local application of antibiotics bypasses any effect of these on microbes in the gut.[45;46] Several UTI-vaccines have been tested or are under development,[47] based on adherence factors, toxins and surface polysaccharides. Most have to date only been tested in mouse or rate models or primates. The ideal UTI-vaccine is probably far in the future.[48] Finally, bacteriophage therapy is currently being investigated, a therapy to remove adhered uropathogens by deliberately infecting the bladder with

viruses. Bacteriophages – viruses or bacteria – adhere to and gain access to the bacterial interior and enter either into a lytic phase resulting in the bacterium bursting open and releasing numerous copies of the bacteriophage, or into a lysogenic phase, integrating into the bacterial DNA. Bacteriophages as therapeutic agents usually are manipulated and competent only for a lytic phase thereby killing the bacteria.[49;50]

Prediction of a complicated course

The course of a bacterial infection like febrile UTI can be unpredictable. Early recognition and therapeutic interventions are of utmost important to prevent progression to life-threatening conditions such as septic shock and multiple organ failure often resulting in death.[51] However, most adults with febrile UTI present with a mild illness, and the vast majority will have a uncomplicated course and can be safely treated at home.[36] In daily clinical practice, the risk of a complicated course and thereby need for clinical observation and hospital-based treatment is based on history, assessment of underlying disease, and on severity of local and vital signs.

Diabetes mellitus is a well-known risk factor for acquisition of febrile UTI.[52;53] It is widely held that a patient with diabetes also has a more complicated course of infection. However, we showed in our prospective multicentre cohort study among 140 diabetic and 718 non-diabetic patients that diabetes is not independently associated with a complicated course of febrile UTI.[54] (**Chapter 4**) The prevalence of complications was indeed higher in diabetic patients but all attributable to concurrent illnesses, especially cardiovascular comorbidities related to diabetes, and a higher age of the diabetic population. This latter is in line with the fact that most of the diabetic patients had type II diabetes.[55] Clinical and microbiological outcomes after 1 month did not differ significantly between diabetic and non-diabetic patients, while they were treated alike. Remarkably, patients with diabetes experienced less flank pain, possibly due to diabetic neuropathy as suggested previously.[56] The lack of flank pain in diabetic patients is an important reminder that flank pain has a low predictive value in the identification of complicated UTI. Instead of that, the presence of fever should be used in suspected UTI as the most reliable distinction between UTIs with and without tissue invasion. Nevertheless, it is important to notice that fever itself is a sign of little specificity to identify a complicated course, as it may reflect the mere presence of local kidney infection but also of a serious impending urosepsis.

So, as indicated above, clinical symptoms and medical history often fail to provide accurate guidance to the clinician which patients will run a complicated course and need hospital admission. Currently, in the Netherlands about 90% of patients presenting with febrile UTI at Emergency Departments are admitted, because the chance of life-threatening complications cannot be reliably estimated. This has major implications for health care costs of febrile UTI.[41] Clinical guidance in risk stratification is urgently needed to better identify patients at risk of complications thus allowing resources be focused to those who need it most.[57]

Inflammatory biomarkers may help to determine the severity of disease. Currently used conventional biomarkers include blood leucocyte count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and procalcitonin (PCT). Because these were shown to have a limited role in the prediction of a complicated course of disease, we evaluated a new biomarker: midregional pro-adrenomedullin (MR-proADM).[57;58] MR-proADM is involved in regulation of complement activity, and has immune modulating, metabolic and bactericidal activity.[59-62]

In a prospective observational multicentre cohort study, as described in **Chapter 5**, we evaluated the prognostic value of the plasma biomarker pro-ADM in predicting bacteraemia, need for hospital admission and a complicated course of infection. The results were compared with those of currently used biomarkers like CRP, leucocyte count and PCT. We chose to recruit patients in both primary care and emergency department. We therewith were able to specifically assess the added value of the biomarker in the various clinical domains. It is important to realize that the predictive value of a certain biomarker in e.g. the Emergency Department (ED) setting is not the same as in primary care, in part due to selection of patients with mostly a more severe illness and a more profound inflammatory reaction. The added value of a biomarker is the highest if the pre-test possibility can chance substantially post-test due to the biomarker test result, and importantly, this pre-test possibility of infection depends on the clinical domain in which the assessment is done, e.g. primary care, ED, hospital ward or intensive care unit (ICU).

In the study 494 febrile UTI patients were included, of which 376 (76%) recruited at the ED. MR-proADM was significantly correlated with bacteraemia and ICU-admission. The discriminating accuracy for predicting 30-day mortality was higher than the more conventional biomarkers ESR, CRP, PCT and leucocyte count. A plasma level of 1.00 nmol/L was the optimal cut-off to stratify 30-day mortality in our study population. Further studies are

needed to confirm and validate the selected cut-off value as a predictor of complicated course. Plasma level of MR-proADM was only measured at presentation. It would be of interest of daily follow-up measurements of MR-proADM will improve diagnostic accuracy, and if they are correlated to clinical recovery.
We compared the prognostic value of MR-proADM to other currently used biomarkers only among the ED patients, because in the Dutch primary care setting it is not a standard to perform routine laboratory research in every patient. For our analysis this presented no objection, because in this way we were able to compare the prognostic value of different biomarkers in the most outspoken clinical manifestation of febrile UTIs. Consistent herewith, we found significantly lower MR-proADM levels and a lack of 30-day mortality in primary care patients treated at home. One could argue whether a rapid MR-proADM measurement, if available in the primary care setting, could be used to select patients with low MR-proADM levels for outpatient treatment considering the favourable clinical course and outcome.
In a previous prospective study we derived a clinical bedside prediction rule, designated the APSI score, which reliably identifies low-risk febrile UTI patients with an acceptable low risk of a complicated course who can be safely treated at home.[63] The present study showed that the plasma levels of MR-proADM correlated well with the APSI score. Whether or not MR-proADM can independently add acumen to the clinical prediction rule, is uncertain. Currently, we evaluate the use of biomarkers like pro-ADM and PCT in guiding the decision which patients with febrile UTI to admit to hospital and which patients to treat at home in a prospective clinical trial in which the clinical prediction rule is implemented at eight Emergency Departments.

Besides systemic plasma inflammatory biomarkers, one could argue that also locally produced biomarkers of host defense could be able to indicate the severity of the infection. In current clinical practice, urine is collected for nitrite test and urine culture. If we could find a bedside biomarker test, which is able to predict a complicated course, that would be of great value. We therefore set up a case-control study in febrile UTI patients to determine the role of certain urinary cytokines and antimicrobial proteins in predicting bacteraemia, i.e. IL-6, IL-8, cathelicidin (LL37), β-defensin 2 and uromodulin. As described in **Chapter 6**, none of these urinary biomarkers were able to distinguish between patients with and without bacteraemia. Noteworthy, the inability to produce uromodulin, present in a few patients, increased the risk of developing bacteraemia substantially (OR 6.0, 95% CI: 1.2-29.2).[64]

Vitamin D is known to play an important role in the first line of host defense against bacterial infection, e.g. by induction of the antimicrobial proteins cathelicidin and β-defensin, biomarkers described above.[65-68] In vitro experiments have shown that bladder epithelium from women taking vitamin D supplements are capable of producing larger amounts of cathelicidin upon infection.[66] In this respect, one might hypothesize that higher vitamin D levels might to some extent have a protective effect against invasive UTI and bacteraemia e.g. via cathelicidin. This was explored in the study described in **Chapter 6**. An association of plasma vitamin D with urinary cathelicidin however was not found. Furthermore, there was no association between either vitamin D level or urinary cathelicidin with the presence of bacteraemia.[64]

The role of genetics in UTI

In most infectious diseases, in addition to exposure, environmental factors and strain virulence, genetic host variations play a role in susceptibility to disease.[69;70] Likely, genetic factors also play in role in UTI, as illustrated by the finding that positive family history is a risk factor for recurrent UTI.[71] In daily clinical practice, it is noticeable that some patients seem to be extremely prone to UTIs, while others (nearly) never encounter a UTI. Possibly, this may be due to variations in genetic host susceptibility, affecting the production of urinary proteins involved in local host defense. To further investigate the attribution of genetic variations to susceptibility to febrile UTI, we genotyped 15 single nucleotide polymorphisms (SNPs) in 12 genes with a known role in the recognition, defense or immune response to uropathogens (**Chapter 6**).[64] None of the SNP alleles or genotypes were found to be associated with susceptibility to febrile UTI when analysing febrile UTI patients versus healthy controls. A significant difference in β-defensin 2 production was found between the *DEFB1* rs1800972 genotypes, as most individuals with a CC or CG genotype did not produce β-defensin 2 while the individuals with a GG genotype did. This was new evidence in the genetic pathways of UTI. No significant correlations were found between the 14 other genotypes and protein production, in contrast to other much smaller studies.[72-75]

In conclusion, this genomic analysis of a large cohort showed that the role of genetics in the susceptibility of febrile UTI is marginally, at least in our cohort. The lack of association in our cohort as opposed to that reported in others may be due a difference in genetic background or due to the selection of elderly patients in our cohort, as at an elderly age comorbidity and an aging immune system may have more effect on susceptibility to UTI than

subtle genetic variations. This is supported by the fact that studies evaluating the role of genetic factors in UTI were mainly successful in children.[72-74;76-78] Moreover, we analysed the largest UTI cohort so far with more than 700 febrile UTI patients, which makes the power to detect an association (if present) greater than in most of the other cohorts. Therefore, our study has gained new insight in the genetics of febrile UTI susceptibility in adults, valuable for future research in genetic pathways and cytokine production.

Future directions of research

Escherichia coli strains need specific virulence factors, like P-fimbriae, to be able to adhere to the renal epithelium to cause a febrile UTI.[20] As the majority of UTIs is caused by *E. coli* strains migrating from a gut reservoir to the urethra followed by ascending to the bladder and kidneys, future research should be directed to the composition and possibilities of alteration of the microbiome of the gut, perineum and vagina. A promising future strategy could possibly be the inhibition of the growth of periurethral uropathogenic bacteria by means of microbiome modulation, for instance by local application of specific lactobacilli strains or by fecal transplant. Herewith, colonisation with uropathogenic strains possessing virulence factors necessary to adhere to the renal epithelium, could be replaced by non-uropathogenic strains. An additional advantage of such a microbiome modulation is that herewith resistant uropathogen are being replaced too, therewith theoretically preventing UTIs with a (multi-)resistant uropathogen.

Another promising strategy in the light of increasing antimicrobial resistance rates could be the local application of antibiotics, e.g. intravesical instillation, because this bypasses any effect of these on microbes in the gut.[45;46] Systemic antibiotics exert a major selecting pressure on gut flora, which leads to the selection of resistant strains and reduction of resident commensal bacteria, facilitating e.g. *Clostridium difficile* infection. Moreover, UTIs are mainly (at least at the start) local infections of the urinary tract, which possibly can be sufficiently treated with local antibiotics only. Future trials should be designed to further investigate this issue in preferably a randomized controlled trial comparing locally applied versus oral antibiotics.

Finally, since clinical estimation of the severity of febrile UTI at first presentation is often not reliable and as the biomarker MR-proADM has shown to be a reliable predictor of a complicated course, further studies should be initiated to confirm and validate the role of MR-proADM in other febrile UTI cohorts. Furthermore, it would be of interest of daily follow-up measurements of MR-proADM will improve diagnostic accuracy, and if they are correlated to

clinical recovery. Ultimately, MR-proADM as a rapidly-available bedside test could then be used to stratify patients in risk categories of clinical course, and therapy could be tailored, e.g. home-based treatment in patients with an expected low risk of complications, or duration of antimicrobial treatment based on the level of MR-proADM.

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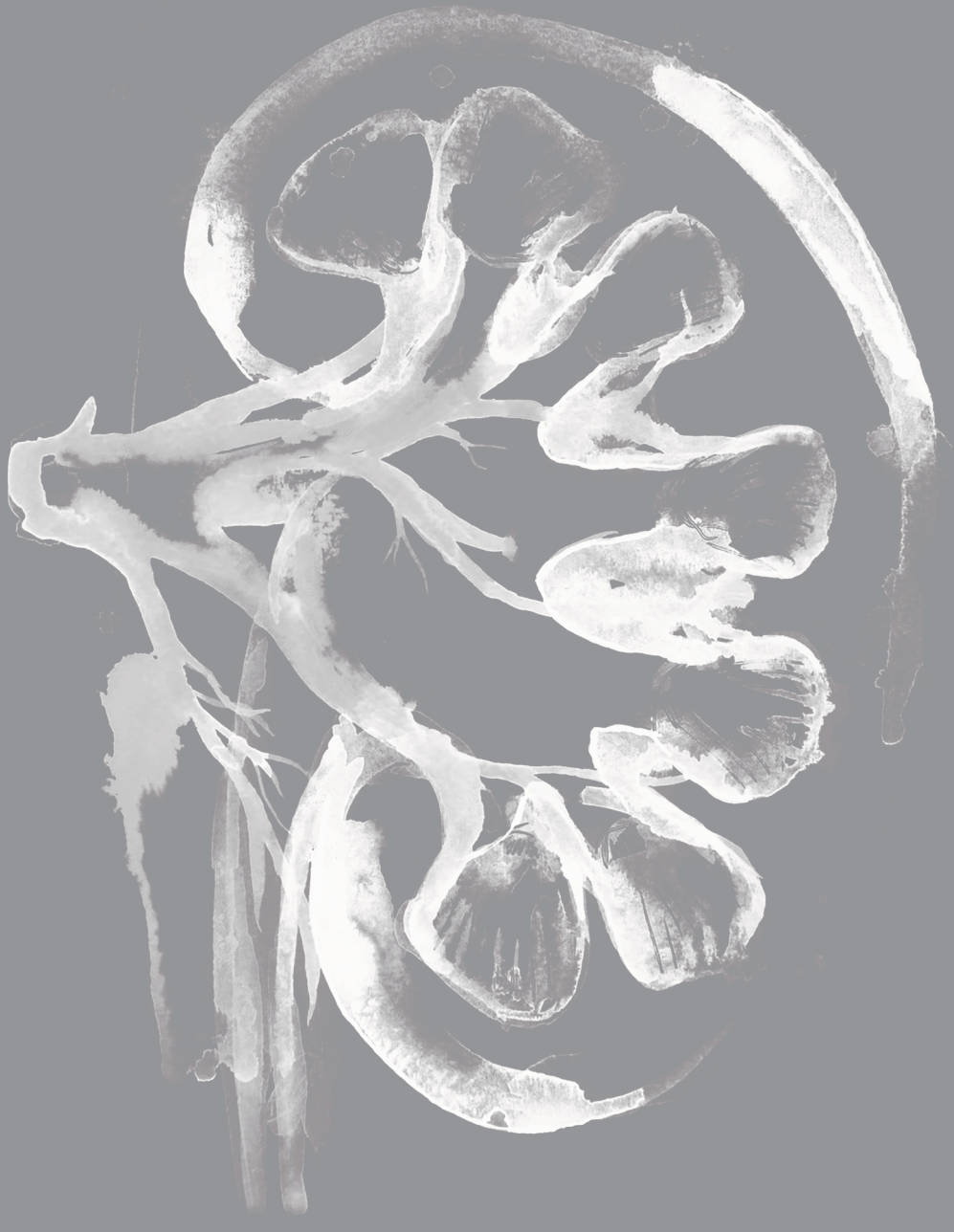
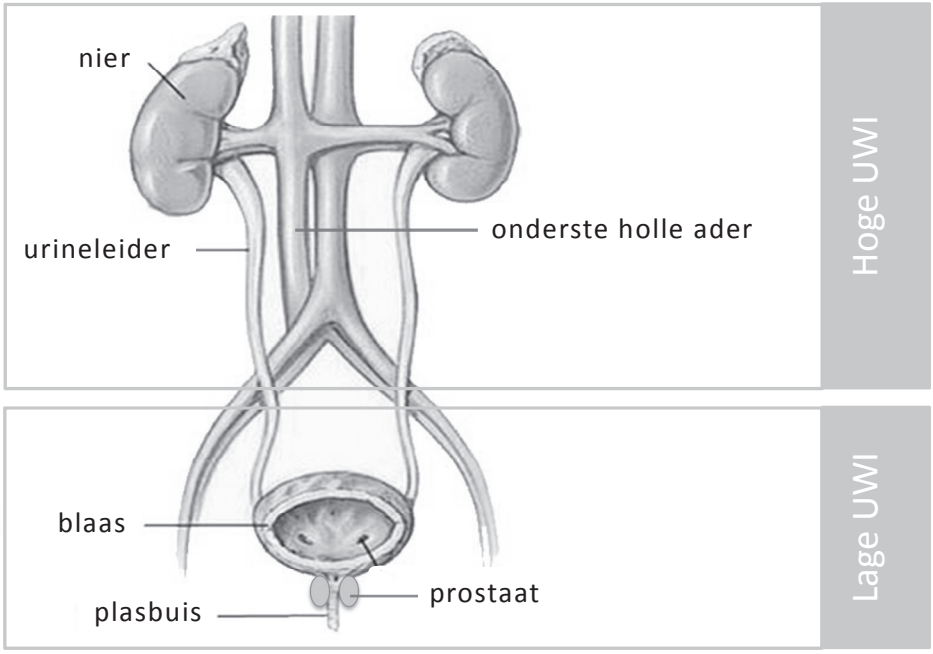


Nederlandse samenvatting

Nederlandse samenvatting

Een urineweginfectie is een ontsteking aan de binnenkant van de urinewegen. De urinewegen zijn de structuren in het lichaam waar urine passeert op weg naar buiten: het nierbekken, de urineleiders, blaas en plasbuis met bij mannen de prostaat (Figuur 1). Een infectie ontstaat wanneer bacteriën, meestal afkomstig vanuit het maagdarmstelsel, zich vasthechten aan de opening van de plasbuis. Van daaruit gaan zij zich vermenigvuldigen, raakt het slijmvlies ontstoken en stijgt de infectie hogerop. Een urineweginfectie die zich beperkt tot de plasbuis en blaas (blaasontsteking) wordt een lage urineweginfectie genoemd. Meestal geeft deze alleen lokale klachten, zoals een branderig gevoel bij plassen, vaak kleine beetjes plassen en troebele, stinkende urine. Een onbehandelde lage urineweginfectie kan soms overgaan in een nierbekkenontsteking (pyelonefritis), een prostaatontsteking bij mannen (prostatitis) of een bloedbaanontsteking met de aanwezigheid van bacteriën vanuit de urine in het bloed (bacteriëmie of urosepsis). Dit noemen we ook wel een hoge urineweginfectie of urineweginfectie met koorts (Figuur 1). Een hoge urineweginfectie gaat naast de lokale verschijnselen namelijk ook gepaard



Figuur 1. Anatomie van de urinewegen met weergave van de locatie van lage en hoge urineweginfecties. (bron: Kaper JB e.a. Pathogenic Escherichia coli. Nat Rev Microbiol 2004;2:123-140)

met koorts, een algeheel ziek zijn en soms met flankpijn en koude rillingen. Een urineweginfectie met koorts heeft, zodra behandeling met antibiotica is gestart, over het algemeen een mild beloop met een voorspoedig herstel. Het kan echter ook een levensbedreigend karakter aannemen, vooral als er sprake is van een bacteriëmie, waarbij zelfs IC-opname noodzakelijk kan zijn en sommige patiënten overlijden.

Ondanks dat een urineweginfectie de meest voorkomende bacteriële infectie is en vele mensen ooit een urineweginfectie zullen doormaken, zijn er nog vrij veel onbeantwoorde vragen over de juiste aanpak en behandeling van de infectie. Zo is het bijvoorbeeld niet bekend wat de optimale duur van behandeling met antibiotica is, en of hierbij een verschil is tussen mannen en vrouwen.
Om hierover meer duidelijkheid te krijgen, is een groot wetenschappelijk onderzoek opgezet in de regio Leiden-Den Haag-Gouda. Aan dit onderzoek deden circa 35 huisartsenpraktijken en 7 Spoedeisende Hulpafdelingen mee. Elke volwassene met klachten van een urineweginfectie met koorts, die zich bij één van deze deelnemende centra meldde, werd gevraagd om deel te nemen aan het onderzoek. Bij toestemming werd bloed en urine afgenomen voor onderzoek en gedurende 3 maanden werd de patiënt meerdere malen bezocht of gebeld om de uitkomst van de urineweginfectie te monitoren. Met de verzamelde gegevens werden meerdere deelonderzoeken verricht, waarvan de resultaten in dit proefschrift beschreven staan.

Escherichia coli is de meest voorkomende bacteriële verwekker van urineweginfecties. Bij een urineweginfectie met koorts moet een antibioticum worden gegeven dat zowel werkt in de urine als ook in het nierweefsel en de bloedbaan. Ciprofloxacine is zo'n antibioticum, dat in tabletvorm kan worden gegeven. Dit middel wordt daarom als eerste keus gebruikt bij personen die thuis worden behandeld. Het probleem is echter dat *Escherichia coli* in toenemende mate ongevoelig (resistent) is voor ciprofloxacine, waardoor ciprofloxacine niet of minder werkzaam wordt. Daarom is het voor een arts belangrijk om in te kunnen schatten bij welke patiënten er rekening gehouden moet worden met zo'n resistente *Escherichia coli*, want dan kan daarmee bij de keuze van het antibioticum rekening worden gehouden. Om de risicofactoren voor ciprofloxacine resistentie te onderzoeken, hebben wij de patiënten met een urineweginfectie met koorts door een resistente *Escherichia coli* vergeleken met diegenen waarbij een gevoelige *Escherichia coli* de oorzaak was. Dit wordt beschreven in **Hoofdstuk 1**. Het onderzoek toont aan

dat het individueel gebruik van fluorchinolonen (de groep antibiotica waar ciprofloxacine toe behoort) in de voorafgaande 6 maanden de belangrijkste risicofactor is voor een urineweginfectie met een resistente *Escherichia coli*.

Hoofdstuk 2 geeft een overzicht van de onderzoeken naar de optimale behandelduur van urineweginfecties met koorts die in het verleden zijn gepubliceerd. Momenteel is de standaardbehandeling meestal een kuur van 10-14 dagen, maar eerdere onderzoeken lieten zien dat 5 tot 7 dagen al voldoende is bij jonge, verder gezonde vrouwen. Opvallend is echter dat er voor andere patiëntgroepen, zoals mannen, ouderen en patiënten met onderliggende aandoeningen, nauwelijks onderzoeken zijn gepubliceerd naar de optimale duur van behandeling. Wellicht profiteren ook zij van een kortere antibiotische behandeling met minder bijwerkingen en kans op resistentie ontwikkeling, maar dat is tot nu toe nooit onderzocht.
Om dit te onderzoeken hebben wij alle patiënten met een urineweginfectie met koorts die zich in een bepaalde periode bij hun huisarts of op de Spoedeisende Hulp meldden, gevraagd om mee te doen aan een onderzoek. Daarbij werd door loting bepaald of zij 7 of 14 dagen behandeld werden met antibiotica. Elke patiënt kreeg 14 dagen tabletten. In de 2^{e} week was de inhoud òf placebo òf het antibioticum ciprofloxacine, maar dit was voor de patiënt en diens behandelaar niet bekend om de resultaten van het onderzoek niet te beïnvloeden. De resultaten van het onderzoek staan beschreven in **Hoofdstuk 3**. Uit ons onderzoek onder 200 patiënten blijkt dat vrouwen veilig en effectief kunnen worden behandeld met een korte kuur van 7 dagen, zelfs als zij een hoge leeftijd hebben en ernstige onderliggende aandoeningen. Mannen echter hebben vaker een recidief urineweginfectie als zij behandeld worden met een 7-daagse kuur, dus voor hen geldt het advies om minimaal 14 dagen te behandelen.

Diabetes mellitus ('suikerziekte') is een bekende risicofactor voor het krijgen van een urineweginfectie. Het is echter controversieel of diabetes er ook voor zorgt dat de infectie ernstiger en met meer complicaties verloopt. Om hier duidelijkheid over te krijgen, hebben wij in een onderzoek het ziektebeloop van de urineweginfectie bij 140 patiënten met diabetes vergeleken met 718 patiënten zonder diabetes. De uitkomsten van dit onderzoek staan beschreven in **Hoofdstuk 4**. Alhoewel diabeten inderdaad vaker complicaties van de infectie hadden, bleek dit niet door de diabetes op zichzelf te worden veroorzaakt, maar door een hogere leeftijd en bijkomende aandoeningen.

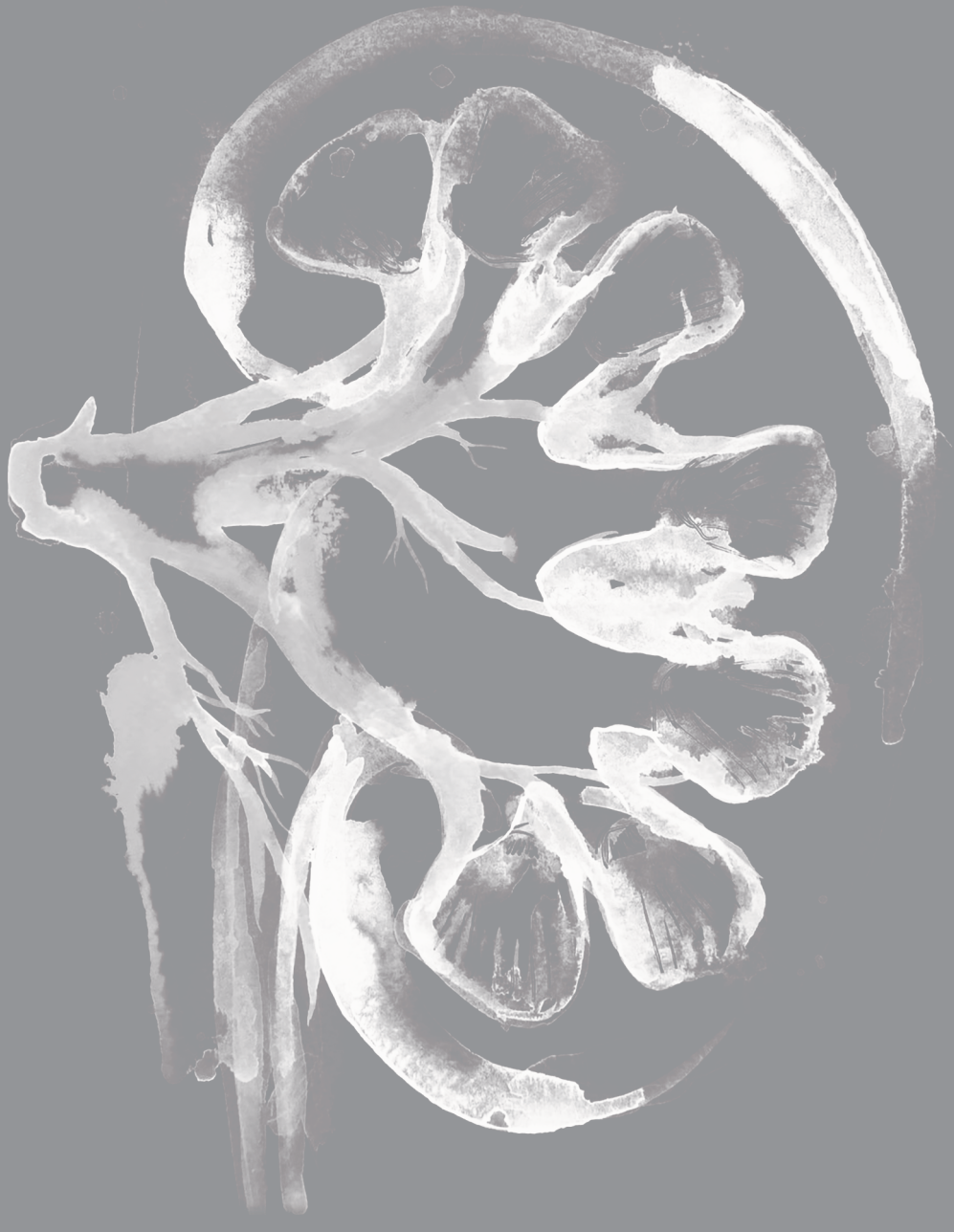
Voor een behandelend arts is het vaak lastig in te schatten welke patiënten met een urineweginfectie met koorts een gecompliceerd beloop zullen krijgen en een ziekenhuisopname nodig hebben. Dat blijkt wel uit het feit dat rond de 90% van alle patiënten die met een urineweginfectie met koorts op de Spoedeisende Hulpafdeling komt, wordt opgenomen omdat de kans op levensbedreigende complicaties niet met voldoende zekerheid kan worden uitgesloten. In de praktijk blijkt de patiënt vaak na enkele dagen weer naar huis te kunnen, zonder dat er complicaties zijn opgetreden. Dit is een veilige aanpak, maar zorgt echter ook voor onnodig hoge zorgkosten. In **Hoofdstuk 5** is daarom onderzocht of de kans op een gecompliceerd beloop kan worden voorspeld met behulp van een bloedtest. De uitslag daarvan zou de arts dan kunnen steunen bij de beslissing een patiënt al dan niet op te nemen in het ziekenhuis. De onderzochte test meet de waarde van het hormoon pro-adrenomedulline in het bloed, een stof die betrokken is bij de afweer tegen bacteriën. Uit het onderzoek blijkt dat pro-adrenomedulline beter dan andere ontstekingsmarkers in staat is een gecompliceerd beloop te voorspellen, met name de kans om binnen 30 dagen te overlijden. Momenteel vindt er vervolgonderzoek plaats, waarbij onderzocht wordt of pro-adrenomedulline samen met een klinische beslisregel (een soort beslisboom) inderdaad van waarde is in het beslisproces op Spoedeisende Hulpafdelingen.

Bij nagenoeg elke patiënt met een urineweginfectie wordt de urine wel onderzocht op aanwijzingen voor de infectie. Indien er een test zou kunnen worden ontwikkeld, die eenvoudigweg in de urine aantoont of er sprake is van een ernstige infectie met complicaties of juist niet, zou dat een waardevolle aanvulling zijn op de meer invasieve bloedtesten. Bij een urineweginfectie worden in de urine door de gastheer bepaalde stoffen (biomarkers) uitgescheiden als reactie op de aanwezigheid van bacteriën in het urinewegstelsel. Het doel hiervan is de bacteriën uit de urinewegen te verwijderen en zo een urineweginfectie te bestrijden. De hoogte van de waarde in de urine zegt mogelijk iets over de ernst en/of uitgebreidheid van de infectie. **Hoofdstuk 6** beschrijft een onderzoek, waarbij wordt gekeken of met behulp van urine biomarkers de aanwezigheid van een bacteriëmie kan worden voorspeld. Dit bleek echter geen relatie met elkaar te hebben.

In **Hoofdstuk 6** werd tevens onderzocht of genetische factoren een rol spelen bij de gevoeligheid voor het krijgen van een urineweginfectie met koorts. In eerdere onderzoeken werd namelijk aangetoond dat het risico op herhaaldelijke urineweginfecties verhoogd is indien urineweginfecties in

de familie voorkomen. Bovendien wekt de dagelijkse praktijk de indruk dat sommige patiënten buitengewoon gevoelig zijn voor een urineweginfectie, terwijl anderen die nauwelijks tot nooit krijgen. Mogelijk spelen hierbij gedragsfactoren, zoals seksuele activiteit, en anatomische urologische afwijkingen een rol, maar het is ook denkbaar dat er een erfelijke component aanwezig is. Wij hebben daarom het DNA van ruim 700 patiënten met urineweginfectie met koorts onderzocht op genetische variaties die betrokken zijn bij de afweerreactie en immuunrespons. Hierbij werden enkele genetische variaties ontdekt, maar de rol van genetische factoren bij urineweginfecties met koorts lijkt concluderend toch klein te zijn.

Tenslotte beschrijft **Hoofdstuk 7** een onderzoek naar het verband tussen de mate van bloedstolling bij urineweginfecties met koorts en het optreden van bacteriëmie. Bacteriëmie of urosepsis, een ernstige vorm van urineweginfectie met koorts waarbij er bacteriën in de bloedbaan aanwezig zijn, zorgt namelijk voor activatie van de stollingscascade, waardoor er vele kleine stolsels in de bloedbaan kunnen ontstaan, met alle vervelende gevolgen van dien. Ons onderzoek toont aan dat hierbij zogenaamde micropartikels met stollingsactiverende eiwitten betrokken zijn, die in de bloedbaan terecht komen als reactie op de aanwezigheid van *Escherichia coli*, de belangrijkste verwekker van urineweginfecties.



Dankwoord

Curriculum vitae

List of publications

Lijst van deelnemende centra

Dankwoord

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Curriculum vitae

Willize van der Starre werd op 2 mei 1986 geboren in Gouda. In 2004 behaalde zij haar Gymnasium diploma aan het Van Lodenstein College in Amersfoort. In datzelfde jaar startte zij met de studie Geneeskunde aan de Universiteit Leiden. Het doctoraal examen en artsexamen behaalde zij in 2010 beide cum laude. Haar eerste onderzoeksactiviteiten vonden plaats in 2010 op de afdeling Infectieziekten in het LUMC onder leiding van prof. dr. J.T. van Dissel en dr. C. van Nieuwkoop. Met haar wetenschapsstage getiteld 'Risicofactoren voor fluoroquinolon-resistente *E. coli* bij gecompliceerde urineweginfecties' werd de basis gelegd voor haar promotie-onderzoek zoals beschreven in dit proefschrift. Zij ontving een promotie-aanstelling via het MD/PhD-traject voor excellente studenten in het LUMC. In september 2013 vervolgde zij haar loopbaan als arts-assistent Interne Geneeskunde in het Medisch Centrum Haaglanden te Den Haag, waarna zij in maart 2014 begon aan de opleiding tot huisarts op de afdeling Public Health en Eerstelijnsgeneeskunde in het LUMC. Haar proefschrift heeft zij in de tussentijd afgerond.

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