Optimizing triage and treatment strategies in urinary tract infection

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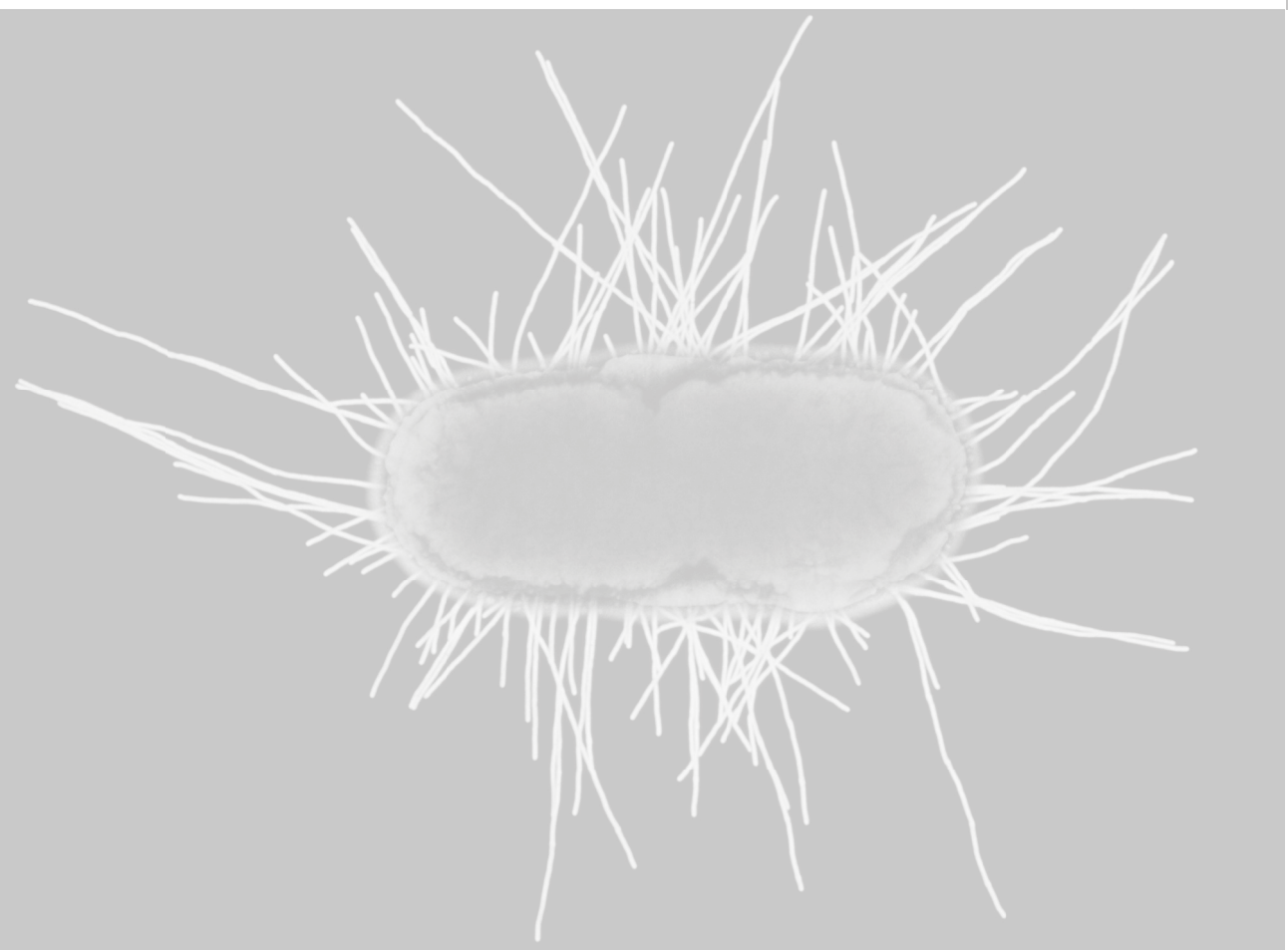

CHAPTER 1

Introduction and outline of the thesis

Janneke E. Stalenhoef, Jaap T. van Dissel, Cees van Nieuwkoop

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INTRODUCTION

Urinary tract infections (UTIs) are a common reason for consultation in the emergency department (ED). The majority of UTI patients present with cystitis that can be diagnosed on clinical grounds only and treated with a short course of oral antibiotics; in case of a resistant causal uropathogen, the bladder infection may still be a self-limiting disease.[1] Although the majority of cystitis episodes are attended at the primary-care settings, they are also a frequent cause of ED consultation.[2] Fever in UTIs represents the presence of tissue invasion and inflammation such as pyelonephritis or prostatitis, both of which may be accompanied by urosepsis syndrome and progress to life-threatening septic shock. In febrile UTI, risk for a resistant uropathogen and complicated course should be evaluated to help guide clinical decisions regarding antimicrobial therapy and hospitalization.

In this chapter, we review the recent evidence for the optimization of diagnosis, triage decisions, and empirical therapy with a focus on febrile UTI at EDs.

DIAGNOSIS OF URINARY TRACT INFECTION

The clinical presentation is the cornerstone of diagnosis of UTIs. Classification of UTIs was recently updated to generalize the approach for clinical and research purposes.[3] Acute cystitis is characterized by dysuria, frequency, urgency and suprapubic pain without fever, and should be distinguished from invasive infection such as acute pyelonephritis, prostatitis, and urosepsis to allow a more rational use of antibiotics and other resources. The diagnosis is confirmed by the presence of a significant number of microbial pathogens within the urinary tract, but unfortunately the results of urine cultures are not immediately available at presentation in the ED to ensure correct diagnosis.

Leukocyturia supports a clinical suspicion of UTIs, but can be deceptive because of its low positive predictive value. A positive nitrite reaction is more accurate in diagnosing UTIs (positive likelihood ratio of 7.5–24.5), but its sensitivity is poor.[4] Particularly in elderly individuals, interpretation of urinary dipstick testing, urinalysis, and even urine culture is challenging because of the high prevalence (up to 60%) of asymptomatic bacteriuria. Additionally, adults aged 65 years and older may present with less clear symptoms. Caterino et al.[5] performed a retrospective cross-sectional analysis of the 2001–2008 National Hospital Ambulatory Medical Care Survey of adult patients visiting the ED with a diagnosis of UTIs, including cystitis and pyelonephritis, representing approximately 25 million ED visits for UTIs. In this study, urinary tract symptoms were identified in 32% of patients aged 18–64 years, in 24% in those aged 65–84 years, and 17% in those aged 85 and older. Age over 65 years and nursing home residence were associated with a lack of urinary tract symptoms.

Although studies of urine culture-proven UTIs in older inpatients showed a similar rate of urinary symptoms of 32%,[6] the absence of classic symptoms in this study raises the question

of correct diagnosis of UTIs in acute care setting. This finding was confirmed in a retrospective chart review, in which 43% of older women (70 and older) diagnosed with UTIs in the ED did not have a positive urine culture and 95% of these women were treated with antibiotics.[7] In these patients, the true cause of their complaints might have been overlooked, and the inappropriate use of antibiotics for presumed UTIs may contribute to the development of resistance. As older patients frequently have asymptomatic bacteriuria,[8] the percentage of patients with true UTIs among the symptomless patients might even be lower. Clearly, an effective strategy is needed to improve the diagnostic accuracy of UTIs in the ED.

ADDITIONAL VALUE OF BLOOD CULTURES

Urine cultures are recommended in case of invasive UTI to tailor antibiotic treatment. Bacteraemia is present in 19–29% of patients presenting with febrile UTI.[9-11]

In a prospective, multicenter study concerning adults with community-onset febrile UTI, the causative uropathogen was isolated from blood cultures only in 5% of patients with negative urine cultures. Antimicrobial pre-treatment [odds ratio (OR), 3.3; 95% confidence interval (CI), 1.5–7.1], an indwelling catheter (OR, 2.8; 95% CI, 1.0–7.5), and malignancy (OR, 2.7; 95% CI, 1.1–6.9) were identified as independent risk factors for these discordant culture results.[10] These findings were confirmed in a recent retrospective study, in which receiving antibiotic therapy at the moment of presentation (OR, 2.06; 95% CI, 1.18–3.61) was the only factor independently associated with discordant culture results (in 7% of patients).[11] These data show that blood cultures are of limited additional diagnostic value in most cases of febrile UTI. However, patients with a urinary catheter or malignancy or those who experience failure of antibiotic therapy for UTIs do have a higher risk for discordant cultures. We therefore recommend obtaining blood cultures for these specific patients.

IMAGING OF THE URINARY TRACT

Clinical studies evaluating the role of radiologic imaging in patients with febrile UTI are scarce. The European Association of Urology recommends ultrasound evaluation to rule out urinary obstruction in all patients with pyelonephritis and additional radiologic testing, such as computed tomography (CT), if fever persists after 72 h of appropriate treatment.[12] A recent prospective study validated a clinical rule to predict the need for radiologic imaging in febrile UTI patients at the EDs.[13] This study advocates performing ultrasonography only in patients with either a history of nephrolithiasis, renal insufficiency (MDRD <40ml/min/1.73m3), or a urinary pH at least 7.0. The absence of these predictors ruled out the presence of pyonephrosis or obstructive uropathy, the findings that were present in 6% of febrile UTI patients. Another retrospective study in bacteraemic UTI patients showed significant urologic abnormalities in

32% of the patients.[14] In this cohort, complicated diabetes, renal disease, pre-existing urologic abnormalities, or nephrolithiasis were significant predictors for the presence of hydronephrosis or renal stones. CT findings of the kidneys might be of prognostic value as it correlates with the disease severity of acute pyelonephritis.[15] However, to date, there are no studies demonstrating that CT findings of the urinary tract significantly alter the therapeutic strategy. On the basis of these findings, we recommend performing an immediate ultrasonography of the urinary tract in febrile UTI patients with either a history of nephrolithiasis, renal insufficiency (MDRD <40 ml/min/1.73m3) or a urinary pH of at least 7.0 and in those who require ICU admission.

ADMISSION POLICY IN FEBRILE URINARY TRACT INFECTION

Fever in patients with UTIs reflects deeper tissue involvement and the presence of pyelonephritis, acute prostatitis, or urosepsis. Febrile UTI usually presents as a mild or moderate infection, which can be treated safely by oral antimicrobials in an outpatient setting; a minority of cases progress to septic shock. Therefore, a critical decision at the ED is whether the patient has to be hospitalized. This decision has consequences for resources, laboratory evaluation, risk for nosocomial infections, possible hospital-acquired disability in elderly patients and thus, healthcare costs.

Currently, admission policy is at the discretion of the emergency physician and guided by history, underlying disease, and on the severity of local and vital signs. Clinical tools helping to classify risks in patients with febrile UTI are being developed to guide triage decision. As a rule, women with uncomplicated acute pyelonephritis can be safely treated with oral antimicrobials at home. Recently, a prospective study in women and men consecutively demonstrated that all patients with febrile UTI with no suspicion of deterioration to severe sepsis as judged 'on gut feeling' by the attending physician can also be safely treated at home with oral antibiotics.[16]. In the current clinical practice, this assessment is based on the clinical parameters such as history, underlying disease, and on the severity of local and vital signs. We evaluated the use of a clinical prediction rule at EDs to help guide triage upon hospital admission policy in patients with febrile UTI. The results of this study are outlined in Chapter 2.

BIOMARKERS AS PREDICTOR OF BACTERAEMIA OR ADVERSE EVENTS

Two compounds that are used as biomarkers are procalcitonin and pro-adrenomedullin.

Procalcitonin

Procalcitonin (PCT) is a prohormone of calcitonin, used as a biomarker for the diagnosis of bacterial infection and sepsis, and for differentiation from viral infection or auto-immune disorders. PCT has been shown to differentiate lower UTI from pyelonephritis in children and

may predict subsequent renal scarring in this population.[17] In adults with febrile UTI, PCT is a marker of bacteraemia.[18] Recently, this finding has been confirmed in a study including women with acute pyelonephritis.

PCT predicted the severity of sepsis and bacteraemia (area under the curve (AUC) of 0.75 and 0.72, respectively), but disease classification systems as Sequential Organ Failure Assessment score (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) performed better in predicting 28-day mortality.[19] Ha et al. showed that PCT outperformed CRP in the prediction of bacteraemia in adults with acute pyelonephritis.[20] Debate on the value of PCT for triage decisions has not yet been closed, as in the study by Lemiale,[21] PCT could not predict a complicated course in outpatients with acute pyelonephritis.

Pro-adrenomedullin

Pro-adrenomedullin (proADM) is a promising inflammatory biomarker that, because of its shorthalf-life in serum, is measured best by its stable midregional fragment of pro-ADM (MR-proADM). Both proADM and MR-proADM levels are elevated in patients with sepsis.[22,23] A prospective multicenter study recently showed that MR-proADM accurately predicts a complicated course as reflected by bacteraemia and need for ICU admission, as well as 30-day mortality, in patients presenting with community-acquired febrile UTI. Predictive value of MR-proADM was superior to PCT and the more conventional biomarkers C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and leukocyte count.[24]

BIOMARKER-GUIDED TRIAGE

As biomarkers are objective, can be readily available in the ED if laboratory infrastructure is organized, and can predict complicated course, they might guide the physician in deciding the location of treatment. Claessens et al. report that biomarkers PCT, CRP, and midregional pro-natriuretic peptide did not help guide physicians to decide upon the need for hospital in patients with acute pyelonephritis.[25] MR-proADM might perform better, as its predictive value was superior to other biomarkers. Litke et al. retrospectively evaluated a virtual triage algorithm including patients with (un)complicated cystitis and febrile UTI.[26] Combination of proADM (>1.5 nmol/l) or urea (>14 mmol/l) with objective clinical admission criteria, such as inability to take oral antibiotics or evidence of serious complications or comorbidity necessitating hospitalization, could have reduced the hospitalization rate by 12% without compromising the safety.

Prospective interventional trials are needed to address this question, especially in patients with invasive UTI. Currently, a prospective, randomized study on pro-ADM-enhanced triage is conducted in Switzerland.[27] As outlined in Chapter 3, we evaluated the potential use of pro-ADM in patients presenting with febrile UTI.

THERAPY OF COMMUNITY-ONSET URINARY TRACT INFECTION

Antibiotic resistance among Gram-negative microorganisms complicates the management of UTI. The WHO published a first global assessment this year that showed alarming resistance rates throughout the world.[28] This report underlined the fact that surveillance of antimicrobial resistance is well organized in Europe and the USA, but remains poor in other regions. Resistance to third generation cephalosporins, most frequently used for the intravenous treatment of febrile UTI, and to fluoroquinolones exceed 50% in five of the six WHO regions. These numbers have to be interpreted with caution, because of variances in sample collection, interpretation of laboratory findings, and regional coverage, but they confirm the previous reports of increasing resistance worldwide.[29,30]

Awareness of the sensitivity rates of *Escherichia coli* isolates in the local region is essential for the appropriate empirical selection of agents to treat UTIs. These data obtained from the regional microbiology laboratories should be incorporated in the local treatment guidelines available in the ED. Restrictive use of antibiotics and adherence to guidelines are essential to reduce the antibiotic pressure worldwide and stop or slow down the development of resistance. The available antibiotics should be preserved for those patients who really need them, highlighting the importance of differentiating asymptomatic bacteriuria from UTI in the ED.

Acute uncomplicated cystitis

Recently updated guidelines from the Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommend the use of nitrofurantoin, fosfomycin, and sulfamethoxazole–trimethoprim (SMX–TMP) as the first-line treatment for acute uncomplicated cystitis.[31] These agents reach adequate levels in the urine and have low propensity for collateral ecological damage. An additional advantage is that these agents usually remain active in the case of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria. The empiric use of SMX–TMP is recommended if local resistance rate of uropathogens does not exceed the threshold of 20% or if the causative pathogen is known to be susceptible.[31] Fluoroquinolones and b-lactam agents (including inhibitor combinations) should be reserved for patients who failed in first-line therapy or with contraindications.

Widespread use of fluoroquinolones contributes to the development of resistance; therefore, it is important to reserve its use for patients with suspected invasive UTIs with a risk of bacteraemia.

Despite these recommendations, ciprofloxacin and levofloxacin are the most common prescribed drugs for acute cystitis in the USA.[32] We endorse antibiotic stewardship to reduce the inappropriate use of antibiotics. Simple interventions in the emergency room, such as the introduction of an electronic ordering set and feedback, have been shown to significantly and sustainably improve the adherence to guidelines (overall adherence to UTI treatment guideline increased from 44 to 82% and prescription of fluoroquinolones for uncomplicated cystitis decreased from 44 to 13%).[2]

Febrile urinary tract infection

In patients with febrile UTI, a urine culture with an antibiogram should be performed, to adjust empirical antimicrobial therapy according to the resistance pattern of the causative pathogen. In areas where the prevalence of resistance to fluoroquinolones is below 10%, ciprofloxacin is the recommended first-line outpatient treatment for acute uncomplicated pyelonephritis.[31] As a result of the high bioavailability, good penetration in renal and prostate tissue and blood, oral ciprofloxacin can be used even in the case of bacteraemia.[33]

In regions with higher level of ciprofloxacin resistance, a dose of a long-acting parental antimicrobial, such as a 1 g dose of ceftriaxone or a 24-h dose of an aminoglycoside, can be given once during the initiation of therapy while culture results are pending.[31]

Patients with systemic symptoms requiring hospitalization should better be started on an intravenous antimicrobial regimen. The optimal treatment differs regionally and should be adapted to local resistance prevalence. Most guidelines advise the use of second-generation cephalosporins with or without an aminoglycoside, third-generation cephalosporins, or carbapenems. Guideline-adherent initial treatment with a broad-spectrum agent should be tailored on the basis of susceptibility results and switched to a culture-directed oral agent as soon as possible, because this has been shown to reduce the length of hospital stay in a recent Dutch multicenter study.[34] This approach might have a favourable impact on the patient outcome and healthcare costs.

Identification of risk factors for resistant uropathogens

To select the appropriate empirical antibiotic treatment, it is essential to estimate the risk of the involved uropathogen resistant to antibiotics. Several recent studies addressed the identification of risk factors for resistance to fluoroquinolones in patients presenting to the ED with community-onset acute pyelonephritis, with reported resistance ranging from 12 to 34%.[35-40] Risk factors for resistance to fluoroquinolones imply prior use of antibiotics and healthcare-associated UTI, including a history of hospital admission within the last 3–6 months, the presence of an indwelling urinary catheter, nursing home residence, or recent invasive urinary instrumentation (Table 1).

Risk factors for community-acquired febrile UTI due to ESBL-producing enterobacteriaceae are similar, as shown in Table 1.[40-42] Interestingly, recent travel to an area where

ESBL is endemic was a strong predictor of ESBL producing uropathogens in Norway, where the prevalence of ESBL is low.[42] The range of prevalence of ESBL-positive isolates in these reports was 1.3–10.3%. Inappropriate initial antibiotic treatment did not lead to more clinical and microbiological failure in patients with ESBL-positive isolates in a Korean study,[41] but a resistant causative uropathogen is associated with a prolonged duration of hospitalization.[37,40,41] We recommend considering the adjustment of empirical therapy in patients presenting to the ED with febrile UTI and risk factors as presented in Table 1.

For patients at risk for fluoroquinolone resistance, intravenous therapy with an extended-spectrum cephalosporin is an option; in case of risk for ESBL-producing bacteria, the rational

choice would be to add an aminoglycoside to the regimen or to use a carbapenem [31]. Severity of disease, possibility of follow-up, and monitoring of treatment failure should be taken into account. In the case of a recent history of UTI with resistant bacteria, alternative treatment should also be considered.

Table 1. Risk factors for FQ-R and ESBL-producing uropathogens in community-onset acute pyelonephritis.

	Risk factor for	Multivariate odds ratio	Reference
Male sex	FQ-R	3.1	37,40
Exposure to antibiotics in the proceeding 3–6 months (fluoroquinolone strongest predictor)	FQ-R ESBL	2.3-17.5 3.1-4.2	35,37-42
Prior hospitalization within 6 months	FQ-R ESBL	2.0-4.8 7.4	35,36,38,40
Indwelling urinary catheter	FQ-R ESBL	3.1 4.4	35,36,41
Isolation of fluoroquinolone resistant *Escherichia coli* in the urine within the preceding 3 months	FQ-R	5.8	39
Nursing home residence	FQ-R	3.1-4.8	38,40
Recent travel to Asia, the Middle East or Africa (within 6 weeks)	ESBL	21	42
Underlying comorbidity such as diabetes, chronic kidney disease, haematological or neurological disease	FQ-R ESBL	2.9 3.2-16.8	37,39-42

ESBL, extended-spectrum beta lactamase; FQ-R, fluoroquinolone resistance.

CONCLUSION

Though UTI is part of the daily practice in EDs, there remain controversies upon its management. Policies to risk stratify patients to guide the choice of empirical antibiotic treatment and decisions upon need for hospitalization and need for microbiologic, radiologic, and urologic diagnostics should be improved. Blood cultures and ultrasound of the urinary tract might be restricted to specific patient groups. Women with acute uncomplicated pyelonephritis can be managed as outpatients, whereas this is less clear in other patient groups. Biomarkers such as pro-ADM might be of use in guiding admission policy. Empirical therapy of febrile UTI should be based on local susceptibility data, taking individual risk factors for resistance into account.

OUTLINE OF THE THESIS

The current thesis aims at optimizing care for patients with urinary tract infections, with a focus on admission policy, diagnostics and treatment duration in patients presenting with febrile UTI, and on UTIs caused by resistant uropathogens.

In **Chapter 2** we validated a clinical severity assessment tool, called the 'Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden' (PRACTICE) in patients with febrile urinary tract infection. Subsequently, the use of the PRACTICE guiding admission policy was evaluated in a stepped wedge cluster randomized trial, enrolling patients presenting to the emergency department.

Chapter 3 focuses on improvement of risk assessment and triage decisions by the use of biomarkers. In this study, we compared the biomarkers MR-proADM, procalcitonin, CRP and the PRACTICE prediction tool in their ability to predict a clinically severe course of disease, initial hospitalization and subsequent readmission during the treatment of febrile UTI.

Chapter 4 describes a randomized placebo-controlled, double-blind, non-inferiority trial on treatment duration of febrile urinary tract infection. 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 hospitalized patients.

In **Chapter 5** we assessed the use of the biomarkers procalcitonin, MR-proADM and CRP to predict clinical cure or failure in these patients with community-acquired febrile urinary tract infection.

The study in **Chapter 6** reports on the effectiveness, the safety and the feasibility of prophylactic intravesical gentamicin treatment for patients with complex recurrent urinary tract infections caused by multidrug-resistant bacteria.

Chapter 7 describes a patient with recurrent urinary tract infections caused by multi-drug resistant *Pseudomonas aeruginosa*, which hampered a planned kidney transplant. He was treated with combined intravesical gentamicin, intravenous colistin and fecal microbiota transfer, and fecal microbiota profiles before and after treatment were analyzed.

In **Chapter 8** extended spectrum cephalosporin-resistant *Escherichia coli* isolates from patients with urinary tract infection, broilers, humans on broiler farms, versus isolates from humans in the general population were compared with respect to virulence factors, phylogenetic groups, and resistance genes.

In **Chapter 9** a retrospective case record study is presented on the use of automated urine microscopy analysis in the clinical diagnosis of urinary tract infection in an academic setting, aiming at defining an optimal diagnostic score based on both clinical and automated urine analysis parameters.

Finally, the results of the thesis are summarized and discussed in **Chapter 10**.

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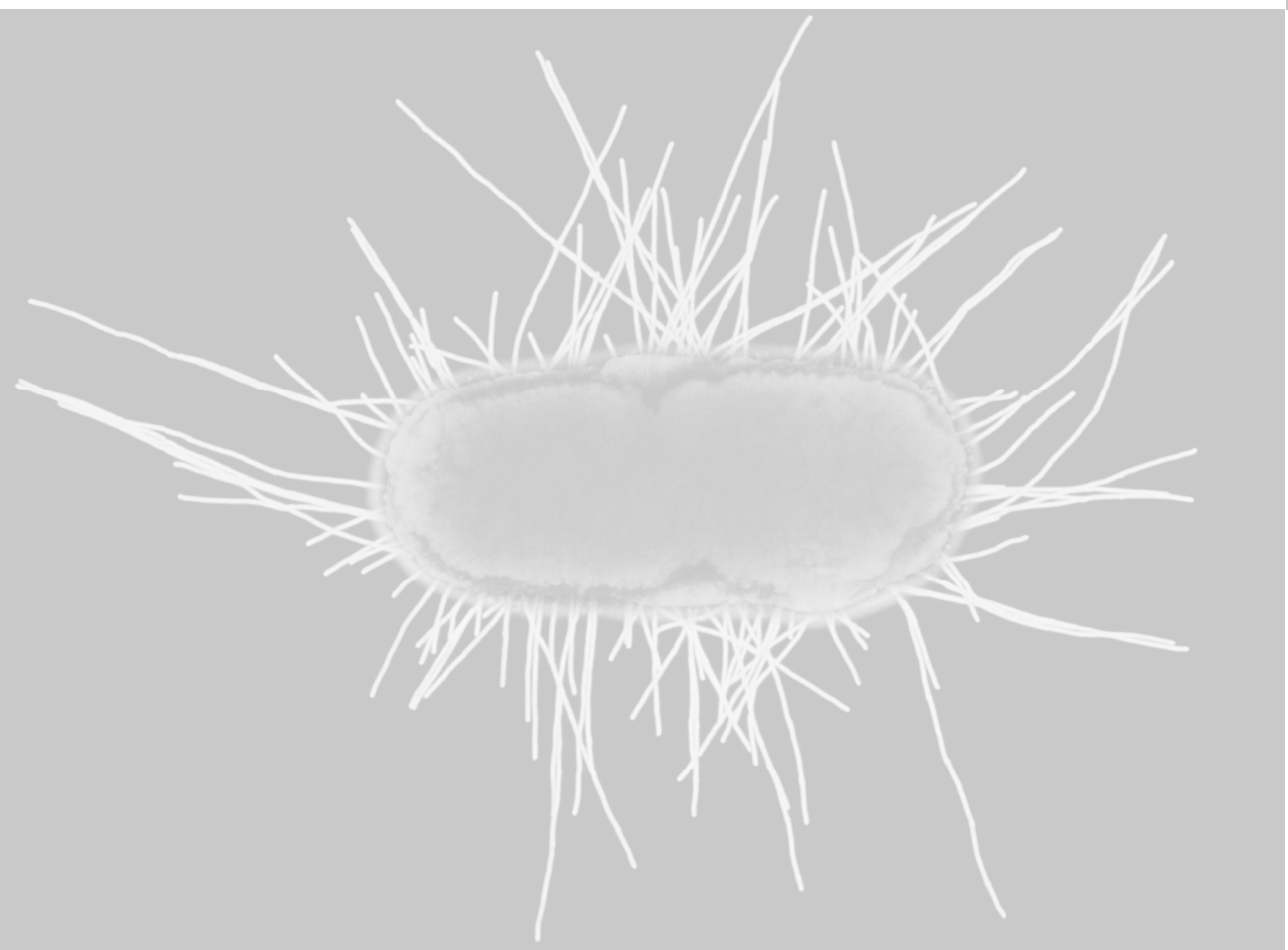

CHAPTER 2

Hospitalization for community-acquired febrile urinary tract infection: validation and impact assessment of a clinical prediction rule

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ABSTRACT

Background

There is a lack of severity assessment tools to identify adults presenting with febrile urinary tract infection (FUTI) at risk for complicated outcome and guide admission policy. We aimed to validate the Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden (PRACTICE), a modified form of the pneumonia severity index, and to subsequentially assess its use in clinical practice.

Methods

A prospective observational multicenter study for model validation (2004–2009), followed by a multicenter controlled clinical trial with stepped wedge cluster-randomization for impact assessment (2010–2014), with a follow up of 3 months. Participants were 1157 consecutive patients with a presumptive diagnosis of acute febrile UTI (787 in validation cohort and 370 in the randomized trial), enrolled at emergency departments of 7 hospitals and 35 primary care centers in the Netherlands.

The clinical prediction rule contained 12 predictors of complicated course. In the randomized trial the PRACTICE included guidance on hospitalization for high risk (>100 points) and home discharge for low risk patients (<75 points), in the control period the standard policy regarding hospital admission was applied. Main outcomes were effectiveness of the clinical prediction rule, as measured by primary hospital admission rate, and its safety, as measured by the rate of low-risk patients who needed to be hospitalized for FUTI after initial home-based treatment, and 30-day mortality.

Results

A total of 370 patients were included in the randomized trial, 237 in the control period and 133 in the intervention period. Use of PRACTICE significantly reduced the primary hospitalization rate (from 219/237, 92%, in the control group to 96/133, 72%, in the intervention group, $p < 0.01$). The secondary hospital admission rate after initial outpatient treatment was 6% in control patients and 27% in intervention patients (1/17 and 10/37; $p < 0.001$).

Conclusions

Although the proposed PRACTICE prediction rule is associated with a lower number of hospital admissions of patients presenting to the ED with presumptive febrile urinary tract infection, further improvement is necessary to reduce the occurrence of secondary hospital admissions.

Trial registration

NTR4480 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4480

BACKGROUND

The majority of adults presenting to hospital with an acute febrile illness suffer from respiratory or urinary tract infections.[1,2] The course of infection may be unpredictable, and fever may reflect the onset of sepsis with potential progression to septic shock and multi organ failure. However, adults with fever of bacterial origin usually present with a mild illness at emergency departments (ED) and respond favourably to antibiotic treatment. It thus appears that the vast majority of these patients can be managed safely as outpatients. In daily clinical practice the need for hospital-based treatment for febrile urinary tract infection (FUTI) is assessed on basis of history, comorbidity and on severity of local and vital signs.

For respiratory tract infection there are validated clinical rules to calculate the mortality risk, such as the *Pneumonia Severity Index* (PSI), which is used to provide guidance on decisions regarding treatment and hospital admission.[3-5] To date, there are no such rules to assess the risk of poor outcome in patients presenting with FUTI.

The risk of complicated course of FUTI increases with age and comorbidity, but the event rate of life-threatening complications is low.[6-8] Physicians tend to apply low thresholds for hospitalization, which suggests that many admissions may be avoidable.[9,10] Therefore, clinical tools that predict prognosis in patients with FUTI are needed to identify those who benefit from hospital admission, and those who may safely be managed as outpatients.

The main predicting factors of mortality in the PSI are not specific for pneumonia such as age, co-morbidity and physical or laboratory signs of sepsis.[3] We therefore considered that this risk assessment might also apply for community-acquired infections other than pneumonia. As our focus was on the evaluation of a practical and bedside available prediction tool, we modified the PSI by erasing all the laboratory variables (Table 1) and changed the name in the *Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden* (PRACTICE).

Table 1. Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden (PRACTICE)

Characteristic	Allocated points[a]
Demographic	
Age (men)	Age (years)
Age (women)	Age (years) - 10
Nursing home resident	+10
Comorbidity[b]	
Malignancy	+30
Congestive heart failure	+10
Cerebrovascular disease	+10
Liver cirrhosis	+20
Renal disease	+10
Signs & Symptoms	
Altered mental status	+20
Respiratory rate ≥ 30/min	+20
Systolic blood pressure < 90 mm Hg	+20
Pulse ≥ 125/min	+10
Temperature ≥ 40 ^{O}C	+15

[a]A total score individual patient score is obtained by summing the points for each characteristic.
[b]Malignancy is defined as any cancer except basal- or squamous-cell cancer of the skin that was active within the previous year of presentation. Congestive heart failure is defined as ventricular dysfunction for which the patient is prescribed medication and/or consults a hospital-based medical specialist. Cerebrovascular disease is defined as a history of stroke or transient ischemic attack. Liver disease is defined as a clinical diagnosis of cirrhosis. Renal disease is defined as a history of chronic renal disease.
According to risk class the following recommendations will apply:

< 75 points	strong recommendation towards home-based management
75-100 points	consider home-based management
>100 points	strong recommendation towards hospital admission

We used data from a prospective observational multi-center cohort study that included 787 consecutive adults with febrile UTI between 2004 and 2009 to validate this PSI derived prediction rule for complicated course in patients with FUTI (all details and methods are described in the Supplementary Data). In this validation cohort, the PRACTICE score identified those at very low risk for 30-day mortality and ICU admission; the area under the curve (AUC) of the receiver operating characteristic curve for these outcomes indicated a good discriminatory power (AUC 30-day mortality: 0.91; AUC 30-day mortality or ICU admission: 0.84). The PRACTICE score was divided in 5 risk categories (see Additional file 1: Table S1), showing that patients with a PRACTICE score < 100 points (n = 636) had a very low risk (<2%) of adverse outcomes; yet 380 (60%) of those were hospitalized. Using a cut-off value of the PRACTICE score ≥ 100 resulted in a negative predictive value for 30-day mortality of 1.00 and for the composite endpoint 'complicated course' (30-day mortality, ICU admission or hospitalization >10 days) of 0.90.

Because the cut-off point was chosen to identify low-risk patients, the positive predictive values (PPV) were low (PVV 0.12 and 0.39, respectively). We assumed that the PRACTICE is a good bedside clinical tool to distinguish patients with FUTI at low risk of complicated course who can be managed as outpatients. The aim of the present study is to validate the PRACTICE in a new prospective cohort to guide the need for hospitalization in patients with FUTI presenting at EDs, with the aim to reduce hospitalization rates without compromising clinical outcome.

METHODS

Trial design

We performed a stepped wedge cluster-randomized trial involving consecutive patients presenting with a presumptive diagnosis of FUTI, at the EDs of 7 hospitals in the Netherlands, between January 2010 and June 2014.[11]

These centers also participated in the validation cohort study (see Supplementary Data). All participating centers started with a control period, in which routine clinical practice with regard to hospitalization policy was applied. The intervention (use of the PRACTICE) was introduced at the participating centers sequentially, in random order.

By the end of the allocation all sites, except one, used the PRACTICE to guide admission policy.

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

pregnancy, haemodialysis or peritoneal dialysis, a history of kidney transplantation or polycystic kidney disease. The study protocol was approved by the local Ethics Committee, and all participants signed an informed consent form prior to enrolment.

Intervention and treatment

The PRACTICE score ranges from 8 to >125. Based on the validation cohort it was divided into three risk classes (low <75 points; intermediate 75–100 points; high >100 points) with corresponding recommendations regarding hospitalization policy (Table 1). During the control period, the decision to treat the patient at home or admission to hospital was made at the discretion of the ED physician. At the start of the intervention period the ED physicians were instructed to calculate the PRACTICE score and, on that basis, decide on hospital-based or home-based treatment. Preferably admission policy was done according to the guidance as described in Table 1, however, the attending physician was responsible for the final decision on treatment location.

Throughout the whole study period the antibiotic therapy was left at the discretion of the treating physician. According to local guidelines, outpatient treatment for FUTI consisted of a 10–14 day course of oral antimicrobials (first choice ciprofloxacin 500 mg b.i.d.).[12] In case of

risk factors for quinolone resistance a single dose of a long-acting parental antimicrobial, e.g. ceftriaxone or an aminoglycoside, at the initiation of therapy was advised while culture results were pending.[13] Admitted patients started with empirical antimicrobials intravenously according to local policy and were switched to an oral antibiotic based on antimicrobial sensitivity testing of the uropathogen cultured.

Study procedures

Within 24–48 h of notification, qualified research nurses collected demographic and clinical data by reviewing the medical record completed with an interview by telephone or in person, using a standardized questionnaire. A midstream-catch urine culture and a set of blood cultures were taken before commencement of antimicrobial therapy.

All patients were contacted in person 3–4 days and 28–32 days after enrolment, and contacted by phone at day 13–15 and day 84–92, to assess clinical outcome. Urine culture was repeated at the 28–32 day follow-up visit. In case of (re) admission during the study period, related data were obtained from the medical record and interview. In case a patient was lost to follow up, survival and readmission were assessed by inquiry with the patient's primary care physicians, hospital chart and/or local governmental mortality registries. Urine and blood cultures were performed using standard microbiological methods at local certified laboratories. Data collection of patients included during the validation period was identical (see Supplementary Data).

Endpoints

The primary endpoints were primary hospital admission rate (the percentage of patients who were directly admitted to hospital) and secondary hospital admission rate (the percentage of patients who needed to be hospitalized for FUTI after initial home-based treatment). Secondary outcome measures were 30- and 90-day all-cause mortality rate, ICU admission rate, the total number of hospitalization days over a 3-month follow-up and clinical- and microbiological cure rate through the 10- to 18-day post-treatment visit.

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.[14] 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 combined with disappearance of leucocyturia).[15]

A Data Safety Monitoring Board (DSMB) monitored the study and prescheduled interim analyses were performed according to predefined stopping rules. For the analysis of secondary hospital admission only low risk patients PRACTICE-score = < 100 points were considered.

Definitions

UTI in men, postmenopausal women and in women with any structural or functional abnormality of the urinary tract were considered 'complicated' whereas in all others it was

considered 'uncomplicated' UTI.[13,15] Comorbidity was defined as the presence of any urinary tract disorder, heart failure, cerebrovascular disease, renal insufficiency, diabetes mellitus, malignancy or chronic obstructive pulmonary disease.

Statistical analysis

The primary endpoints were analysed on the intention-to-treat (ITT) and per-protocol (PP) population Evaluable patients for ITT analysis included all patients who met the inclusion criteria and had at least 1 follow up visit. The PP population consisted of cases in which PRACTICE-hospitalization recommendations were actually followed in the intervention period and all cases in the control period. Binomial or categorical outcome measures were analysed using Chi-square tests (Pearson's or Fisher's). Risk difference with 95% confidence interval (CI) was used to compare the differences of categorical outcomes. Tests of significance were at 0.05 level, two-tailed, for primary hospital admission rate.

A study sample size of 326 patients in both arms was calculated on the basis of secondary hospital admission rate, which was estimated to be approximately 5%, based on our previous study on FUTI,[16] to have a power of 90% to show that the secondary admission rate in the intervention period (PRACTICE-guided management) is at least as low as the control period. As we were only interested in non-inferiority and not in equivalence in secondary hospital admission rate, the sample size calculation was based on a one-tailed alpha of 0.025. This implies that the 90% CI of a two-tailed Chi-square test should not cross the predefined risk difference of 2.5% higher secondary admission rates. All analyses were performed using SPSS 20.0 (SPPS Inc., USA).

RESULTS

Study participants

A total of 370 patients was included, 237 in the control period and 133 in the intervention period (see the flowchart in Fig. 1). In the ITT-population, baseline demographic characteristics were similar in the two groups (Table 2), except for a difference in history of cerebrovascular and chronic renal disease. Patients in who PRACTICE recommendations were followed (the PP-analysis) were significantly older, had more comorbidity and more often suffered complicated UTI than control patients (Table 2).

Figure 1. Patient inclusion flow chart

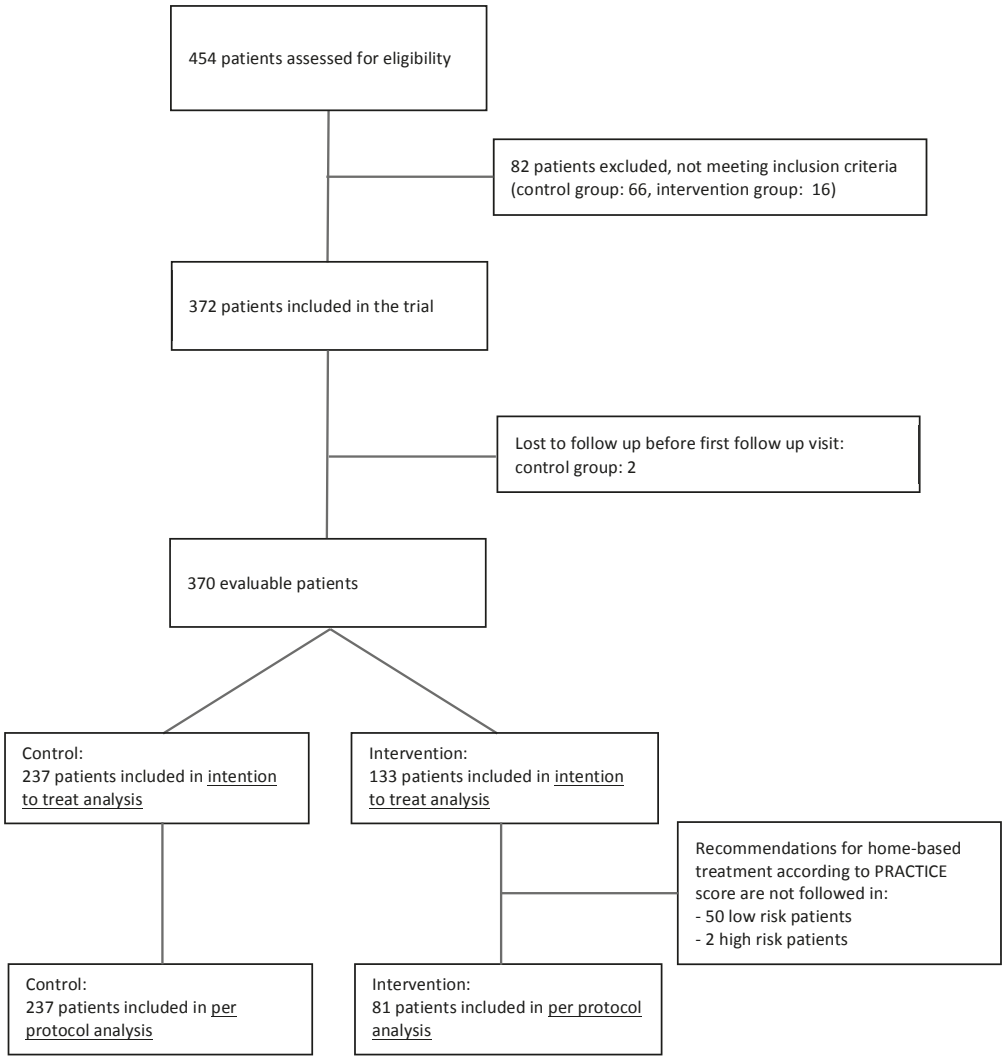

Table 2. Patients' demographics

	Control group	Intervention group		*p*	
	ITT = PP	ITT	PP	Control vs ITT	Control vs PP
	n=237	*n*=133	*n*=81		
Age in years; median, (IQR)	60 (30)	61 (34)	71 (26)	ns	<0,01
Sex – female	148 (62)	74 (56)	33 (41)	ns	<0,01
Febrile uncomplicated UTI	54 (23)	30 (23)	9 (11)	ns	0,02
Antimicrobial treatment at inclusion	90 (38)*	44 (33)	22 (27)	ns	ns
Urologic history					
Present urinary catheter	17 (7)	9 (7)	8 (10)	ns	ns
History of urinary tract disorder[a]	73 (31)	33 (25)	29 (36)	ns	ns
Recurrent UTI[b]	30 (13)[†]	11 (9)	5 (7)	ns	ns
Comorbidity					
Any	124 (52)	77 (58)	57 (70)	ns	<0,01
Diabetes mellitus	36 (15)	29 (22)	25 (31)	ns	<0,01
Malignancy	13 (5)	11 (8)	10 (12)	ns	ns
Heart failure	32 (13)	12 (9)	11 (14)	ns	ns
Cerebrovascular disease	17 (7)	20 (15)	18 (22)	0.02	<0,01
Cirrhosis	1 (0)	2 (1)	1 (1)	ns	ns
Renal insufficiency	12 (5)	20 (15)	18 (22)	<0.01	<0,01
Immunocompromised	19 (8)	10 (8)	5 (6)	ns	ns

Data are presented as n (%) unless otherwise stated. ITT intention to treat analysis, PP per protocol analysis, IQR interquartile range, ns not significant (at 0,05 level), UTI urinary tract infection. [a]Urinary tract disorder: presence of any functional or anatomical abnormality of the urinary tract excluding the presence of a urinary catheter. [b]Recurrent UTI: two or more episodes in the last 6 months or three or more episodes of UTI in the last year. cUTI history was unknown in 13 subjects in control period and 6 subjects in intervention period.

Fifteen patients who were included in the study by ED-physicians did not completely meet the predefined inclusion criteria, but discharge diagnosis as concluded by the attending physician was FUTI in all cases. On hospital presentation, ten of these patients had no specific symptoms of UTI, 8 of these 10 patients had cultures of blood (3) and/or urine (5) positive with significant growth of an uropathogen, 2 had negative urine cultures, and 1 of them used antibiotics at inclusion. The other 5 patients did not have or report fever at inclusion, 1 of them was on TNFα-inhibitors. Follow up was not completed in 37 patients in the control group and in 13 patients in the intervention group. Based on review of medical charts and governmental records these patients were all alive and without secondary admission, and included as such in the analysis.

Cultures

The results of urine cultures, performed in 347 (93%) patients are shown in Table 3; 125 (36%) urine cultures were either sterile or contaminated of which 65% were obtained during antibiotic (pre)treatment. Blood cultures, performed in 357 (96%) patients, revealed bacteraemia in 97 (27%) cases (Table 3). Rate of bacteraemia was similar in intervention and control group.

Table 3. Bacteria isolated from baseline cultures

	Control period n=237	Intervention period n=133
Urine cultures		
Escherichia coli	126 (56)	51 (42)
Klebsiella spp	12 (5)	7 (6)
Proteus spp	5 (2)	3 (2)
Enterococcus spp	3 (1)	-
Pseudomonas aeruginosa	-	1 (1)
Staphylococcus aureus	1 (0)	1 (1)
Other	7 (3)	6 (5)
Contaminated / mixed flora	26 (12)	24 (20)
Total positive urine cultures	154/225 (68)	69/122 (57)[a]
Blood cultures		
Escherichia coli	56 (25)	21 (68)
Klebsiella spp	4 (6)	4 (13)
Proteus spp	-	1 (3)
Enterobacter spp	1 (1)	-
Pseudomonas aeruginosa	1 (1)	-
Staphylococcus aureus	1 (1)	2 (6)
Beta haemolytic streptococcus	1 (1)	2 (6)
Citrobacter spp	1 (1)	-
Bacteroides fragilis	1 (1)	-
Salmonella paratyphi	-	1 (3)
Total positive blood cultures	66/228 (29)[b]	31/129 (24)[b]

Data are presented as n (%). [a]Urine cultures were not performed in 12 patients in the control period and 11 patients in the intervention period. [b]Blood cultures were not obtained in 9 patients in the control period and 4 patients in the intervention period.

Outcome

The mean PRACTICE scores in the control and intervention groups (ITT analysis) were 62 (95% CI: 57.7 to 65.4) and 64 (95% CI: 58.3 to 69.7), respectively. Mean PRACTICE score in the PP population was 76 (95% CI: 69.0 to 83.3; $p < 0{,}01$).

Use of the PRACTICE significantly reduced primary hospitalization rate, 96 (72%) patients in the intervention group were admitted in the hospital versus 219 (92%) in the control period ($p < 0.01$) (Table 4). The hospitalization rate was further reduced to 57% in the PP population.

The attending physician overruled the PRACTICE rule in 50 out of 153 patients categorized as low risk, who were admitted to the hospital because of 'sick appearance' (n = 9), severe flank pain (n = 2), antibiotic treatment at presentation (n = 7), comorbidity (n = 5), nausea (n = 3), uncertain diagnosis (n = 4), unknown (n = 7) or other reasons (n = 13). On the other hand, two patients categorized as high risk were treated at home because they insisted on home based treatment.

The median number of hospitalization days over a 3-month follow-up was 5 days (95% CI 5.6 to 7.0) vs 4 days (95% CI 4.4 to 6.7) for the control and intervention period, respectively.

Clinical and microbiological cure on day 30 did not differ significantly between both groups (Table 4). The clinical outcomes according to risk class are outlined in Table 5.

Table 4. Patients' outcomes

	Control period n=237	Intervention period ITT n=133	Intervention period PP n=81
Hospitalization			
Primary hospitalization	219 (92) *	96 (72)*	46 (57)*
Low risk	136	50	0
Intermediate risk	58	29	29
High risk	25	17	17
Secondary admission (all risk classes)	2/18 (11)	10/37 (27)	10/35 (29)
Low risk	1/17	6/29	6/29
Intermediate risk	0/0	4/6	4/6
High risk	1/1	0/2	0/0
Need for ICU admission	8 (3)	1 (1)	1 (1)
Hospital admission > 10 days	15 (6)	10 (8)	9 (11)
Total number of hospitalization days in 90 days of follow up [median, CI]	5 [5,6-7,0]	4 [4,4-6,7]	4 [4,2-7,6]
Bacteraemia	66/228 (29)	31/129 (24)	21/77 (27)
Mortality			
30-day all-cause mortality	3 (1)	3 (2)	2 (2)
90-day all-cause mortality	7 (3)	5 (4)	4 (5)
Cure at day 30			
Clinical cure	182/209 (87)	98/121 (80)	59/73 (81)
Microbiological cure	170/190 (89)	107/113 (95)	61/65 (94)

Data are presented as n (%) unless otherwise stated. CI confidence interval, ITT intention to treat analysis, PP per protocol analysis, ICU intensive care unit. *$p = < 0.001$

Table 5. Clinical outcome of febrile urinary tract infection according to PRACTICE risk class; control and intervention groups combined.

PRACTICE score (points)	Low risk Class I-II (<75)			Intermediate risk Class III (76-100)			High risk Class IV-V (>100)			Total
	control	*intervention*	*all*	*control*	*intervention*	*all*	*control*	*intervention*	*all*	
No. of patients	153	79	232	58	35	93	26	19	45	370
Clinical outcome										
30-day mortality, %	0	0	0	3 (5)	1 (3)	4 (4)	3 (11)	2 (10)	5 (11)	9 (2)
90-day mortality, %	0	0	0	3 (5)	3 (9)	6 (6)	4 (15)	2 (10)	6 (13)	12 (3)
ICU admission, %	3 (2)	0	3 (1)	2 (3)	0	2 (2)	3 (11)	1 (5)	4 (9)	9 (2)
Length of hospital stay										
Median no. of days [IQR]	4.0 [2]	3.0 [4]	4.0 [3]	6.0 [4]	4.0 [4]	5.0 [4]	6.5 [4]	6.0 [6]	6.0 [4]	5.0 [3]

Data are presented as n (%) unless otherwise stated, IQR interquartile range, ICU intensive care unit.

Safety

In the control period, 18 patients were treated at home (1 high risk and 17 low risk patients), of which 1 low risk patient was admitted 5 days after start of home treatment because of flank pain shown to be due to renal vein thrombosis.

Of the 37 patients in the intervention group who received initial home-based treatment (29 low risk, 6 intermediate risk and 2 high risk patients), 10 patients (27%) had a secondary hospital admission. These 10 patients (7 females; median age 61, range 18–85 years) had a low or intermediate risk for adverse events according to the PRACTICE-score (6 low, 4 intermediate), and were treated with oral ciprofloxacin (n = 9) or amoxicillin-clavulanic-acid (n = 1). Four out of 10 patients consulted the ED for re-evaluation on their own initiative because of worsening of symptoms such as fever or nausea. Six patients (60%) were contacted by phone by the treating physician to return to the hospital because of positive results of blood cultures, which grew *Escherichia coli* (n = 2, both ciprofloxacin sensitive), *Salmonella paratyphi* (n = 1), *Staphylococcus aureus* (n = 1) and Streptococcus Lancefield group A (n = 1) and G (n = 1). Median hospital stay was 2 days (range 1–14 days). In none of these secondary admissions intensive care treatment was required, and no complications were noted.

The first interim analysis, that took place after inclusion of 133 patients in the intervention group, showed an absolute risk difference in secondary hospital admission rate between intervention and control cohort of 23% (10/35 (29%) subjects in the intervention cohort, vs 1/17 (6%) in the control group). Because the difference in secondary admission rate exceeded the predefined stopping criterion of 20%, the DSMB advised to stop the trial.

DISCUSSION

We assessed the clinical use of a prediction rule, the PRACTICE, that stratifies patients presenting with FUTI into three risk groups for short-term mortality or admission to the ICU, and is based on bed-side available patient characteristics.

Our hypothesis that the use of this prediction rule would reduce hospitalization rate was confirmed in this study, as shown by a 20% absolute reduction. The impact of the PRACTICE on admission policy could have been bigger, because in 33% of low risk patients PRACTICE recommendations were overruled by the attending physician, possibly because of unfamiliarity with the decision rule. Patients in the PP population were older, had more comorbidity and thus a higher PRACTICE score, reflecting the fact that physicians were more likely to follow PRACTICE guidance when admission was recommended. The secondary admission rate of 29% exceeded the predefined stopping criterion (of a 20% absolute increase over that in the control group), and the study was stopped accordingly.

This real world study underlines the importance of the validation of clinical prediction rules in a new cohort to ensure its predictive value and usefulness in clinical setting, but there are some limitations. The PRACTICE was adapted from the *Pneumonia Severity Index* (PSI). Selecting candidate predictors for prognostic modelling is generally done by logistic regression analysis. In order to have sufficient power, as a rule of thumb, we need at least ten outcomes per candidate predictor.[17] Predicting 30-day mortality rate of FUTI, which was estimated to be 2–5%, and considering analysis of 20 candidate predictors this implies a sample size of at least 4000-10,000 patients to obtain sufficient power.

Based on previous studies, we realized such a large prospective study would be infeasible. Since the PRACTICE score was validated in a prospectively collected broad population of 787 patients and its impact was subsequently analysed in a randomized intervention trial, our study was conducted according to guidelines for development of clinical prediction rules.[18,19] As the PRACTICE predicts the composite outcome of complicated course (30-day mortality, ICU-admission and prolonged hospitalisation), according to the rule of thumb (one predictor for 10 or more outcomes), the validation cohort has sufficient power for reliable statistical analyses.[17]

The trial was stopped because of safety concerns, since secondary hospital admission reached our predefined stopping rule. We note that all secondary admitted patients were discharged after a short and uncomplicated hospital stay. Two readmissions because of E coli bacteraemia might have been avoided, because ciprofloxacin has been shown to be equally effective orally as intravenously in bacteraemic UTI.[20] Among secondary admissions were patients with primary bacteraemia caused by salmonella, staphylococci and streptococci, in whom presenting aspecific symptoms, e.g. fever and back pain, were mistaken for pyelonephritis, and sent home. Apparently, these patients were 'misdiagnosed' at first consultation as having FUTI, and subsequently were treated for other diagnoses at secondary admission. We included these patients in our analysis because the attending physicians at the EDs enrolled the patients in the current trial on a presumptive diagnosis of FUTI and we believe that these diagnostic errors reflect every day patient care.[21]

Acute pyelonephritis and urosepsis are common conditions seen in the ED, and it is of importance to be aware that other unusual diseases can mimic its general symptoms.

Other studies support our observation that the accuracy of UTI diagnosis may be suboptimal in the ED.[22,23] Apparently the diagnosis of FUTI is not as straightforward as the diagnosis of pneumonia, where the presence of an infiltrate on chest X-ray is both definitive and confirmative and clinical decision rules such as the PSI have been implemented successfully in daily practice.[3] The PSI was derived from a large cohort of >14,000 patients and validated in almost 40,000 patients, and studies prospectively addressing its use in clinical practice found secondary admission rates of 4–9%.[24-27] The fact that we found higher secondary admission rates in FUTI, might also be explained by a different pathway leading to failure of home treatment in these two infections. Whereas respiratory distress is probably the main cause of secondary hospitalization of pneumonia patients; inability to take oral medication and need for volume resuscitation is more important for FUTI patients. These factors might be underrepresented in the composite outcome of complicated course of FUTI as predicted by the PRACTICE.

Differences in validation and intervention trial cohorts in this study might have attributed to the difference in secondary admission rate. In the historical cohort patients were recruited not only in EDs, but (a minority) also in the practice of general practitioners. The main difference with the historical cohort is the higher percentage of complicated UTI (or in some cases, an alternative diagnosis made on basis of blood culture findings) in the current cohort, which cannot be explained by a difference in sex or age. Other demographic parameters and outcome such as ICU admissions and mortality were comparable in the historical and current cohort.

Our patient group reflects the daily practice of patients presenting with community acquired FUTI, as both men and women, and patients with comorbidity were included.

A previous study on women with acute pyelonephritis identified factors associated with hospital admission using a risk stratification model.[28] Age > 65 years, chills, segmented neutrophils >90%, creatinine >1.5 mg/dL, CRP >10 mg/dL and albumin 3.3 g/dL were independent risk factors for patient admission. Since details on mortality or complications are not given, no conclusion can be made on the actual risk for poor outcome. Furthermore, this model was not validated in a prospective cohort. In contrast, our PSI derived predictor variables can be readily assessed at the bedside level on the basis of history and physical examination.

How can the prediction rule for admission policy be optimized? The cut-off value of 75 points had a negative predictive value for predicting 30-day mortality of 100% in the intervention cohort. Lowering the threshold for admission policy in the intervention phase would hypothetically have led to a hospitalization rate of 77% (102/133), but would still have resulted in a secondary hospitalization rate of 19% (6/31). The effect of the acute host response

might be underrepresented in the PRACTICE, because it is based on the 30-day mortality in the validation cohort.

Prognosis of the patient presenting with severe febrile illness consist of two factors. Firstly, the severity of the acute host response to the infection and inflammatory cascade eventually leading to shock and multi organ failure is best reflected by the hyperacute mortality. Secondly, the

patient's general health condition, mainly defined by age and comorbidity, that determines the 30-day mortality in patients who survive the first days of illness. Addition of a plasma biomarker reflecting the severity of sepsis, such as procalcitonin or midregional pro-adrenomedullin,[29] might improve the decision rule in identifying patients who benefit from hospital-based treatment in the acute phase and lower the secondary admission rate. Furthermore, improved diagnosis of UTI is necessary to ensure safe implementation of prediction tools regarding clinical decision making.

CONCLUSION

Implementation of the PRACTICE rule could decrease the number of hospital admissions of patients presenting to the ED with febrile urinary tract infection by 20%, at the expense of a high secondary admission rate.

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SUPPLEMENTARY DATA

Practice validation cohort

Methods

We conducted a prospective observational multi-center cohort study. The participating centers were 35 primary health care centers (PC) and emergency departments (ED) of 7 hospitals, all clustered into a single area of the Netherlands. Recruitment of consecutive patients who presented with febrile UTI took place from January 2004 to December 2009. The study was approved by the local ethics committees. All participating patients gave written informed consent.

Inclusion and exclusion criteria

Inclusion criteria were age of 18 years or above, fever (≥ 38.2°C) and/or a history of fever and chills including 24 hours before presentation, at least one symptom of UTI and leukocyturia. Exclusion criteria were present treatment for urolithiasis or hydronephrosis, pregnancy, receipt of haemodialysis or peritoneal dialysis, a history of kidney transplantation or a history of polycystic kidney disease.

Evaluation

Baseline patient characteristics were collected by qualified research nurses. Data were collected from the medical record and an interview at the bedside or by telephone using a standardized questionnaire within 24 hours after notification. Collection of data included the predictors that compromise the PRACTICE score. Missing values of categorical variables were considered to indicate the absence of that characteristic. This was applied for diabetes mellitus (n = 2), urinary tract disorder (n = 2) and renal disease (n = 1). In case the medical record reported the respiratory rate to be 'normal' or 'no tachypnea' (n = 494) this was considered to indicate a respiratory rate < 30/minute. For missing continuous variables the mean of the study population was imputed. This was applied for blood pressure (n = 23), pulse rate (n = 20) and temperature (n = 1).

Blood and urine cultures were taken before commencement of antimicrobial therapy and were performed using standard microbiological methods. All patients were contacted 28-32 days and 84-92 days after enrolment to assess clinical outcome.

Study outcome

Our primary outcome was all-cause mortality 30 days after presentation with febrile UTI. Secondary outcomes were need for ICU admission, hospital admission > 10 days, 90-day mortality and a combination of these outcome measures. Survival was assessed using patient or proxy interviews. In case the patient was lost to follow-up, survival was assessed using interview from patient's primary care physicians and/or hospital chart review and/or local governmental mortality registries. Survival could thus not be assessed with certainty in 12 patients after 30 days

and in 17 patients after 90 days. These patients (13 acute uncomplicated pyelonephritis, 4 acute complicated pyelonephritis) were all considered to be alive.

Statistical analysis

Descriptive statistics included frequencies, percentages, medians and means. We calculated the area under the receiver operating characteristic curves (AUC) with 95% confidence intervals (CI) to assess a rule's discriminatory power to predict the outcome. Cut-off values were considered according to sensitivity, specificity, positive and negative predictive values (PPV, NPV) for low- versus high-risk patients. All analyses were performed using SPSS 17.0 (SPPS Inc, Chicago, IL, USA).

Results

Of 879 patients screened for eligibility, 787 patients met the inclusion criteria, provided informed consent and were included in the study. 189 (24%) patients were included by PCs and 598 (76%) by EDs. The median age was 67 years and 37% were men. The majority of the patients had comorbidity (Table A).

The results of urine cultures, performed in 742 (94%) patients, were: 421 (54%) *Escherichia coli*, 31 (4%) *Klebsiella* species, 18 (2%) *Proteus* species, 18 (2%) *Pseudomonas aeroginosa*, 16 (2%) *Staphylococcus* species, 13 (2%) *Enterococcus* species, and 26 (4%) other uropathogens; 199 (27%) urine cultures were either sterile or contaminated of which 52% were obtained during UTI treatment. Blood cultures, performed in 743 (94%) patients, revealed bacteraemia in 176 (24%) cases; 76% of those grew *E. coli* and 24% other uropathogens.

The median score of the PRACTICE score (range 18-180 points) was 74 (IQR: 48-95). The 30-day mortality rate was 3%. AUC for prediction of 30-day mortality was 0.91 [95% CI: 0.85-0.96], Figure A. Dividing the PRACTICE score into five risk categories, the different clinical outcomes according to risk class are outlined in Table B. The median age across the different PRACTICE score classes were: 32 [IQR 23-40] years for class I, 61 [56-68] for class II, 76 [69-81] for class III, 81 [76-86] for class IV and 86 [80-89] for class V. Across the risk classes the percentages of males were 12, 41, 43, 60 and 53 percent for class I through V respectively. The rates of any co-morbidity were: 29 percent for class I, 51 percent for class II, 81 percent for class III, 91 percent for class IV and 100 percent for V. Mortality, need for ICU admission and duration of hospital stay increased with higher PRACTICE score risk. Though adverse outcomes were exceedingly low for PSI risk class I, II and III, yet a large number of patients within these low risk classes were hospitalized. This suggests that these patients might have been safely treated at home and presumably 40% (class I and II) to 74% (380 of 516 hospitalized patients) (class I, II and III) of the admissions were potentially avoidable. Dichotomizing the PRACTICE score as low risk versus high risk, using a cut-off value of the PRACTICE score ≥ 100 points (class IV and V), resulted in a negative predictive value for predicting 30-day mortality of 100% (95% CI: 99-100%). Because the cut-off point was chosen to identify low-risk patients, the positive predictive value was low: 12% (95% CI: 7-18%). The corresponding sensitivity, specificity and the predictive value for predicting 90-day mortality, need for ICU admission and prolonged hospitalization are outlined in Table C.

Figure and tables

Figure A. The receiver operating characteristics curve of the PRACTICE score for predicting 30 day mortality in adults with febrile UTI.

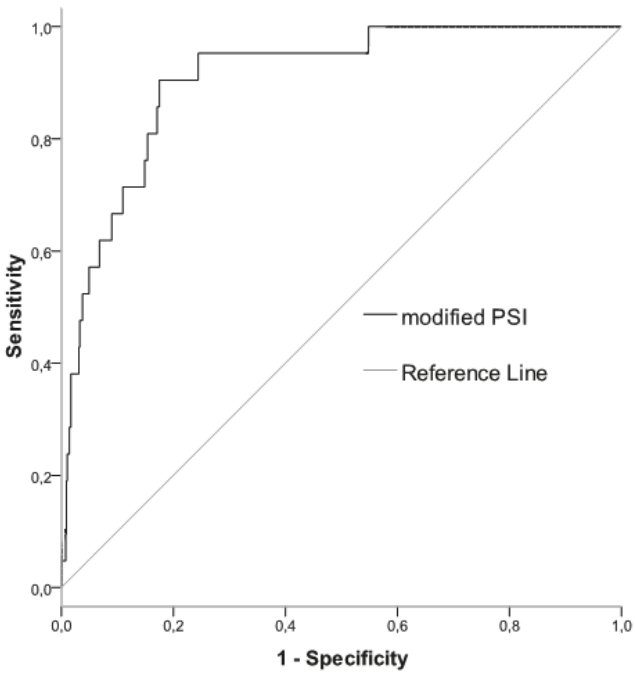


Table A. Patients' demographics and outcomes

Patients (n = 787)	
Site	
Primary health care centers	189 (24)
Emergency departments	598 (76)
Age years; median, (IQR)	67 (46-78)
Sex	
Men	291 (37)
Women	496 (63)
Diagnosis	
Acute uncomplicated UTI/pyelonephritis	420 (53)
Acute complicated UTI/pyelonephritis	367 (47)
Antimicrobial treatment for UTI	231 (29)
Urologic history	
Present urinary catheter	52 (7)
History of urinary tract disorder	215 (27)
Any history of UTI	391 (51) *
Recurrent UTI	189 (25) *
Co-morbidity	
Any	493 (63)
Diabetes mellitus	126 (16)
Malignancy	84 (11)
Heart failure	124 (16)
Cerebrovascular disease	105 (13)
Renal insufficiency	73 (9)
Immunocompromised	107 (14)
Treatment	
Outpatient	271 (34)
Inpatient	516 (66)
Outcomes	
30-day mortality	21 (3)
Need for ICU admission	28 (4)
Hospital admission > 10 days	92 (12) †
90-day mortality	33 (4)

Data are presented as n (%) unless otherwise stated. IQR interquartile range, UTI urinary tract infection. Urinary tract disorder: presence of any functional or anatomical abnormality of the urinary tract excluding the presence of a urinary catheter. * UTI history unknown in 21 patients; † 3 missing values.

Table B. Clinical outcome of febrile urinary tract infection according to PRACTICE score risk class.

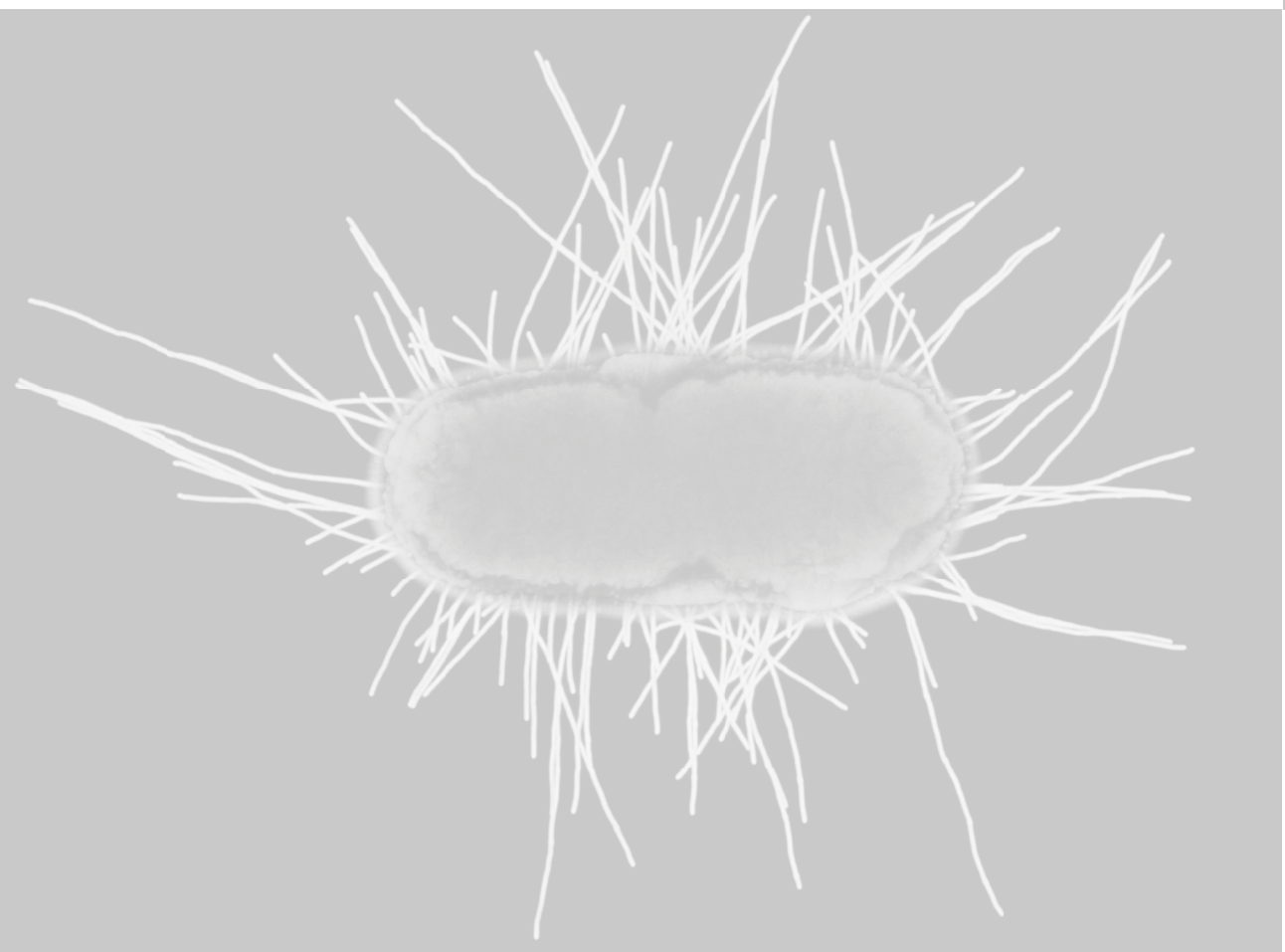
PRACTICE score (points)	Class I (<50)	Class II (51-75)	Class III (76-100)	Class IV (101-125)	Class V (>125)	Total
No. of patients	211	188	237	105	46	787
Management						
Outpatient, No (%)	104 (49)	88 (47)	64 (27)	11 (11)	4 (9)	271 (34)
Inpatient, No (%)	107 (51)	100 (53)	173 (73)	94 (89)	42 (91)	516 (66)
Clinical outcome						
30-day mortality, %	0.0	0.5	0.8	6.7	23.9	21 (2.7)
90-day mortality, %	0.5	0.5	2.5	10.5	30.4	33 (4.2)
ICU admission, %	0.9	1.1	2.5	6.7	23.9	28 (3.6)
Length of hospital stay						
Median no. of days [IQR]	1 [0-4]	2 [0-6]	5 [0-8]	7 [4-11]	9 [5-14]	4 [0-7]
≤ 3 days, %	67.8	57.2	36.3	21.9	8.6	47.1
4-10 days, %	30.3	34.8	46.6	54.9	45.7	39.3
> 10 days, %	1.9	8.0	17.1	30.2	45.7	13.6

ICU intensive care unit

Table C. Predictive value of PRACTICE score ≥ 100 for different clinical outcomes in adults with febrile urinary tract infection

Clinical outcome (n =787)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	AUC of ROC (95% CI)
30-day mortality (n = 21)	0.86 (0.63-0.96)	0.83 (0.79-0.85)	1.00 (0.99-1.00)	0.12 (0.07-0.18)	0.84 (0.75-0.93)
90-day mortality (n = 33)	0.76 (0.57-0.88)	0.83 (0.80-0.86)	0.99 (0.97-0.99	0.17 (0.11-0.24)	0.80 (0.71-0.88)
30-day mortality and/or ICU admission (n = 41)	0.71 (0.54-0.83)	0.84 (0.81-0.86)	0.98 (0.97-0.99)	0.19 (0.13-0.27)	0.77 (0.69-0.86)
30-day mortality and/or ICU admission and/or > 10 days hospitalization (n = 122)	0.48 (0.39-0.57)	0.86 (0.83-0.89)	0.90 (0.87-0.92)	0.39 (0.31-0.47)	0.67 (0.62-0.73)

CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value; AUC of ROC; area under the curve of receiver operating characteristic; ICU: intensive care unit.



CHAPTER 3

Biomarker guided triage can reduce hospitalization rate in community acquired febrile urinary tract infection

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ABSTRACT

Objectives

Febrile urinary tract infections (fUTI) can often be treated safely with oral antimicrobials in an outpatient setting. However, a minority of patients develop complications that may progress into septic shock. An accurate assessment of disease severity upon emergency department (ED) presentation is therefore crucial in order to guide the most appropriate triage and treatment decisions.

Methods

Consecutive patients were enrolled with presumptive fUTI across 7 EDs in the Netherlands. The biomarkers mid-regional proadrenomedullin (MR-proADM), procalcitonin (PCT), C-reactive protein (CRP), and a clinical score (PRACTICE), were compared in their ability to predict a clinically severe course of fUTI, initial hospital admission and subsequent readmission using area under the receiver operating characteristic (AUROC) curves.

Results

Biomarker concentrations were measured in 313 patients, with 259 (83%) hospitalized upon ED presentation, and 54 (17%) treated as outpatients. Of these outpatients, 12 (22%) were later hospitalized. MR-proADM had the highest diagnostic accuracy for predicting a complicated fUTI (AUROC [95% CI]: 0.86 [0.79-0.92]), followed by PCT (AUROC [95% CI]: 0.69 [0.58-0.80]). MR-proADM concentrations were unique in being significantly elevated in patients directly admitted and in outpatients requiring subsequent hospitalization, compared to those completing treatment at home. A virtual triage algorithm with an MR-proADM cut-off of 0.80 nmol/L resulted in a hospitalization rate of 66%, with only 2% secondary admissions.

Conclusion

MR-proADM could accurately predict a severe course in patients with fUTI, and identify greater patient numbers who could be safely managed as outpatients. An initial assessment on ED presentation may focus resources to patients with highest disease severities.

INTRODUCTION

Urinary tract infections (UTI) are amongst the most common infectious diseases found in the emergency department (ED), and usually result in a mild, low severity illness. Nevertheless, these conditions may rapidly develop in a minority of patients into a life-threatening condition, such as septic shock or multiple organ failure. Due to this potential risk, many patients are initially hospitalized, leading to a potential over treatment of low severity patients and increased healthcare costs.[1,2] Previous studies, however, have found that uncomplicated pyelonephritis in women can be safely treated at home with oral antibiotics,[3] whilst elderly patients, men and those with comorbidities may also be potentially eligible for outpatient treatment.[4]

It is therefore surprising that no tools have been established to rapidly identify UTI disease severity on ED admission, unlike the specialized scores such as CURB-65 and PSI developed for community acquired pneumonia.[5] Recently, we assessed the use of a clinical score - the Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden (PRACTICE) - to guide admission policy in a randomized clinical trial of fUTI patients. Although implementation of this score resulted in a decrease in hospital admissions, a subsequent readmission rate of more than 25% was observed in patients who were initially discharged.[6] Consequently, more accurate tools of disease severity are required to not only assess the requirement for initial hospitalization, but to also prevent subsequent readmissions.

The use of blood biomarkers has shown considerable promise in resolving this unmet clinical requirement in several infectious diseases. In adults with community acquired UTI, procalcitonin (PCT) has been shown to be an accurate marker of bacteremia,[7-9] whilst mid-regional proadrenomedullin (MR-proADM) has been shown to strongly predict a complicated course of treatment, the need for ICU admission, as well as identifying patients at risk of mortality.[10,11] Consequently, a combination of these biomarkers, or their use in isolation, may aid in determining the most appropriate setting for treatment.

This study therefore enrolled patients presenting to the emergency department with febrile urinary tract infections (fUTI), and aimed to compare the performance of biomarkers (MR-proADM, PCT and CRP) with the existing clinical score (PRACTICE) in order to (i) assess initial fUTI disease severity, (ii) predict the requirement for hospitalization, and (iii) predict the readmission rate in patients initially selected for outpatient treatment.

METHODS

Design and study population

This was a secondary analysis of the Hospitalization for community-acquired febrile urinary tract infection: validation and impact assessment of a clinical prediction rule study [6]; a stepped wedge cluster-randomized trial involving consecutive patients presenting with a presumptive diagnosis of fUTI at the emergency departments of 7 hospitals in the Netherlands, between January 2010 and June 2014.

All participating centers started with a control period, in which routine clinical practice regarding hospitalization policy was applied. The intervention (use of the PRACTICE) was introduced at the participating centers sequentially, in random order. By the end of the allocation all sites, except one, used the PRACTICE to guide admission policy. The PRACTICE is a prediction rule allocating points to age, sex, nursing home residency, comorbidities, and vital signs at presentation (see Supplementary Table 1). The score ranges from 8 to >125 points and is divided into the following risk classes: low <75 points (recommendation towards ambulant care); intermediate 75 – 100 points (consider ambulant care); high > 100 points (recommendation towards hospital admission), based on the validation cohort.[6]

Inclusion criteria were age ≥18 years, fever (≥38.0°C) and/or a history of fever or shaking chills within 24 hours before presentation, a positive nitrite dipstick test or leukocyturia, and at least one symptom of UTI (dysuria, perineal pain or flank pain). Exclusion criteria included pregnancy, haemo- or peritoneal-dialysis, and a history of kidney transplantation or polycystic kidney disease. In the current analysis, only patients with blood samples available for biomarker analysis were included (Supplementary Appendix Figure S1). The study protocol was approved by the local ethical committee, and written informed consent was obtained from all participants. The original study was monitored by a data safety monitoring board and was stopped prematurely on their advice, due to the rate of secondary admissions in the interventional group exceeding the predefined stopping criterion.[6]

Biomarker and clinical score measurements

CRP was measured at the local laboratories upon patient enrolment using an immunoturbidimetric assay, with cut-offs varying from 5 - 10 mg/L. Surplus EDTA plasma samples were additionally collected, centrifuged and stored at -80°C within 2 hours of patient enrolment. MR-proADM and PCT were batch-measured in a blinded fashion by TRACE technology (Time Resolved Amplified Cryptate Emission) using a new sandwich immunoassay (Kryptor Compact Plus Analyzer, BRAHMS, Hennigsdorf, Germany), with a limit of detection of 0.05 nmol/L and 0.02 ng/L, respectively. The PRACTICE score (Supplementary Appendix Table S1) was calculated in the total patient population, regardless of whether they were enrolled as part of the control or interventional group in the original study.[6]

Endpoints

Severe course of febrile urinary tract infection was defined as a composite of all-cause 30-day mortality, intensive care unit (ICU) admission, and extended hospitalization (>10 days). Patient

disposition was noted upon initial ED presentation, and classified as either being (i) admitted for hospital treatment, (ii) discharged for outpatient treatment, or (iii) admitted for treatment after initial outpatient therapy.

Statistical analysis

Descriptive statistics are expressed as counts (percentage), means (standard deviation) or medians [first quartile - third quartile], as appropriate. Biomarker values were log-normalized before analysis. Univariate analysis was performed using ANOVA, Student's t-test or Mann-Whitney U test for continuous variables, and Chi-square test for categorical variables. Area under the receiver operating characteristics (AUROC) curves with 95% confidence intervals [95% CI] were used to compare the predictive value of the biomarkers and clinical score. Differences between AUROCs were assessed using DeLong's test for significance.[12]

Based on disease severity observations, biomarker suitability for guiding triage decisions was further investigated. Biomarker concentrations in relation to predetermined cut-offs allowed patients to be allocated to either virtual hospitalization or outpatient treatment groups. Patients allocated to outpatient care who were later hospitalized were counted as readmissions. The virtual admission and readmission rates, as well as instances of bacteraemia, ICU admission and 30-day mortality were subsequently calculated. A p-value of <0.05 was considered statistically significant. SPSS software (SPSS Inc. Chicago, version 23.0) was used for statistical analysis.

RESULTS

A total of 313 patients with a presumptive diagnosis of fUTI were analysed (details provided in the Flowchart in the Supplementary Appendix Figure S1). Patient characteristics in terms of urologic history, comorbidities and presenting symptoms are outlined in Table 1. The 30-day mortality rate across the total population was 2% (N = 5), with 114 (36%) patients undergoing existing antimicrobial treatment prior to ED presentation. Patients had an average age of 58 (40 - 75) years, with females comprising the majority of enrolled patients (N = 186; 59%).

Upon presentation to the ED, 259 (83%) patients were hospitalized, with 54 (17%) selected for outpatient treatment. Of these outpatients, 12 (22%) subsequently re-presented to the ED and were hospitalized. Bacteraemia was found in 74 (24%) patients (Supplementary Appendix Table S2), and 9 (3%) patients were admitted onto the ICU. Median biomarker concentrations across the total patient population were as follows: MR-proADM: 1.0 [0.71 - 1.54] nmol/L; PCT: 0.60 [0.16 - 2.5] mg/mL; and CRP: 115 (52 - 199) mg/L. Both MR-proADM and PCT were significantly correlated to the PRACTICE score ($p < 0.001$), albeit weakly ($R^2 = 0.28$ and 0.05, respectively; Supplementary Appendix Figure S2). There was no significant correlation between the PRACTICE score and CRP concentrations.

Table 1. Patient characteristics and outcome.

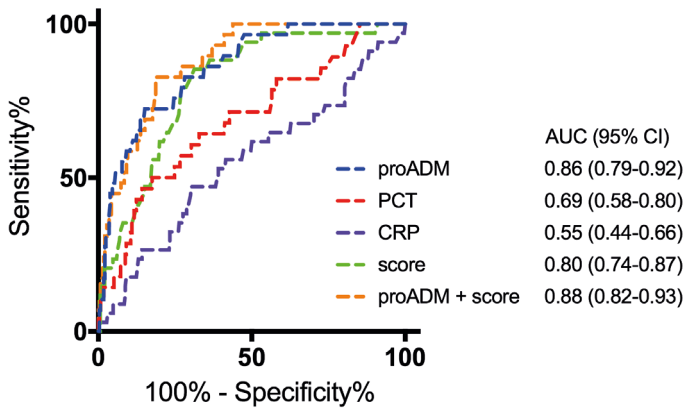
Patient characteristics	Control group (N = 185)	Intervention group (N = 128)	Total (N = 313)
Age in years; median (IQR)	58 (40-73)	61 (42-76)	58 (40-75)
Sex – female	117 (63)	69 (54)	186 (59)
Febrile uncomplicated UTI	45 (24)	28 (22)	73 (23)
Antimicrobial pre-treatment at inclusion	73 (39)	41 (32)	114 (36)
Urologic history			
Present urinary catheter	11 (6)	9 (7)	20 (6)
History of urinary tract disorder	58 (31)	33 (26)	91 (29)
Co-morbidities			
Any	94 (51)	76 (59)	170 (54)
Diabetes mellitus	24 (13)	29 (23)	53 (17)
Malignancy	10 (5)	11 (9)	21 (7)
Heart failure	22 (12)	12 (9)	34 (11)
Cerebrovascular disease	10 (5)	20 (16)	30 (10)
Cirrhosis	1 (0)	2 (2)	3 (1)
Renal insufficiency	8 (4)	20 (16)	28 (9)
Immunocompromised	11 (6)	10 (8)	21 (7)
Presentation			
Shaking chills	124 (67)	92 (72)	216 (69)
Systolic BP (mmHg), mean ± SD	130 ± 22	132 ± 22	130 ± 22
Diastolic BP (mmHg), mean ± SD	71 ± 14	74 ± 14	72 ± 14
Heart rate (b.p.m.), mean ±SD	96 ± 18	98 ± 18	97 ± 14
Fever duration at presentation, median hours [IQR]	28 [12-72]	24 [12-48]	24 [12-72]
Need for percutaneous nephrostomy	5 (3)	4 (3)	9 (3)
Outcome			
Hospitalization			
Total hospitalization	169 (91)*	102 (80)*	271 (87)
- Primary admission	167 (90)*	92 (72)*	259 (83)
- Outpatient treatment	18 (10)*	36 (28)*	54 (17)
- Readmission	2/18 (11)	10/36 (28)	12/54 (22)
Mortality			
- 30-day mortality	2 (1)	3 (2)	5 (2)
- 90-day mortality	3 (2)	5 (4)	8 (3)
Need for ICU admission	8 (4)	1 (1)	9 (3)
Hospital admission > 10 days	11 (6)	10 (8)	21 (7)
Length of hospital stay [median; IQR]	5; 4-7	5; 3-6	5; 4-7
Severe course of fUTI	22 (9)	12 (9)	34 (9)
Bacteraemia	44/177 (25)	30/125 (24)	74/302 (24)
Clinical cure	146/165 (79)	94/117 (80)	240/282 (85)
Microbiological cure	139/154 (90)	102/108 (94)	241/262 (92)

Data are presented as n (%) unless stated otherwise. BP: blood pressure. SD: standard deviation. Bpm: beats per minute. IQR: interquartile range. Readmission: after initial outpatient treatment. ICU: intensive care unit.
* $p < 0.05$. Severe course: composite of 30-day mortality, need for ICU-admission or >10 days hospitalization. Clinical and microbiological cure: assessed at day 30. * $p < 0.01$.

Disease severity: the prediction of severe course of fUTI

The performance of individual biomarkers and the PRACTICE score in predicting a severe course of treatment was assessed using AUROC analysis (Figure 1). MR-proADM exhibited the strongest performance (AUROC [95% CI]: 0.86 [0.79 - 0.92]), which was significantly greater than that of PCT (AUROC [95% CI]: 0.69 [0.58 - 0.80]; $p < 0.001$) and CRP (AUROC [95% CI]: 0.55 [0.44 - 0.66]; $p < 0.001$). There were no significant differences between the performance of MR-proADM and the PRACTICE score (AUROC [95% CI]: 0.80 [0.74 - 0.87]). The combination of MR-proADM, PCT or PRACTICE with one another did not significantly increase predictive ability more than the use of MR-proADM alone (e.g. MR-proADM + PRACTICE: AUROC [95% CI]: 0.88 [0.82 - 0.93]; Supplementary Appendix Table S3).

Figure 1. Biomarker and clinical score accuracy in the prediction of a severe course of fUTI



proADM: Mid-regional proadrenomedullin; PCT: Procalcitonin; CRP: C-reactive protein; fUTI: febrile urinary tract infection; AUC: area under the curve.

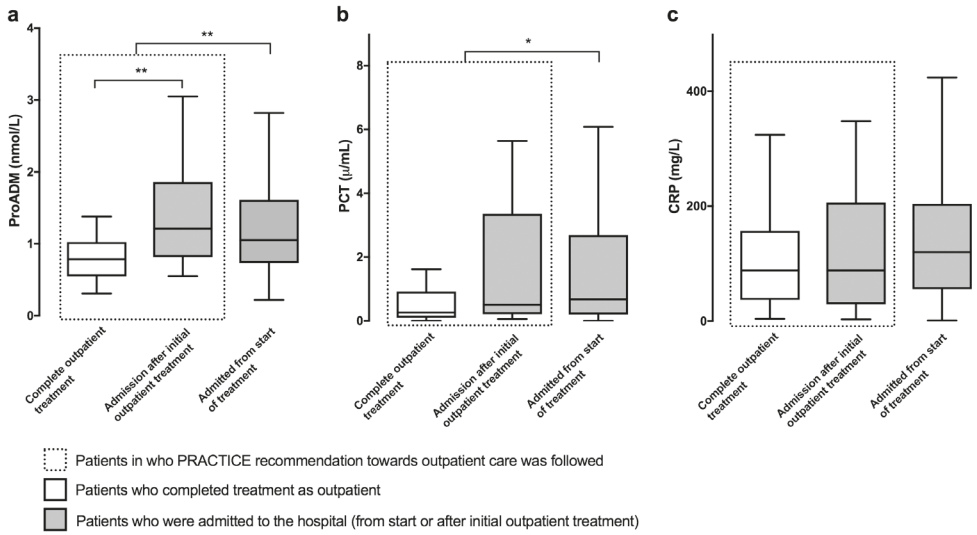
Prediction of the need for hospital admission in the total population

Biomarker measurements upon presentation to the ED (Figure 2) found significantly higher concentrations of MR-proADM and PCT in patients who were hospitalized compared to those who were treated as outpatients (MR-proADM: 1.05 [0.73 – 1.61] vs. 0.83 [0.57 – 1.15] nmol/L, $p < 0.01$; PCT: 0.68 [0.20 – 2.69] vs. 0.29 [0.13 – 1.07] mg/mL, $p < 0.05$). Conversely, there were no significant differences in CRP concentrations between the two groups.

AUROC analysis indicated that the PRACTICE score had the highest accuracy in predicting the need for hospitalization (AUROC [95% CI]: 0.72 [0.64 - 0.79]), although there were no significant differences compared to the performance of either MR-proADM or PCT (AUROC [95% CI]: 0.68 [0.60 - 0.76] and 0.63 [0.54 - 0.72], respectively; Supplementary Appendix Figure S3). Furthermore, there were no significant improvements in accuracy when MR-proADM, PCT or the PRACTICE score were combined in any order (Supplementary Appendix Table S4).

Figure 2. Biomarker concentrations in different patient treatment settings

Distribution of (a) MR-proADM, (b) PCT and (c) CRP in patients treated as who completed treatment as an outpatient, patients who were hospitalized after initial outpatient treatment, and patients who were hospitalized from the start of treatment. * p



<0.05; ** p <0.01.

Prediction of hospitalization in the outpatient population

Interestingly, in the subgroup of patients that were initially treated as outpatients but who later re-presented to the emergency department and were hospitalized, MR-proADM concentrations were significantly elevated upon initial presentation (1.21 [0.81 – 1.86] nmol/L) compared to those who completed outpatient treatment at home (0.78 [0.55 – 1.02] nmol/L; p < 0.01). There were no significant differences in either PCT or CRP concentrations between the two groups.

AUROC analysis for the prediction of hospitalization in patients who were initially deemed suitable for outpatient treatment found that MR-proADM had the greatest performance (AUROC [95% CI]: 0.74 [0.58 - 0.90]) followed by the PRACTICE score (AUROC [95% CI]: 0.72 [0.52 - 0.91]), although differences were not significant (Supplementary Appendix Figure S4). There was no significant association using either PCT or CRP.

Potential effects on triage decisions

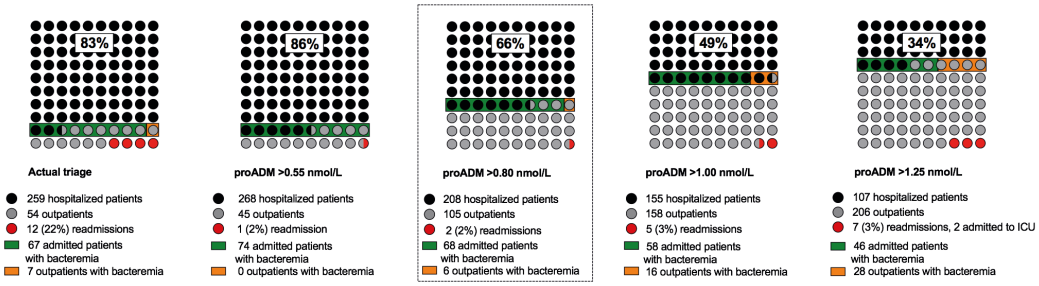
Based on the previous analysis, MR-proADM was chosen for the virtual biomarker guided treatment allocation. Four different cut-off values were subsequently used based on those found in the literature, which included: 0.55 nmol/L;[13] 0.80 nmol/L; 1.0 nmol/L;[10] and 1.25 nmol/L.

The potential impact of this virtual triage algorithm on both hospitalization and outpatient treatment decisions is shown in Figure 3. Compared to the actual hospitalization rate of 83% (N = 259), a decreased hospitalization rate of 66%, 49% and 34% could be found at MR-proADM cut-offs of 0.80, 1.0 and 1.25 nmol/L, respectively. Only at the lowest cut-off of 0.55 nmol/L, did the hospitalization rate (86%) exceed that of the actual hospitalization rate. Interestingly, the secondary admission rate at all MR-proADM cut-offs did not exceed 3%, compared to the actual

readmission rate of 22%. PCT and CRP had less value in the virtual triage, since commonly used cut-off points did not lower the primary admission rate when compared to MR-proADM, without assigning outpatient treatment to patients with actual ICU admission or mortality within 30 days.

Figure 3. 10x10 dot plot of virtual triage based on MR-proADM at different cut off levels

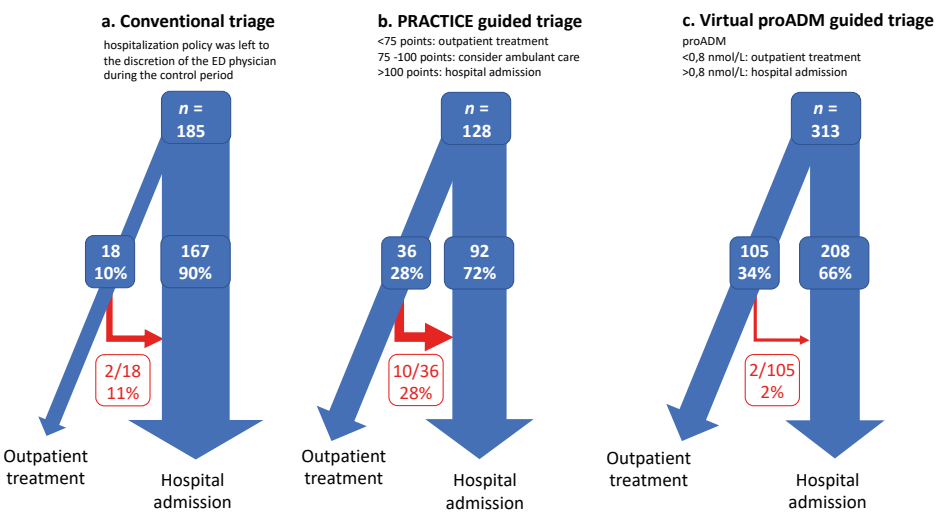
Data are presented as N. Admission: hospitalization after initial outpatient treatment; N = 12 in all patients. Bacteraemia: N = 74 in all patients. ICU: admission on Intensive Care Unit, N = 9 in all patients. Mortality: assessed at day 30; N = 5 (all admitted to hospital in each of the triage scenarios). proADM: mid-regional proadrenomedullin.



In comparison to the conventional hospital triage and interventional PRACTICE guided triage arms of the original study, MR-proADM guided triage at a cut-off of 0.80 nmol/L could decrease initial hospital admissions from 90% and 72%, respectively, to 66% (Figure 4). Furthermore, outpatient readmissions could also be decreased from 11% in the conventional triage and 28% in the PRACTICE guided triage, to 2% in the virtual MR-proADM guided triage.

Figure 4. Comparisons of hospital admission and outpatient admission in different triage models

a. Conventional triage in control period. b. PRACTICE guided triage. c. Virtual triage algorithm based on an MR-proADM with cut off 0.8 nmol/L. ED: emergency department. proADM: mid-regional proadrenomedullin.



DISCUSSION

This study highlights the ability of MR-proADM in accurately predicting a severe course of febrile urinary tract infection (fUTI) in patients presenting to the emergency department (ED), and in turn, demonstrates its potential use in safely decreasing emergency department admissions, increasing outpatient numbers, and lowering subsequent outpatient hospitalization.

Urinary tract infections are the second most frequent infection diagnosed within the ED,[14] and many patients with low disease severities are hospitalized due to concerns regarding infectious progression towards sepsis, septic shock and multiple organ failure. Indeed, 20 - 30% of all sepsis cases originate in the urogenital tract,[15] and despite relatively low mortality rates compared to other origins of septic shock, deaths due to urosepsis can still reach up to 60% in specific patient groups.[16] Conversely, the unnecessary hospitalization of low disease severity patients can result in potential overcrowding and overtreatment issues, subsequently leading to an increase in associated clinical costs. An accurate assessment of initial disease severity and likelihood of disease progression, therefore, are crucial in order to facilitate a more personalized patient treatment strategy at the most appropriate setting.

This study therefore compared the use of commonly used biomarkers, such as procalcitonin (PCT) and C-reactive protein (CRP), and a pre-established clinical score (PRACTICE),[6] with that of mid-regional proadrenomedullin (MR-proADM) in order to predict a severe course of fUTI, and provide an appropriate model of triage. MR-proADM was found to be the most accurate parameter in identifying patients at risk of a severe course, which was significantly greater than that of either PCT or CRP. Similar results in a previous study of fUTI patients found that MR-proADM performance was also greater than that of either PCT or CRP in predicting 30 day mortality, and indeed, confirm the lack of prognostic ability of CRP found within this study.[10]Whilst only a limited number of studies have investigated MR-proADM performance in urinary tract infections, numerous studies have been conducted in patients with lower respiratory tract infections (LRTI). In accordance with the findings of our study, the use of MR-proADM as a stand-alone parameter in LRTI patients has been shown to have either a greater or comparable accuracy in predicting mortality or the development of adverse events compared to established clinical scores, such as CURB-65 or PSI.[17-27] Numerous clinical scores have now been developed for assessing severity in several infectious diseases, with the recent addition of qSOFA in sepsis patients.[28] The use of a single biomarker to provide a simple and rapid assessment of disease severity across all infectious disease subsets, independently on the aetiology of the infectious source, may therefore be of significant clinical value.

This study also found that MR-proADM may play a significant role in the triage of fUTI patients. Using a cut-off of 0.80 nmol/L, MR-proADM guided triage could decrease ED admissions and allow a higher proportion of patients to be safely treated as outpatients. Indeed, an additional 80 (25.6%) patients could have been treated on an outpatient basis as opposed to being hospitalized. Furthermore, the use of such a cut-off resulted in only 2% of outpatient re-presentations to the ED, as well as no mortalities within 30 days and no requirement for ICU admission. Despite

decreases in initial hospitalization numbers, results in the original study using the PRACTICE score found an unacceptably high admission rate in patients who were initially deemed suitable for outpatient treatment.[6] This failure of the PRACTICE guided triage was also partially due to 4 "misdiagnosed" patients with primary bacteraemia from another source other than the urinary tract. These subjects were initially treated as outpatients, but later re-presented to the ED and were hospitalized. All of these patients with primary bacteraemia would have been admitted if the MR-proADM cut-off was set at 0.80 nmol/L. We therefore consider MR-proADM to be the optimal biomarker for UTI triage, and 0.80 nmol/L the optimal cut-off concerning patient safety, which should be further explored in any future clinical interventional trial.

To our knowledge, only one previous study addressed the use of MR-proADM for triage decisions in urinary tract infections. Litke et al described a virtual treatment algorithm combining a MR-proADM level of 1.5 nmol/L with clinical criteria in UTI patients, and found a non-significant 7% decrease in hospitalization without a corresponding increase in adverse events.[1] The primary admission rate of 78% in this cohort of 123 patients was high, although 33% of these patients were diagnosed with cystitis, possibly due to a higher age and comorbidities as compared to our cohort. Application of their cut off on our population could have further decreased the hospitalization rate. In our cohort, 4 out of 9 patients in need for ICU admission and 7 out of 12 patients who were readmitted after being send home from the ED had an MR-proADM level below 1.5 nmol/L. It is unknown whether these patients would have met their clinical criteria for hospitalization.

It should be noted that in a Dutch clinical setting most patients with acute febrile UTI consult their general practitioner first, and are subsequently referred to the ED if required. Based on the early kinetic profile of MR-proADM in infectious patients,[29,30] MR-proADM may also be of use in the general practitioner's office in order to provide guidance concerning hospital referrals. This in turn could lead to the more efficient use of hospital resources and a considerable reduction in costs. Indeed, Dutch general practitioners are familiar with point-of-care CRP testing since its introduction in primary care, in order to reduce antibiotic administration in respiratory tract infections.[31] In this study, we show that CRP is not a reliable marker in patients with febrile UTI concerning severity, thus, point-of-care testing in a primary setting should be expanded to MR-proADM.

The strength of this study lies within its prospective design, in which both men and women presenting with presumptive community acquired fUTI were included, thus reflecting the full spectrum of invasive UTI found at the emergency department. Detailed clinical and microbiological information was recorded in each patient, allowing for the adjustment of final diagnosis. A retrospective analysis found that some patients meeting the inclusion criteria of presumptive fUTI were in fact diagnosed with infections other than UTI, but were nevertheless included in our analysis, since such diagnostic errors are reflective of real-life patient care. Indeed, the use of clinical judgement only can often be deceptive in patients with unspecific symptoms such as fever and back pain. If these patients could be identified by the use of MR-proADM as being bacteraemic and separate from the remainder of patients that could be safely managed as outpatients, the biomarker could be of great use in clinical guidance.

Our study also has a number of limitations. We included the PRACTICE score in the analysis for the prediction of hospitalization, but acknowledge the fact that this endpoint is influenced by the use of the PRACTICE score in the interventional patient group of the original study. Implementation of the PRACTICE score, on the other hand, will not have affected the prediction of a severe course of fUTI. A composite endpoint was subsequently created due to the low number of mortality and ICU admission events within this study, therefore making direct comparisons with end points from other studies difficult. Finally, biomarker guided triage can only be considered as hypothesis generating, and potential adverse events that would have led to outpatient hospitalization might have been prevented by inpatient care.

We did not include any clinical parameters in our virtual triage, because reasons for (re) admission were diverse and addition of manageable number of parameters criteria did not improve our virtual triage. Optimally, a tool to guide triage designed for the ED should be easy to use. Furthermore, any decision based on a triage algorithm should be critically appraised for the use in an individual patient. Clinical conditions such as comorbidity, patients' preference, compliance, lack of family support cannot easily all be incorporated in a practicable decision tool. For example, in the current era of rising antimicrobial resistance, the likelihood of a causative resistant uropathogen will also influence where and how to manage fUTI.[32]

In conclusion, we show that the use of MR-proADM can accurately predict the development of severe febrile urinary tract infections compared to either PCT or CRP. Consequently, MR-proADM guided triage can identify patients who may benefit from a period of hospitalization from those with a low severity infection who can be managed as outpatients. Accordingly, resources can be focused towards patients with the greatest clinical requirements.

ACKNOWLEDGEMENTS

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FUNDING SOURCES

This study was supported by unrestricted grands by ZonMW (projectnumber 171101003), the Bronovo Research Foundation and the Franje1 Foundation. There was no role of the funding organizations in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation or approval of the manuscript; or decision to submit the manuscript for publication.

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SUPPLEMENTARY DATA

Supplementary Figures

Figure S1. Workflow of enrolled patients

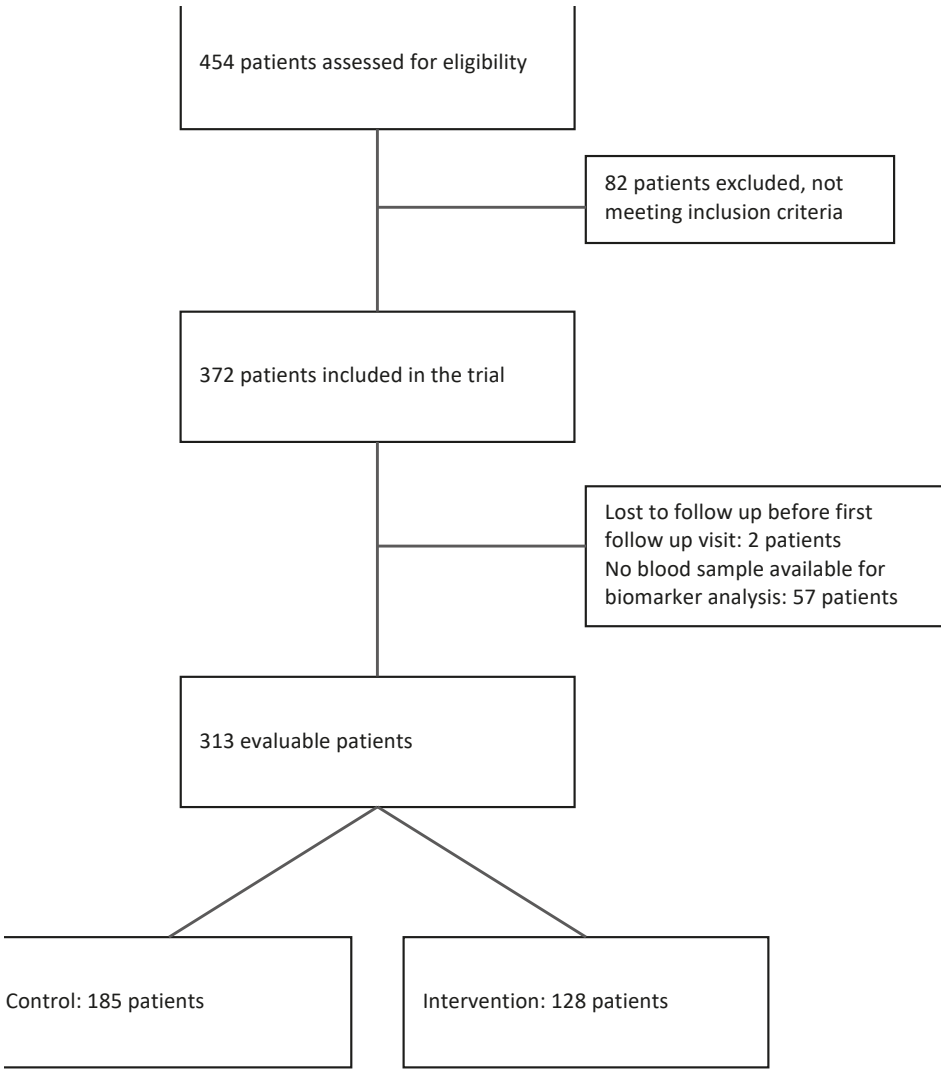


Figure S2. Relationship between MR-proADM, PCT and CRP with the PRACTICE score

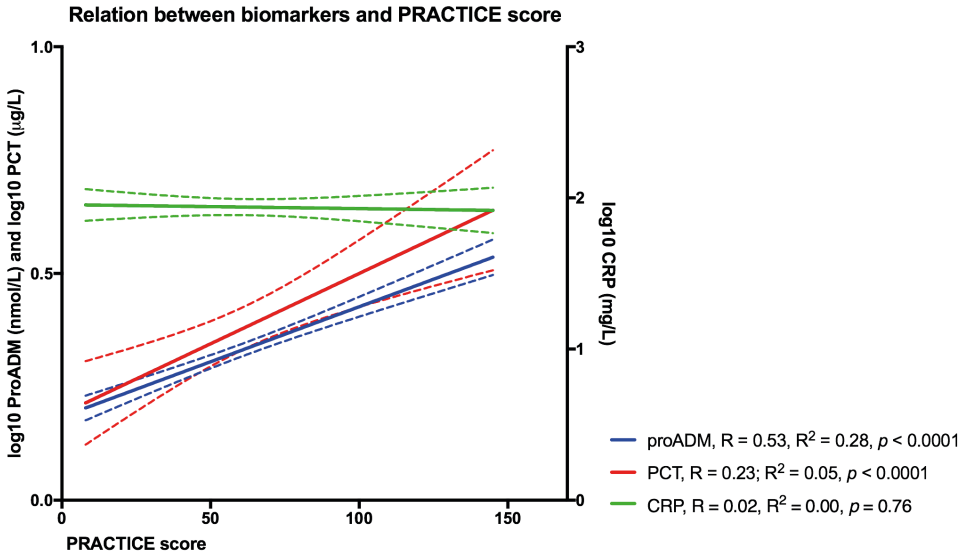
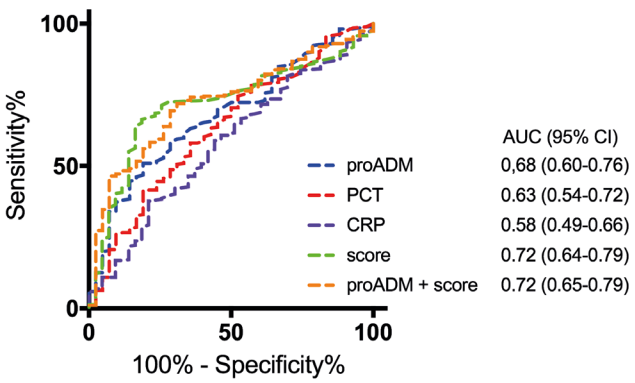
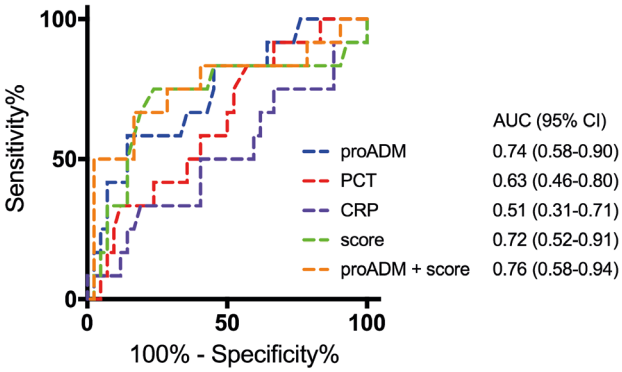


Figure S3. Prediction for hospitalization upon initial ED presentation



MR-proADM: Mid-regional proadrenomedullin; PCT: Procalcitonin; CRP: C-reactive protein

Figure S4. Prediction for hospitalization in patients originally treated as outpatients



MR-proADM: Mid-regional proadrenomedullin; PCT: Procalcitonin; CRP: C-reactive protein

Supplementary Tables

Table S1. Composition of the PRACTICE score

Characteristic	Allocated points*
Demographic	
Age (men)	Age (years)
Age (women)	Age (years) - 10
Nursing home resident	+10
Comorbidity †	
Malignancy	+30
Congestive heart failure	+10
Cerebrovascular disease	+10
Liver cirrhosis	+20
Renal disease	+10
Signs & Symptoms	
Altered mental status	+20
Respiratory rate ≥ 30/min	+20
Systolic blood pressure < 90 mm Hg	+20
Pulse ≥ 125/min	+10
Temperature ≥ 40 ^{O}C	+15

*A total score individual patient score is obtained by summing the points for each characteristic.
†Malignancy is defined as any cancer except basal- or squamous-cell cancer of the skin that was active within the previous year of presentation. Congestive heart failure is defined as ventricular dysfunction for which the patient is prescribed medication and/or consults a hospital-based medical specialist. Cerebrovascular disease is defined as a history of stroke or transient ischemic attack. Liver disease is defined as a clinical diagnosis of cirrhosis. Renal disease is defined as a history of chronic renal disease.

According to risk class the following recommendations will apply:
< 75 points strong recommendation towards home-based management
75-100 points consider home-based management
>100 points strong recommendation towards hospital admission

Table S2. Bacteria isolated from baseline cultures

	Control period (N = 185)	Intervention period (N = 128)
Urine cultures		
Escherichia coli	94 (53)	49 (42)
Klebsiella spp	11 (6)	7 (6)
Proteus spp	5 (3)	4 (3)
Enterococcus spp	2 (1)	-
Pseudomonas aeruginosa	-	1 (1)
Staphylococcus aureus	1 (1)	1 (1)
Other	7 (4)	5 (4)
Contaminated / mixed flora	20 (11)	24 (20)
Total positive urine cultures	140/176 (79)*	91/117 (78)*
Blood cultures		
Escherichia coli	36 (20)	21 (17)
Klebsiella spp	4 (2)	4 (3)
Proteus spp	-	1 (1)
Enterobacter spp	1 (1)	-
Staphylococcus aureus	1 (1)	2 (2)
Beta haemolytic streptococcus	1 (1)	2 (2)
Citrobacter spp	1 (1)	-
Salmonella paratyphi	-	1 (1)
Total positive blood cultures	44/177 (25)#	31/125 (25)#

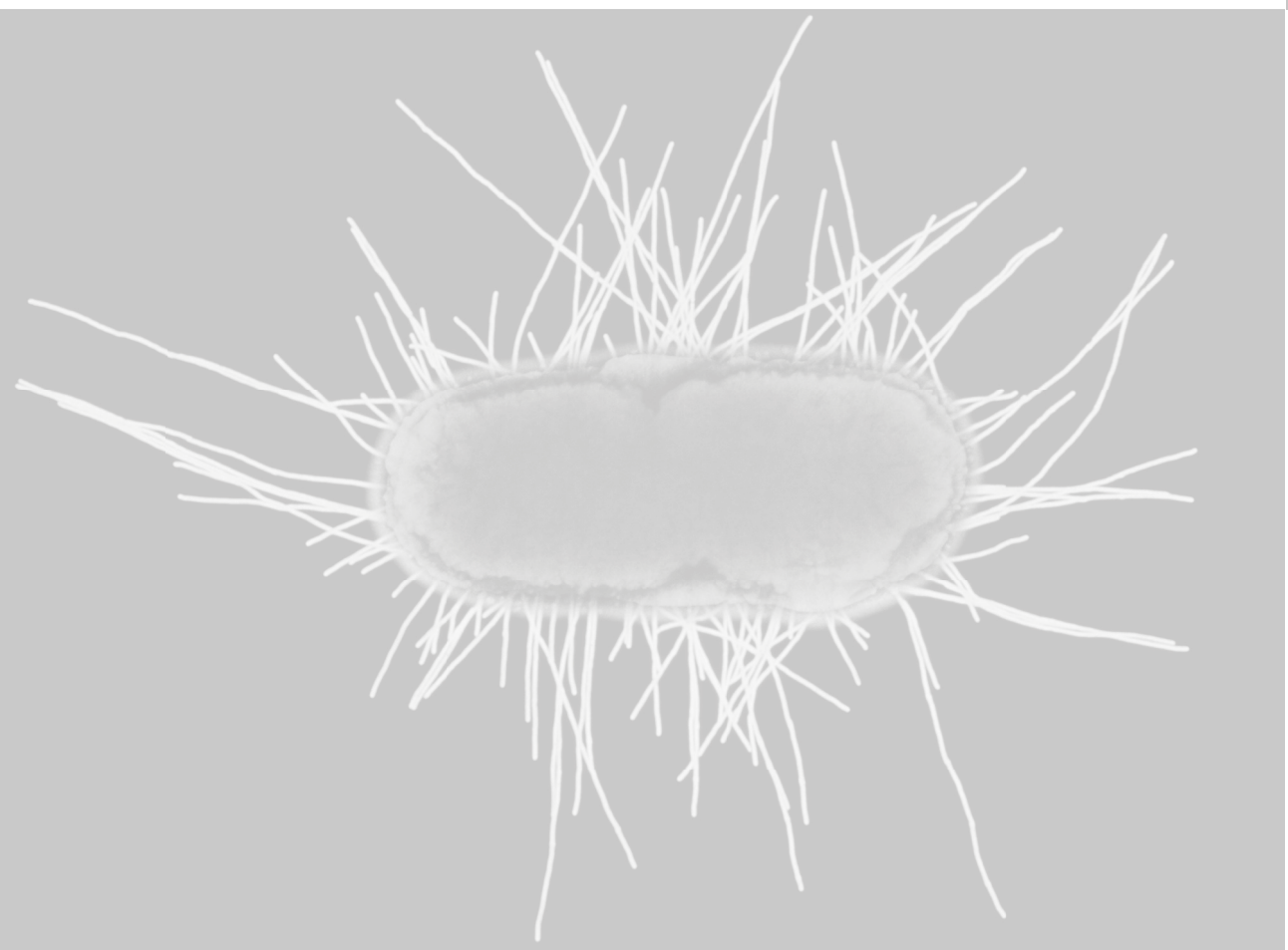
Data are presented as N (%). *Urine cultures were not performed in 9 patients in the control period and 11 patients in the intervention period. #Blood cultures were not obtained in 8 patients in the control period and 3 patients in the intervention period.

Table S3. ROC values for predicting a severe course of fUTI

Variable	AUC	SE	95% CI
MR-proADM	0.86	0.035	0.79 - 0.92
PCT	0.69	0.056	0.58 - 0.80
PRACTICE	0.80	0.039	0.74 - 0.87
MR-proADM + PRACTICE	0.88	0.028	0.82 - 0.93
PCT + PRACTICE	0.82	0.035	0.77 - 0.86
MR-proADM + PCT	0.86	0.034	0.81 - 0.90
MR-proADM + PCT + PRACTICE	0.86	0.033	0.82 - 0.90

Table S4. ROC values for predicting the need for hospital admission

Variable	AUC	SE	95% CI
MR-proADM	0.68	0.042	0.60 - 0.76
PCT	0.63	0.047	0.54 - 0.72
PRACTICE	0.72	0.038	0.64 - 0.79
MR-proADM + PRACTICE	0.72	0.037	0.65 - 0.79
PCT + PRACTICE	0.72	0.038	0.67 - 0.77
MR-proADM + PCT	0.68	0.042	0.62 - 0.73
MR-proADM + PCT + PRACTICE	0.72	0.039	0.66 - 0.77



CHAPTER 4

Treatment duration of febrile urinary tract infection: a pragmatic randomized, double-blind, placebo-controlled non-inferiority trial in men and women

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*both authors contributed equally

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ABSTRACT

Background

In adults with febrile urinary tract infection (fUTI), data on optimal treatment duration in patients other than non-pregnant women without comorbidities are lacking.

Methods

A randomized placebo-controlled, double-blind, non-inferiority trial among 35 primary care centers and 7 emergency departments of regional hospitals in the Netherlands. Women and men aged ≥ 18 years with a diagnosis of fUTI were randomly assigned to receive antibiotic treatment for 7 or 14 days (the second week being ciprofloxacin 500 mg or placebo orally twice daily). Patients indicated to receive antimicrobial treatment for at least 14 days were excluded from randomization.

The primary endpoint was the clinical cure rate through the 10- to 18-day post-treatment visit with preset subgroup analysis including sex. Secondary endpoints were bacteriologic cure rate at 10–18 days post-treatment and clinical cure at 70–84 days post-treatment.

Results

Of 357 patients included, 200 were eligible for randomization; 97 patients were randomly assigned to 7 days and 103 patients to 14 days of treatment. Overall, short-term clinical cure occurred in 85 (90%) patients treated for 7 days and in 94 (95%) of those treated for 14 days (difference –4.5%; 90% CI, –10.7 to 1.7; $P_{\text{non-inferiority}} = 0$.

072, non-inferiority not confirmed). In women, clinical cure was 94% and 93% in those treated for 7 and 14 days, respectively (difference 0.9; 90% CI, –6.9 to 8.7, $P_{\text{non-inferiority}} = 0.011$, non-inferiority confirmed) and, in men, this was 86% versus 98% (difference –11.2; 90% CI –20.6 to –1.8, $P_{\text{superiority}} = 0.025$, inferiority confirmed).

The bacteriologic cure rate was 93% versus 97% (difference –4.3%; 90% CI, –9.7 to 1.2, $P_{\text{non-inferiority}} = 0.041$) and the long-term clinical cure rate was 92% versus 91% (difference 1.6%; 90% CI, –5.3 to 8.4; $P_{\text{non-inferiority}} = 0.005$) for 7 days versus 14 days of treatment, respectively. In the subgroups of men and women, long-term clinical cure rates met the criteria for non-inferiority, indicating there was no difference in the need for antibiotic retreatment for UTI during 70–84 days follow-up post-treatment.

Conclusions

Women with fUTI can be treated successfully with antibiotics for 7 days. In men, 7 days of antibiotic treatment for fUTI is inferior to 14 days during short-term follow-up but it is non-inferior when looking at longer follow-up.

BACKGROUND

In the last decade, treatment of urinary tract infections (UTIs) has become more complicated by the 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 the 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 the ecological environment, including reduction of antibiotic resistance development.[3] Therefore, the focus upon treatment duration of common infections should be that shorter is better.[4] With respect to febrile UTI (fUTI) or acute pyelonephritis, trials upon treatment duration have usually focused on previously healthy young women 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.[5] As such, recommendations upon optimal treatment duration of UTIs in men, the elderly, hospitalized patients, and patients with comorbidities or bacteremia, remain unclear.[5-7]

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.[8] 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 antibiotics for 7 or 14 days. The aims of the study were to compare clinical and bacteriological cure at both the short 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.[9] Consecutive women and men aged 18 years or older with a presumptive diagnosis of community-acquired fUTI established by a primary care physician or on presentation at the hospital's emergency department were screened for enrollment. 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 more than five 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 study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center (protocol P08.65). In addition, the independent scientific boards of all participating hospitals assigned for local participation. The trial was registered at ClinicalTrials.gov (NCT00809913; December 16, 2008) and trialregister.nl (NTR1583; December 19, 2008).

Randomization, antimicrobial treatment, and microbiological methods

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. A computer-generated randomization list, including the numbers 1 to 500, with 125 permuted blocks of four was made. The list and corresponding treatment (placebo or ciprofloxacin) was saved in an independent database with restricted access by an independent pharmacist.

Randomization, and thus treatment allocation, was done once the results of the urine culture became available at the third or fourth day after inclusion. The first week of treatment was open label. In the second week, treatment was continued double-blinded, with either ciprofloxacin 500 mg or placebo orally twice daily (identical capsules for placebo and ciprofloxacin were used), 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 participating centers: a β-lactam antibiotic ± aminoglycoside). These patients were switched as soon as deemed possible to open label oral ciprofloxacin (non-blinded) up to the seventh day after inclusion (equal to day of presentation with febrile UTI and start of antibiotic treatment). The decision whether to treat as outpatient or inpatient was made by the attending physician based on clinical judgment. In case the urine culture was negative or contaminated, patients were only randomized if the attending physician indicated the patient should be further treated for fUTI and no alternative diagnosis was made. Cultures were analyzed according to standard microbiological methods at local certified laboratories. For urine cultures, 10 µL of uncentrifuged urine was inoculated onto culture media. Plates were investigated for growth after 18–24 h of aerobic incubation at 37 °C. The amount of growth was assessed and scored from < 100 CFU/mL (no growth) to $>10^5$ CFU/mL.

A positive urine culture was defined as ≥ 10^4 CFU/mL of urine in women, or ≥ 10^3 CFU/mL of urine in men, or ≥ 10^2 CFU/mL of urine collected during antibiotic treatment of UTI.[9] Further details on randomization, trial medication, microbiological methods, and study procedures have been previously published.[9]

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 0–5 point severity score scale), 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- to 84-day post-treatment visit (cumulative clinical cure), all-cause mortality, adverse event rate determined at 10–18 days and 70–84 days posttreatment, and rate of UTI relapses. In addition, outcome measures were analyzed as stratified by specific predefined subgroups (men, patients with complicated UTI, older age, patients with bacteraemic 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 leukocyturia).[10]

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 (on the eighth day of UTI treatment), 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 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.[11,12] We adopted 10% as the margin of non-inferiority as suggested previously.[13] As we were 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 $\chi 2$ 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.[14] Interim analyses were done after randomization of 100 and 200 patients. After the second interim analysis, there was no reason to stop the trial for safety reasons. However, the principal investigators, who obviously were still blinded with respect to treatment allocation, noted that the overall cure rate was 92%. This is comparable with the results of a recently published similar trial in women comparing 73 to 83 patients treated over 7 or 14 days, respectively [8]. As we had indeed included a larger sample size of 200 patients, we estimated that our study would likely have already met the criteria for non-inferiority while still having a power of 0.80 with a type 1 error of 0.05. As we were confronted with an almost empty budget and a dropping inclusion rate after almost 5 years of participation, we considered continuation of the trial was no longer realistic and thus we decided to stop the trial at this point.

Descriptive statistics were used to describe the baseline characteristics in each arm with $\chi 2$ 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). Confidence intervals around the risk difference were calculated using Episheet (www.krothman.org) and P values for non-inferiority were calculated accordingly. Interaction between predefined covariables and treatment was tested by calculating a *P*-value for difference in risk differences between subgroups.

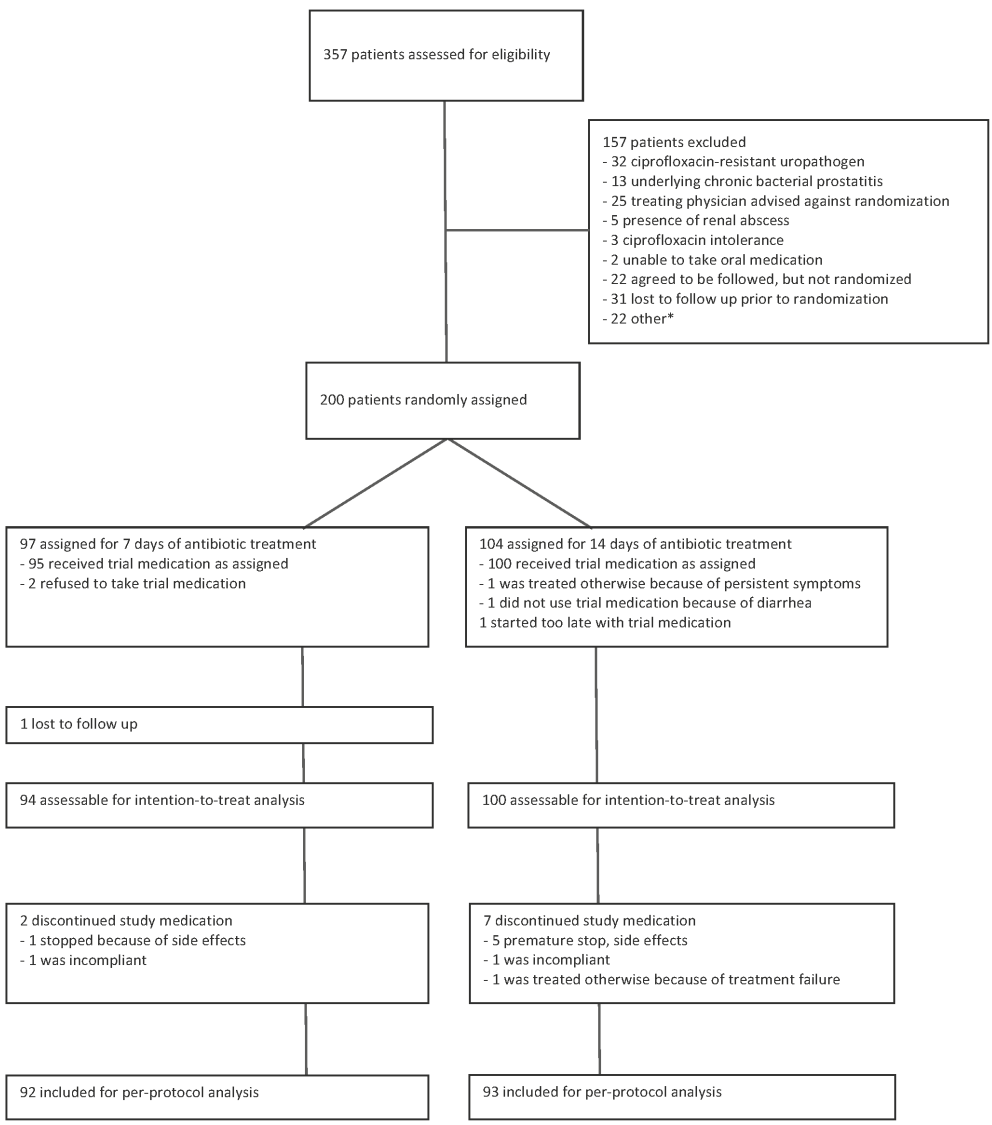
RESULTS

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 antimicrobial treatment for 7 (n = 97) or 14 (n = 103) days.

Reasons for exclusion from randomization, ITT, and PP analyses are listed in Fig. 1.

Of the 157 non-randomized patients, 119 (76%) were evaluable for short-term efficacy and 116 (74%) for cumulative efficacy.

Figure 1. Trial profile



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

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 frequently referred to the emergency department. Additional details are listed in the Supplementary Data. Baseline urine cultures were performed in 341 patients (96%) (Table 2).

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

	Randomized (n=200)			
	Antibiotic treatment for 7 days (n=97)	Antibiotic treatment for 14 days (n=103)	Not randomized (n=157)	p-value[c]
Age (years)	60 (48-72)	61 (40-73)	63 (49-75)	0.277
Male sex	44 (45%)	42 (41%)	58 (37%)	0.247
Body mass index (kg/m^2, mean, SD)	26.3 (5.2)	25.8 (4.5)	26.1 (4.9)	0.969
Urologic history				
Indwelling urinary catheter	3 (3%)	2 (2%)	12 (8%)	0.024
Urinary tract disorder[a]	28 (29%)	28 (27%)	52 (33%)	0.296
Recurrent UTI[b]	19 (20%)	19/100 (19%)	47/147 (32%)	0.007
Comorbidity				
Diabetes mellitus	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
Signs and symptoms at presentation				
Presentation at ED	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
Shaking chills	63/97 (65%)	60/101 (59%)	102/149 (70%)	0.256
Temperature > 38^{0}C	66 (68%)	76 (74%)	121 (77%)	0.226
Systolic blood pressure (mmHg, mean, SD)	132 (19)	132 (22)	129 (20)	0.324
Pulse rate (beats/minute)	93 (17)	94 (19)	97 (19)	0.360
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). COPD chronic obstructive pulmonary disease, UTI urinary tract infection, ED emergency department.
[a]Any functional or anatomical abnormality of urinary tract except urinary catheter.
[b]Three or more UTIs in past 12 months or two or more UTIs in past 6 months.
[c]Randomized (both 7 and 14 days ciprofloxacin) vs. not-randomized patients.

Table 2. Urine culture results at entry[a]

	Randomized		Not randomized
	Antibiotic treatment for 7 days	Antibiotic treatment 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[b]	3 (3%)	3 (3%)	8 (5%)
None or contaminated culture	22 (23%)	32 (29%)	45 (27%)

Data presented as number (%). Urine culture performed in antibiotic treatment for 7 days: 91 (94%), antibiotic treatment 14 days: 100 (97%), non-randomized: 150 (96%).
[a]Some patients had multiple isolates; antibiotic treatment 7 days: n = 6, antibiotic treatment 14 days: n = 10, not randomized n = 17.
[b]Antibiotic treatment 7 days: *Proteus mirabilis* (n = 1), *Citrobacter sedlakii* (n = 1), *Citrobacter koseri* (n = 1), *Candida* spp. (n = 2); Antibiotic treatment 14 days: *Morganella morganii* (n = 1), β-hemolytic streptococci (n = 2); Not randomized: *Serratia marcescens* (n = 1), β-hemolytic streptococci group B (n = 1), *Enterobacter cloacae* (n = 1), *Streptococcus bovis* (n = 1), *Citrobacter koseri* (n = 1), *Morganella morganii* (n = 1), *Proteus mirabilis* (n = 1), β-hemolytic streptococci (n = 1).

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 (group randomized to 7 days: 13 (59%); group randomized to 14 days: 20 (63%)); a similar percentage pertained to those not randomized (n = 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%).

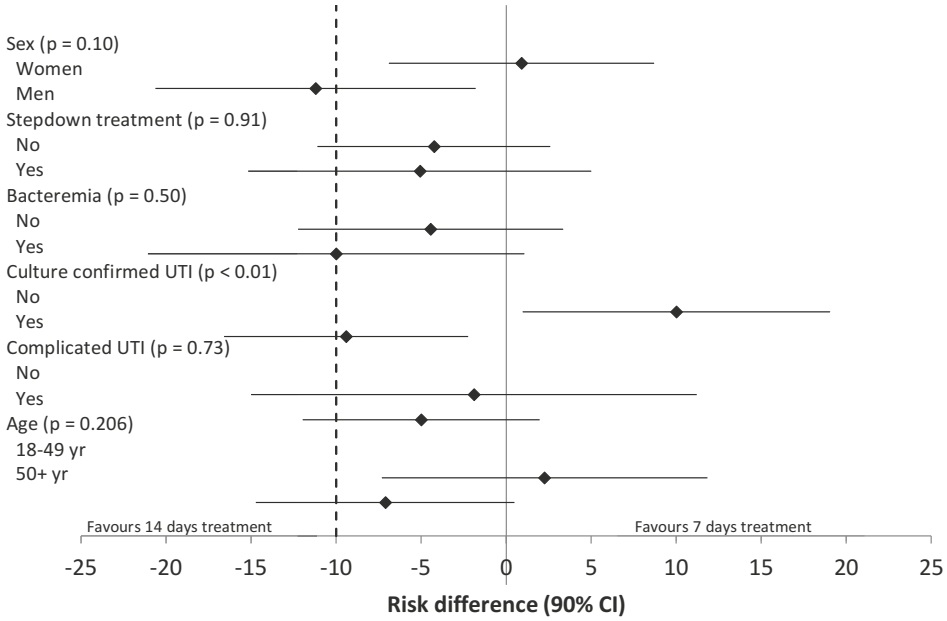
Table 3. Clinical and bacteriologic outcomes in the intention-to-treat and per-protocol population.

	Randomized				
	Antibiotic treatment for 7 days	**Antibiotic treatment for 14 days**	**Difference (90% CI)**	**Non-inferiority test *P*-value**	**Not randomized population**
Intention-to-treat population	(n=94)	(n=99)			
Short-term efficacy[a]	(n=94)	(n=99)			(n=119)
Clinical cure[b]	85 (90.4%)	94 (94.9%)	4.5% (-1.7 to 10.7)	0.114	101 (85%)
Bacteriologic cure[c]	86/93 (92.5%)	89/92 (96.7%)	4.3% (-1.2 to 9.8)	0.101	94/109 (86%)
Cumulative efficacy[d]	(n=94)	(n=94)			(n=116)
Clinical cure[b]	87 (92.6%)	86 (91.5%)	-1.1% (-7.6 to 5.5)	0.394	88 (76%)
Per-protocol population	(n=92)	(n=92)			
Short-term efficacy[a]	(n=92)	(n=92)			NA
Clinical cure[b]	83 (90.2%)	87 (94.6%)	4.3% (-2.1 to 10.8)	0.135	
Bacteriologic cure[c]	84/91 (92.3%)	83/86 (96.5%)	4.2% (-1.5 to 10.0)	0.114	
Cumulative efficacy[d]	(n=92)	(n=87)			
Clinical cure[b]	85 (92.4%)	79 (90.8%)	-1.6% (-8.5 to 5.3)	0.352	

Data presented as number (%), unless otherwise indicated. NA: not applicable.
[a]Short-term efficacy: endpoints assessed at 10- to 18-days post-treatment visit.
[b]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.
[c]Bacteriologic cure: elimination of study entry uropathogen or pathogen growth < 10^4 CFU/mL (women) or <10^3 CFU/mL (men) combined with disappearance of leukocyturia.
[d]Cumulative efficacy: endpoint assessed at 70- to 84-days post-treatment visit.

Both treatment regimens resulted in a high clinical cure rate at short-term follow-up in the ITT population (90% vs. 95% in patients treated 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, –10.7 to 1.7, $P_{non\text{-}inferiority} = 0.072$). Accordingly, the criteria for non-inferiority were not met as the 90% CI exceeded 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 antimicrobial treatment, 2 (IQR, 1–3) days in 14-day antimicrobial treatment. Short-term clinical cure was 85% in non-randomized patients, whereas median time to defervescence amounted to 2 (IQR, 1–3) days. Among all analysis performed, there were no significant differences between ITT and PP analysis. Therefore, within the following, only outcomes of ITT analysis are presented.

Figure 2. Difference in clinical cure rates (10- to 18-days post-treatment) of febrile UTI treated for 7 days versus 14 days in specific subgroups. Stepdown treatment implies initial empiric intravenous antibiotic treatment.



UTI urinary tract infection; CI confidence interval. *P* values represent test for interaction. Data presented from intention to treat analysis.

Short-term clinical cure rates were analyzed in preset subgroups of patients. In women, short-term clinical cures for the 7- and 14-day arms were 47 of 50 (94%) versus 54 of 58 (93%), respectively. The difference in cure rate was 0.9% (90% CI, –6.9 to 8.7, $P_{\text{non-inferiority}} = 0.011$, non-inferiority confirmed). In men, clinical cure rates differed significantly between those treated for 7 or 14 days (38 of 44; 86% vs. 40 of 41; 98%) (Fig. 2). The difference in cure rate was –11.2 (90% CI, –20.6 to –1.8, $P_{\text{non-inferiority}} = 0.417$, $P_{\text{superiority}}$ 2-sided = 0.050, superiority of 14-days treatment confirmed). The large difference in risk differences for age was predominantly determined by men. Therefore, and for explorative reasons, an additional subgroup analysis was also performed within the group of men and women. The results are presented in the Supplementary Data: Figure S1 and Figure S2, respectively.

For stepdown treatment, bacteremia, and complicated UTI, the risk differences were similar between the subgroups and in all subgroups, non-inferiority was not shown.

For cumulative clinical cure rate (70 to 84 days posttreatment), 94 patients were evaluable in each treatment arm. Clinical cure rates were high: 93% vs. 91% in patients treated for 7 or 14 days; difference 1.1% (90% CI, –5.5 to 7.6, $P_{\text{non-inferiority}} = 0.005$, non-inferiority confirmed) (Table 3). Criteria for non-inferiority for cumulative clinical cure rate were also met for the subgroups men and women (Supplementary Data: Table S1).

Both treatment regimens post-randomization, were well tolerated with no differences in side effects (Supplementary Data). Post-treatment urine cultures (at days 28–32) were obtained in 93 of 94 (99%) patients assigned to 7 days, in 92 of 99 (93%) patients assigned to 14 days of antimicrobial treatment, 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 for 14 days, and 86% in non-randomized patients (Table 3). More details on clinical and microbiological outcomes are listed in the Supplementary Data.

DISCUSSION

Our findings show that community-acquired fUTI can be safely and efficaciously treated with antimicrobial treatment for 7 days in women as it is non-inferior to 14 days of therapy. However, in men with fUTI, 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, prostatitis, and the urosepsis syndrome. Over 55% of patients were initially hospitalized because of fUTI, likely because of presumed urosepsis syndrome, and a relative high number of patients had bacteremia. Of note, the findings hold for both the ITT and the PP analyses, 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. Finally, the clinical cure rate at 90 days after initial presentation with fUTI was evaluable in 188 (94%) patients and, though characterized as a secondary outcome measure, for the whole group as well as the subgroups of men and women, they all met the criteria for non-inferiority.

There are, however, also some limitations. First of all, the diagnosis of fUTI was not confirmed by cultures for all patients. Nevertheless, it should be noted that the attending physicians still made a clinical diagnosis of fUTI is such cases, and no alternative diagnosis for fever or urinary tract symptoms was made. Secondly, our study lacks statistical power to draw confident conclusions on the various subgroups because of the limited number of patients enrolled. However, it should be noted that all subgroups analyzed were predefined in the study protocol. Finally, it should be noted that several patient categories (e.g., ciprofloxacin-resistant uropathogen, renal transplant, pregnancy, indication for antimicrobial treatment for at least 14 days) were excluded from randomization. Thus, our findings might not be generalizable to all patients with fUTI.

Our findings extend recent findings of a highly similar controlled randomized Swedish study performed in women with acute pyelonephritis, showing non-inferiority of 7- to 14-days

of antimicrobial treatment.[8] Compared to our study, their patient group was younger, had less comorbidities, and fewer had complicated UTIs.

In men, our results indicate an increase in the rate of clinical and bacteriological treatment failure after the 7-day treatment as compared to 14 days. Likely, though chronic bacterial prostatitis was an exclusion criterion, this is due to prostatic involvement of the infection as this is known to be a cause of recurrent UTI, even after appropriate antimicrobial treatment.[15] There is a lack of studies on optimal treatment duration of fUTI in men.[5] 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.[16] Similarly, a randomized, double-blind trial in Sweden lent support for the efficacy of 14-day treatment with fluoroquinolones in men.[17] 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 [18]. 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,[19] this may have influenced our results and may possibly 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.

Nevertheless, it should be noted that there were similar clinical cure rates between 7 and 14 days of treatment during longer follow-up (70–84 days post-treatment) and this holds both for women and men. In others words, the need for additional antibiotic UTI treatment during longer follow-up is similar irrespective of whether the initial treatment of fUTI was 7 or 14 days. Given the principles of antimicrobial stewardship, this is an interesting finding because, even in men with fUTI, this might be an argument to treat them for 7 days. Indeed, our study indicates that a further study upon treatment duration of men with fUTI should be performed, including outcome measures being set at 3 months or even longer instead of the traditional 2–6 weeks.

Given the consistency of our findings and those of the recent study in Sweden [8], we conclude that women with fUTI, irrespective of disease severity and comorbidities, can be treated orally with 7 days of adequately dosed fluoroquinolones. 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 uropathogens, making it a drug of choice in both outpatient as well as hospital settings. As a surplus, activity against perineum and vagina colonizing Enterobacteriaceae may help to prevent early recurrences [20]. An important concern has been the rise of ciprofloxacin resistance in the community, i.e., up

to 15% of Enterobacteriaceae are currently resistant in The Netherlands, that may preclude 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 40–50%.[21,22] In countries with concurrent high rates of trimethoprim-sulfamethoxazole resistance in Enterobacteriaceae, there may be no oral antibiotic option left for general practitioners to treat fUTI at home, raising healthcare costs. These findings underscore the importance of controlling antimicrobial resistance through antibiotic stewardship, including the administration of antibiotics with optimal duration.[4,23]

CONCLUSIONS

Women with fUTI can be successfully treated with antibiotics for 7 days, including those who initially were treated with β-lactams intravenously. In men, 7 days of antibiotic treatment for fUTI is inferior to 14 days when looking at short-term clinical cure. During long-term follow-up, even in men, 7 days of antibiotic treatment is non-inferior to 14 days. It should be considered that the primary outcome measures on future trials on antibiotic treatment duration of fUTI in men, should be set at 3 months instead of the traditional 2 weeks.

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SUPPLEMENTARY DATA

Additional baseline characteristics, clinical outcomes and microbiological outcomes

Baseline characteristics

In the 7-day treatment arm, 23 (24%) patients had been pretreated for presumptive UTI with: norfloxacin (n=1, 4%), nitrofurantoin (n=5, 22%), trimethoprim ± sulfamethoxazole (n=3, 13%), amoxicillin ± clavulanic acid (n=12, 52%), fosfomycin (n=1, 4%) and others (n=1, 4%). Of those randomized to 14 days antimicrobial treatment, 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%), fosfomycin (n=1, 2%), others (n=4, 7%) and unknown (n=5, 9%).

About half of the patients were initially treated with intravenous antibiotics. This did not differ between the two treatment arms: in the 7-day treatment arm, 48 (50%) patients (cefuroxime n=21, 44%; cefuroxime + gentamicin n=22, 46%; other n=5, 10%) and in the 14-day treatment arm, 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 antibiotic treatment 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 antibiotic treatment, one patient had an acute cystitis at day 30 and four patients had recurrent fUTI at day 8, 9, 19 and 20.

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 antibiotic treatment 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.

For the primary outcome measure, additional subgroup analysis within the group of men

and women are outlined in Figure S1 and S2, respectively. There were no apparent differences within all subgroups except for men without culture confirmed UTI and men < 50 years old, in which 7 days of antibiotic treatment is non-inferior to 14 days (see Figure S1). 12 patients were aged < 50 years. The clinical cure rate (10-to 18-days post treatment) in these men was 100% for both the 7-day and 14-day treatment arm (see Figure S1). For men aged ≥ 50 years, the clinical cure rate was 85% versus 97% for 7 days versus 14 days of treatment ($p_{superiority}$ = 0.027). For women aged < 50 years the clinical cure rates were 95% and 92 % for the 7-day and 14-day treatment arm respectively. For women aged ≥ 50 years this was 93% and 94%. Other outcome measures for men and women are outlined in Table S1.

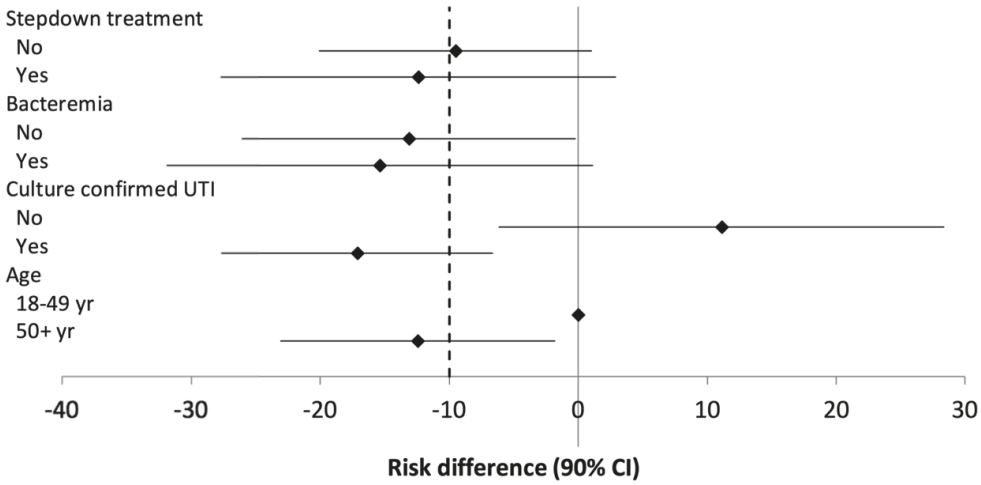
During the study period, no patients treated for 7 days died. One patient, a 84-year old man assigned to 14 days antimicrobial treatment, died on day 92 due to pneumonia and sepsis. Five non-randomized patients died during follow-up due to concurrent medical problems. None of the patients developed pyonephrosis or renal stones requiring additional drainage. Ten patients were temporarily treated with a bladder catheter; 2 patients in the 14-day treatment arm and 8 patients in the 7-day treatment arm.

With respect to side effects, one patient who received placebo, discontinued the 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 the 7 versus 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

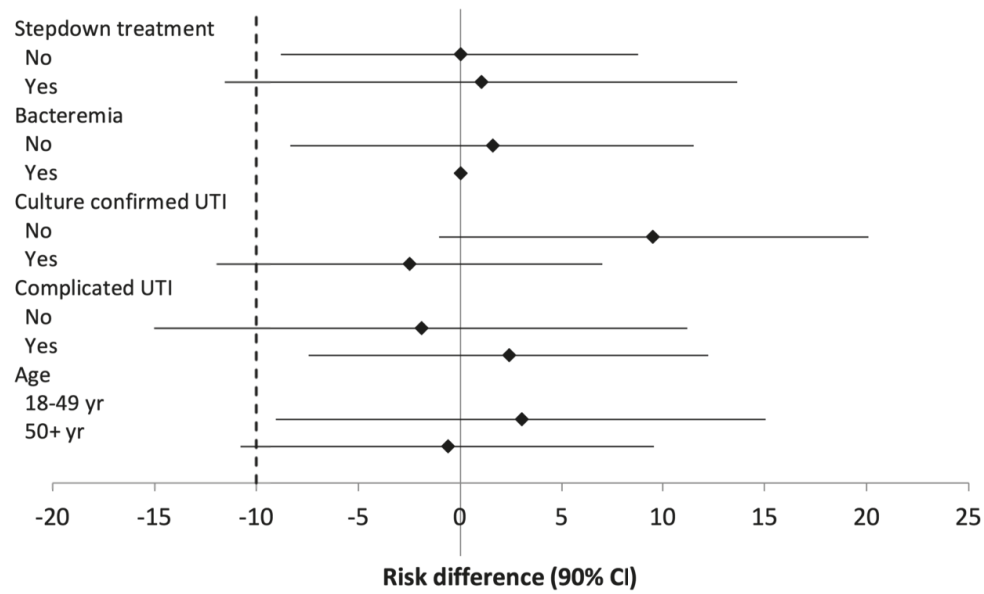
In the group assigned to 7 days of ciprofloxacin, seven patients had asymptomatic bacteriuria at short-term follow-up (five with *E. coli*, one with *Klebsiella oxytoca* and one with *Enterococcus faecalis*). Three patients treated with ciprofloxacin for 14 days had asymptomatic bacteriuria at short-term 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*.

Figure S1. Difference in clinical cure rates (10- to 18-days post-treatment) of febrile UTI treated for 7 days versus 14 days in specific male subgroups.



Stepdown treatment implies initial empiric intravenous antibiotic treatment. UTI: urinary tract infection; CI: confidence interval.

Figure S2. Difference in clinical cure rates (10- to 18-days post-treatment) of febrile UTI treated for 7 days versus 14 days in specific female subgroups.

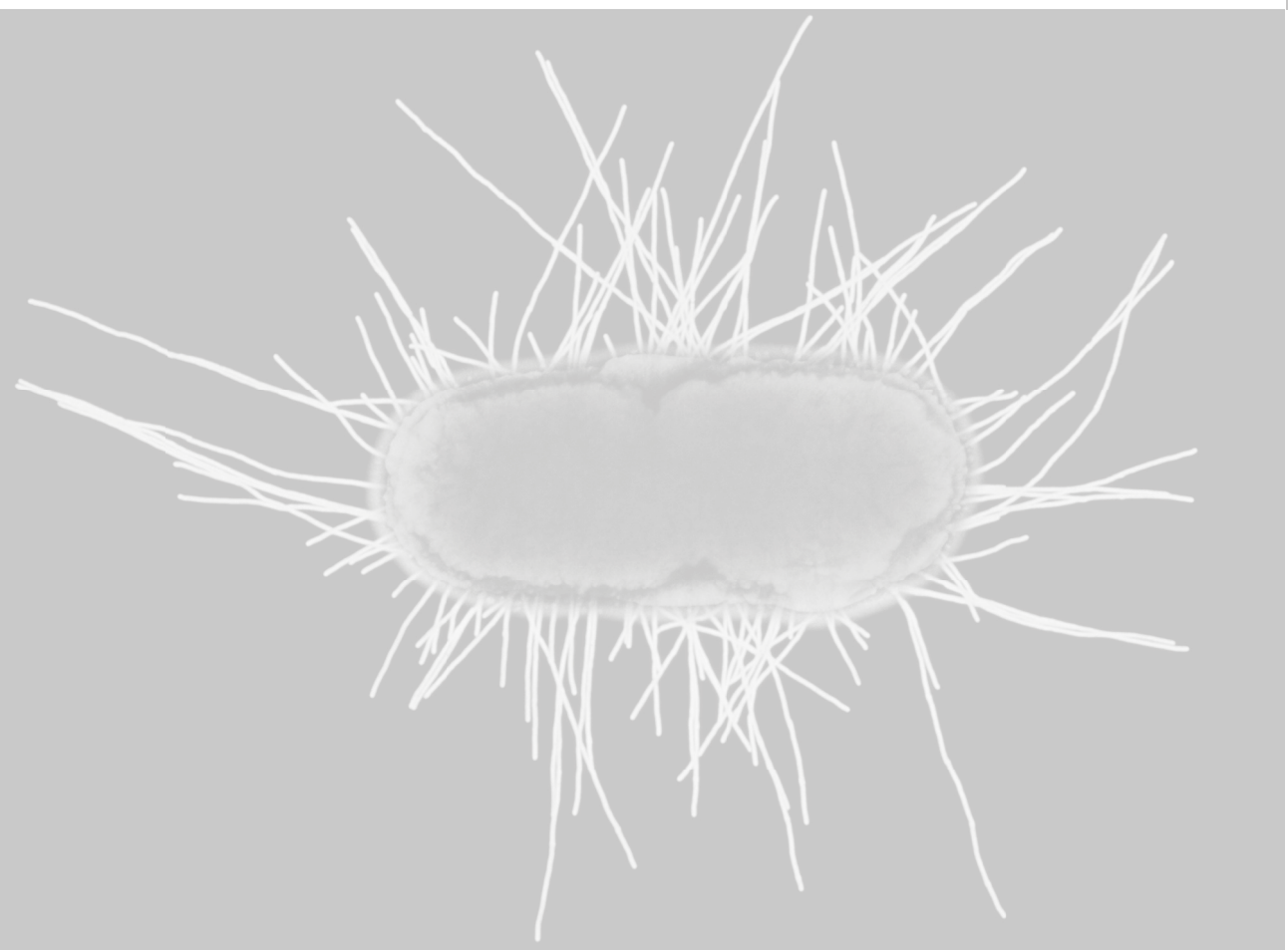


Stepdown treatment implies initial empiric intravenous antibiotic treatment. UTI: urinary tract infection; CI: confidence interval.

Table S1. Difference in outcome measure of 7 days antimicrobial treatment for febrile UTI versus 14 days in men and women.

	Difference (90% CI)	
	Men	**Women**
Short-term efficacy		
Clinical cure	-11.2 % (-20.6 to -1.8), p = 0.417$^{\pi}$	0.9 % (-6.9 to 8.7), p = 0.011
Bacteriologic cure	-4.0 % (-13.2 to 5.3), p = 0.140	-4.2 % (-10.7 to 2.2), p = 0.070
Cumulative efficacy		
Clinical cure	0.9 % (-8.6 to 10.3), p = 0.029	1.1 % (-7.9 to 10.1), p = 0.021

Short term efficacy represents 10- to 18-days post-treatment of febrile UTI
Cumulative efficacy represents 70- to 84-days post-treatment of febile UTI
Clinical cure is defined as absence of UTI symptoms and fever without any additional antibiotic treatment
p values represent test for non-inferiority
$^{\pi}$p value for superiority = 0.025



CHAPTER 5

Procalcitonin, mid-regional proadrenomedullin and C-reactive protein in predicting treatment outcome in community-acquired febrile urinary tract infection.

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ABSTRACT

Background

A reduction in duration of antibiotic therapy is crucial in minimizing the development of antimicrobial resistance, drug-related side effects and health care costs. The minimal effective duration of antimicrobial therapy for febrile urinary tract infections (fUTI) remains a topic of uncertainty, especially in male patients, those of older age or with comorbidities. Biomarkers have the potential to objectively identify the optimal moment for cessation of therapy.

Methods

A secondary analysis of a randomized placebo-controlled trial among 35 primary care centers and 7 emergency departments of regional hospitals in the Netherlands. Women and men aged ≥18 years with a diagnosis of fUTI were randomly assigned to receive antibiotic treatment for 7 or 14 days. Patients indicated to receive antimicrobial treatment for more than 14 days were excluded from randomization. The biomarkers procalcitonin (PCT), mid-regional proadrenomedullin (MR-proADM), and C-reactive protein (CRP) were compared in their ability to predict clinical cure or failure through the 10-18 day post-treatment visit.

Results

Biomarker concentrations were measured in 249 patients, with a clinical cure rate of 94% in the 165 randomized and 88% in the 84 non-randomized patients. PCT, MR-proADM and CRP concentrations did not differ between patients with clinical cure and treatment failure, and did not predict treatment outcome, irrespective of 7 or 14 day treatment duration (ROC_{AUC} 0.521; 0.515; 0.512, respectively). PCT concentrations at presentation were positively correlated with bacteraemia (τ=0.33, $p<0.001$) and presence of shaking chills (τ=0.25, $p<0.001$), and MR-proADM levels with length of hospital stay (τ=0.40, $p<0.001$), bacteraemia (τ=0.33, $p<0.001$), initial intravenous treatment (τ=0.22, $p<0.001$) and time to defervescence (τ=0.21, $p<0.001$). CRP did not display any correlation to relevant clinical parameters.

Conclusions

Although the biomarkers PCT and MR-proADM were correlated to clinical parameters indicating disease severity, they did not predict treatment outcome in patients with community acquired febrile urinary tract infection who were treated for either 7 or 14 days. CRP had no added value in the management of patients with fUTI.

Trial registration:

The study was registered at ClinicalTrials.gov [NCT00809913; December 16, 2008] and trialregister.nl [NTR1583; December 19, 2008].

Keywords

Urinary tract infections; Pyelonephritis; Biomarkers; Treatment duration; Antibiotic therapy; Antibiotic stewardship

BACKGROUND

Febrile urinary tract infections (UTI), including acute pyelonephritis and prostatitis, are relatively common in adults, but data on the optimal duration of treatment are limited, especially within men, the elderly and patients with comorbidities. Emerging bacterial resistance calls for more efficient efforts to shorten the duration of antibiotic treatment.

Our previous findings in the FUTIRST trial have shown that patients with community-acquired febrile urinary tract infections, such as women and elderly patients with severe comorbidities, can be safely and efficaciously treated with oral ciprofloxacin for 7 days, irrespective of disease severity upon presentation.[1] In men, however, a short course of therapy can lead to significantly more clinical failures compared to a 14-day course of oral ciprofloxacin.

Previous studies have shown that procalcitonin (PCT) can provide useful guidance for antimicrobial treatment in patients with respiratory tract infections and sepsis.[2-4] However, little is known concerning PCT guided-therapy in patients with urinary tract infections. An earlier subgroup analysis of a randomized trial performed in the intensive care unit (ICU) has shown that the duration of antibiotic treatment was significantly shorter for 24 UTI-patients receiving procalcitonin-guided treatment compared to 18 UTI control-group patients.[4] However further investigations are scarce. Other biomarkers may also be of interest, such as mid-regional proadrenomedullin (MR-proADM), which has been shown to be increased in the early stages of progression towards multiple organ failure,[5] and is of value in predicting a complicated course of treatment and the requirement for ICU admission.[6-8]

This secondary analysis of the earlier FUTIRST trial[1] therefore hypothesized that procalcitonin measurement on days 0 and 3 could more accurately identify patients at risk of treatment failure and in need a prolonged course of antibiotics compared to either MR-proADM or C-reactive protein.

METHODS

Design and study population

This was a secondary analysis of a randomized, placebo-controlled trial involving patients presenting with febrile urinary tract infection (fUTI) at the emergency departments of 7 hospitals and 32 primary health care centres in the Netherlands, between November 2008 and May 2013, as described previously.[1,9] Consecutive women and men aged 18 years or older with a presumptive diagnosis of community-acquired fUTI established by a primary care physician or on presentation at the hospital's emergency department (ED) were screened for potential enrolment. Eligible patients had 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 more than

five leucocytes per high-power field in a centrifuged sediment). Exclusion criteria for study entry were as follows: a known allergy to fluoroquinolones, pregnancy or lactation, polycystic kidney disease, permanent renal replacement therapy and kidney transplantation. Patients enrolled with fUTI but not randomized to trial medication remained in the observational part of the study to assess outcome. In the current study, all patients participating in both observational and interventional part of the trial were included, except those without a baseline blood sample available for biomarker analysis, without a 10-18 days post-treatment visit, and patients who needed prolonged treatment because of chronic bacterial prostatitis.

The study protocol was approved by the local Ethical Committee, and written informed consent was obtained from all participants. The trial was registered at ClinicalTrials.gov (NCT00809913; December 16, 2008) and trialregister.nl (NTR1583; December 19, 2008).

Procedures

Within 24-48 hours of notification, qualified research nurses collected clinical data and laboratory values by standardized questionnaires. The decision whether to treat as outpatient or inpatient was made by the attending physician based on clinical judgement. Outpatients started with the first week of open label ciprofloxacin 500 mg twice daily. In hospitalized patients, the treating physician could administer empirical intravenous antibiotics at the start of treatment according to local policy (in all participating centres: a ß-lactam antibiotic ± aminoglycoside). These patients were switched to open label ciprofloxacin as soon as was deemed possible. Randomization between the second week ciprofloxacin and placebo twice daily was initiated once the results of the urine culture became available on the third or fourth day after inclusion. In patients who could not be randomized (e.g. due to ciprofloxacin resistance), the choice of antibiotic agent and treatment duration was left at the discretion of the treating physician.[1]

All patients were contacted in person on day 3 (3-4 days after start of treatment) and day 30 (10-18 days post-treatment) after enrolment, and by phone on day 90 (70–84 days post-treatment) to assess clinical outcome. EDTA plasma samples were collected, centrifuged and stored at -80°C within 2 hours of patient enrolment. MR-proADM and PCT were batch-measured in a blinded fashion by TRACE technology (Time Resolved Amplified Cryptate Emission) using a new sandwich immunoassay (Kryptor Compact Plus Analyzer, BRAHMS, Hennigsdorf, Germany), with a limit of detection of 0.05 nmol/L and 0.02 ng/L, respectively. Further details on randomization, trial medication and study procedures have been previously published.[1,9]

Outcome measure

The biomarkers PCT and MR-proADM were evaluated for their ability to predict the clinical cure. Clinical cure was assessed on the day 30 visit (10-18 days post-treatment), and was defined as survival with absence of fever and a resolution of UTI symptoms (either absence of symptoms or at least 2 points improvement on a 0–5 point severity score scale), without additional antimicrobial therapy (for relapse of UTI).[1] Long term clinical cure was assessed at the 90-day post-treatment interview.

Statistical analysis

Descriptive statistics are expressed as frequencies (percentage), means with standard deviation (SD) or as medians with interquartile range (IQR), as appropriate. Univariate analysis was performed using ANOVA, student's t-test or Mann-Whitney U test where appropriate for continuous variables and Chi-square test for categorical variables. Non-parametric tests were used to analyse biomarkers.

Analyses were performed in the total patient population and in 2 subgroups based on treatment duration. Kendall's rank tau-b was used to investigate correlations between biomarker levels and clinical parameters. Finally, area under the receiver operating characteristic (AUROC) curves with 95% confidence intervals (CI) were calculated to assess the prognostic ability of PCT and MR-proADM.

A p-value of <0.05 was considered to indicate statistical significance. SPSS software (SPSS Inc. Chicago, version 23.0) was used for statistical analysis.

RESULTS

A total of 249 patients with a presumptive diagnosis of fUTI were analysed (details provided in Figure 1). Patient characteristics in terms of urologic history, comorbidities and presenting symptoms are outlined in Table 1. Of these, 165 (66%) were randomly assigned to receive antimicrobial treatment for either 7 (N=85) or 14 (N=80) days; in the remaining patients the treatment duration was left at the discretion of the treating physician. The majority of patients (N=175; 70%) were included at the ED, and 73 (29%) patients undergoing existing antimicrobial treatment prior to presentation. Patients had an average age of 60 (45 - 73) years, with females comprising the majority of enrolled patients (N=148; 59%).

The clinical cure rate was high (N=229; 92%) and did not differ significantly between randomized and non-randomized patients (94% vs. 88%, respectively). A total of 20 patients did not reach the endpoint of clinical cure, due to persistence or recurrence of UTI symptoms (N = 8), or to the use of additional antibiotics for relapse of UTI (N = 12), assessed on the day 30 visit. No significant differences were seen in the clinical characteristics between patients with treatment success or failure. In addition, mean treatment duration was similar (11 days) in both patients with clinical cure and clinical failure (Table 2).

Median biomarker concentrations across the total patient population were as follows: PCT: 0.40 [0.12 - 1.54] ug/mL; MR-proADM: 0.89 [0.63 - 1.28] nmol/L; and CRP: 118 (52 - 205) mg/L.

Figure 1. Flow of patients.

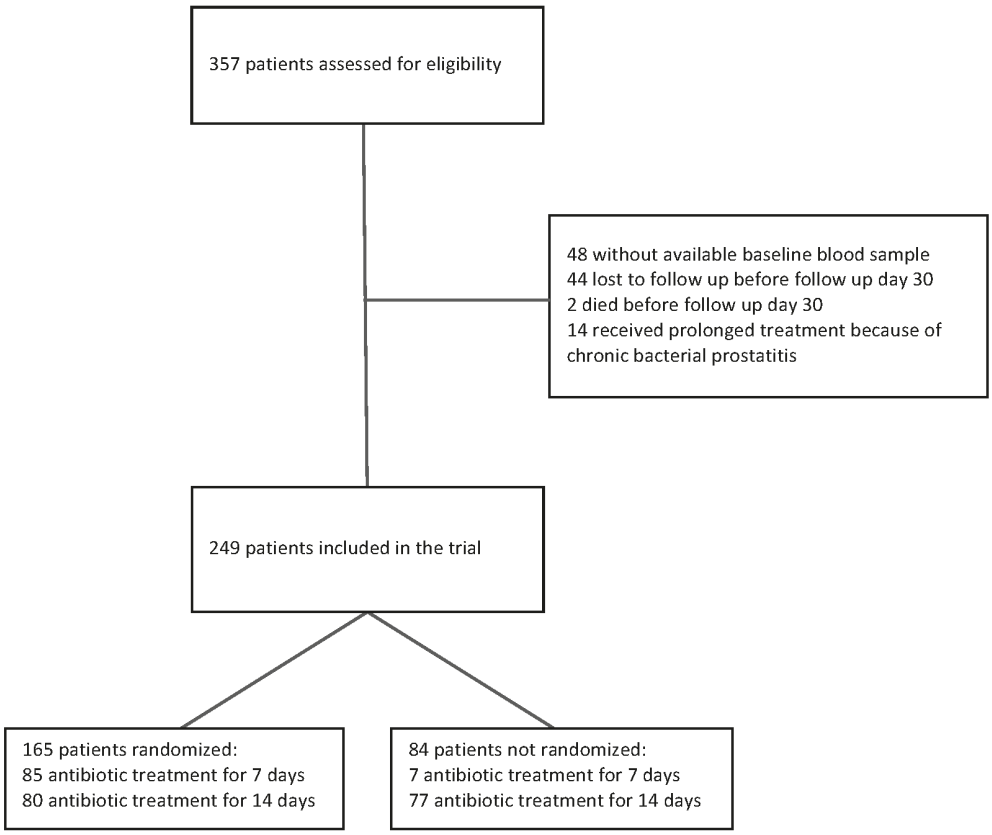


Table 1. Baseline characteristics of 249 patients with febrile urinary tract infection.

All patients (n=249)		
	Age (years)	60 (45-73)
	Male sex	101 (41)
Urologic history		
	Indwelling urinary catheter	7 (3)
	Urinary tract disorder[a]	75 (30)
	Recurrent UTI[b]	57 (23)
Comorbidity		
	Diabetes mellitus	33 (13)
	Malignancy	17 (7)
	Heart failure	21 (8)
	Cerebrovascular disease	13 (5)
	Chronic renal insufficiency	9 (4)
	COPD	27 (11)
	Immunocompromised	15 (6)
Presentation		
	At emergency department	175 (70)
	Antibiotic pretreatment	73 (29)
	Fever duration, hours	36 (19-72)
	Dysuria	188/243 (77)
	Flank pain	159/245 (65)
	Suprapubic pain	127/242 (52)
	Perineal pain	11/241 (5)
	Systolic BP, mmHg	130 (116-146)
	Diastolic BP, mmHg	74 (64-84)
	Heart rate, beats/minute	94 (80-107)
Cultures		
	Positive urine culture	171 (69)
	Escherichia coli	143/171 (84)
	Positive blood culture	45/240 (19)
	Positive urine and/or blood culture	183 (73)

Data presented as number (%) or median (IQR).
BP: blood pressure. AB: antibiotics; TMP/SMX: trimethoprim-sulfamethoxazole.
[a]any functional or anatomical abnormality of urinary tract except urinary catheter
[b]≥3 UTIs in past 12 months or ≥2 UTIs in past 6 months

Table 2. Characteristics of patients with clinical cure and clinical failure.

		Clinical cure (n=229)	Clinical failure (n=20)	*p* value
	Age (years)	60 (45-73)	56 (46-71)	0.412
	Male sex	94 (41)	7 (35)	0.597
Urologic history				
	Indwelling urinary catheter	7 (3)	0	0.428
	Urinary tract disorder[a]	71 (31)	4 (20)	0.304
	Recurrent UTI[b]	50 (22)	7 (35)	0.191
Comorbidity				
	Diabetes mellitus	32 (14)	1 (5)	0.256
	Malignancy	16 (7)	1 (5)	0.735
	Heart failure	21 (9)	0	0.157
	Cerebrovascular disease	12 (5)	1 (5)	0.963
	Chronic renal insufficiency	8 (3)	1 (5)	0.729
	COPD	27 (11)	2 (10)	0.899
	Immunocompromised	14 (6)	1 (5)	0.841
Presentation				
	At emergency department	160 (70)	15 (75)	0.630
	Antibiotic pretreatment	71 (31)	2 (10)	0.048
	Fever duration, hours	36 (18-72)	48 (24-120)	0.279
	Dysuria	175/224 (78)	13/19 (68)	0.332
	Flank pain	142/225 (63)	17 (85)	0.049
	Suprapubic pain	116/222 (52)	11 (55)	0.814
	Perineal pain	11/222 (5)	0/19 (0)	0.321
	Systolic BP, mmHg	130 (116-146)	130 (115-150)	0.753
	Diastolic BP, mmHg	74 (63-85)	72 (68-83)	0.585
	Heart rate, beats/minute	93 (80-107)	96 (78-110)	0.695
Cultures				
	Positive urine culture	158 (69)	13 (65)	0.712
	Escherichia coli	133/158 (84)	10/13 (77)	0.497
	Positive blood culture	42/220 (19)	3 (15)	0.654
	Positive urine and/or blood culture	169 (74)	14 (70)	0.712
Treatment				
	Short antimicrobial treatment (7 days)	82 (36)	10 (50)	0.207
	Days of AB, mean (SD)	11 (3.3)	11 (3.5)	0.252
	Ciprofloxacin	208 (91)	18 (90)	0.902
	Amoxicillin (± clavulanic acid)	11 (5)	2 (10)	0.316
	TMP/SMX	5 (2)	0	0.504
	Other[c]	5 (2)	0	0.504
	Initial intravenous dose(s) of AB	135 (59)	12 (60)	0.927
	Randomized	155 (68)	10 (50)	0.109
	Outpatient treatment	89 (39)	7 (35)	0.733

Data presented as number (%) or median (IQR).
BP: blood pressure. AB: antibiotics; TMP/SMX: trimethoprim-sulfamethoxazole.
[a]any functional or anatomical abnormality of urinary tract except urinary catheter
[b]≥3 UTIs in past 12 months or ≥2 UTIs in past 6 months
[c]cefuroxime iv n=2, meropenem iv n=1, moxifloxacin n=1, flucloxacillin n=1.

PREDICTION OF TREATMENT OUTCOME

Total patient population

Concentrations of PCT, MR-proADM and CRP, measured at presentation, did not differ between patients with clinical cure or treatment failure (Figure 2 and Table 3). As shown in Table 3, biomarker levels also did not predict treatment failure when measured after 3 days of treatment. We also assessed cut-offs for PCT as previously used in other studies, such as 0.25 μg/mL[10,11] and a decrease of PCT concentration by 80%.[4]

ROC analyses were further performed to define the prognostic accuracy of the different biomarkers for predicting treatment outcome. Based on the calculated AUCs, none of the biomarkers had any predictive value for treatment success (Figure 3). Findings were similar in a selection of patients with culture proven UTI (N=183); and regarding the long term clinical cure in all patients (data not shown).

Patients treated for 7 days

We assessed the group of patients who received 7 days of antimicrobial treatment separately, because treatment success was lower as compared to those treated for 14 days (89% versus 94%, respectively, p=0.21). Again, no difference was seen in levels of PCT, MR-proADM and CRP between patients with clinical cure or treatment failure (Table 3).

Figure 2. Levels of PCT, MR-proADM and CRP in patients with clinical cure and failure.

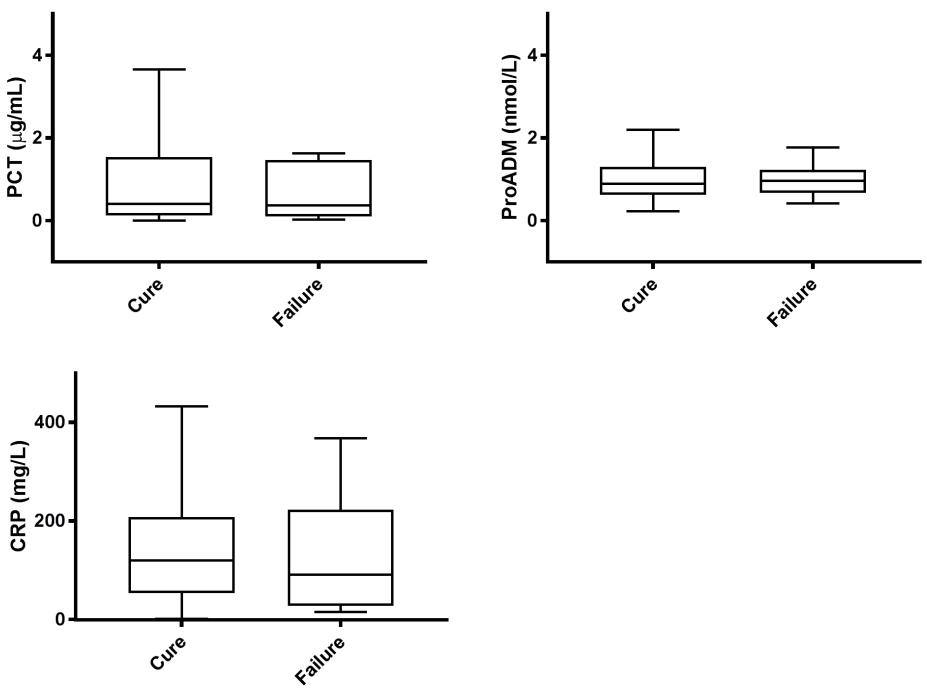
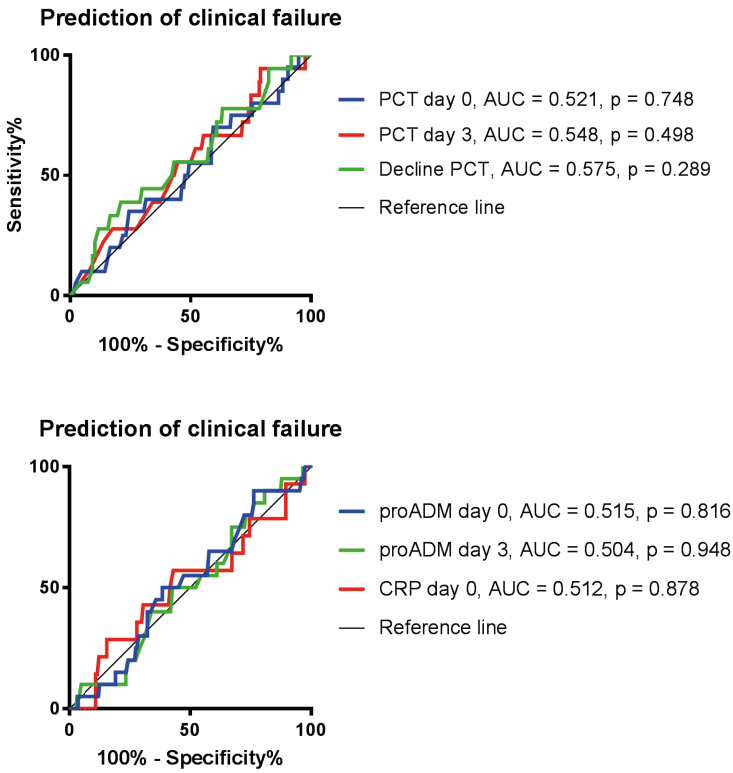


Figure 3. Biomarker accuracy in the prediction of treatment outcome.



CORRELATION BETWEEN BIOMARKERS AND CLINICAL PARAMETERS

Procalcitonin

PCT concentrations at presentation were positively correlated with bacteraemia (τ=0.33, p<0.001) and presence of shaking chills (τ=0.25, p<0.001). Although significant, correlations with initial intravenous treatment, length of hospital admission, time to defervescence, ICU admission, temperature, heart rate and confusion at presentation were weak (τ<0.20). Furthermore, PCT increased slightly with age (τ=0.12, p<0.01) and was not correlated to comorbidity. Median PCT concentrations measured after 3 days of treatment showed a similar trend, whereas the decrease of PCT concentration was not correlated to clinical parameters.

Mid-regional proadrenomedullin

MR-proADM levels at presentation were positively correlated with length of hospital stay (τ=0.40, p<0.001), bacteraemia (τ=0.33, p<0.001), initial intravenous treatment (τ=0.22, p<0.001)

and time to defervescence (τ=0.21, p<0.001). Weak, though significant correlation was seen with temperature, need for ICU admission, confusional state at presentation and the presence of shaking chills (τ<0.20). Compared to PCT, MR-proADM showed a stronger correlation with age (τ=0.49, p<0.001), and was weakly correlated to pre-existing comorbidity (diabetes mellitus, urinary tract disorder and chronic renal insufficiency). Again, after 3 days of treatment, correlations were similar. Decreasing MR-proADM concentrations between presentation and day 3 were correlated to bacteraemia (τ=0.22, p<0.001) and the presence of shaking chills at presentation (τ=0.22, p<0.001).

C-reactive protein

CRP was only weakly positively correlated to time to defervescence (τ=0.13, p=0.02) and weakly negatively correlated to temperature (τ=-0.11, p=0.04) and mean arterial pressure (τ=-0.13 p=0.02). No correlations were found between CRP and other parameters, such as age or comorbidity.

Table 3. Biomarkers in patients with clinical cure and clinical failure.

		Clinical cure	Clinical failure	P value
	All patients (n=249)	n=229	n=20	
At presentation				
	PCT	0.40 (0.13-1.54)	0.36 (0.10-1.46)	0.749
	PCT > 0.25	136 (59%)	12 (60%)	0.957
	CRP	120 (53-211)	90 (27-223)	0.851
	proADM	0.89 (0.62-1.30)	0.86 (0.67-1.20)	0.948
Day 3				
	PCT	0.18 (0.07-0.87)	0.12 (0.04-0.69)	0.667
	PCT ≤ 0.25	122 (60%)	12 (67%)	0.568
	PCT ≤ 0.25 or PCT decline ≥ 80%	144 (71%)	14 (78%)	0.519
	proADM	0.66 (0.50-0.91)	0.63 (0.51-0.78)	0.667
Short treatment (n=92)		**n=82**	**n=10**	
At presentation				
	PCT	0.34 (0.10-1.68)	0.36 (0.11-1.79)	0.980
	PCT > 0.25	47 (57%)	6 (60%)	0.871
	CRP	123 (53-194)	77 (15-142)	0.231
	proADM	0.76 (0.56-1.04)	0.85 (65-1.25)	0.360
Day 3				
	PCT	0.12 (0.06-0.42)	0.13 (0.09-0.63)	0.794
	PCT ≤ 0.25	51 (66%)	7 (78%)	0.484
	PCT ≤ 0.25 or PCT decline ≥ 80%	62 (80%)	8 (89%)	0.542
	proADM	0.56 (0.47-0.76)	0.59 (0.53-0.74)	0.679
Long treatment (n=157)		**n=147**	**n=10**	
At presentation				
	PCT	0.48 (0.15-1.49)	0.48 (0.09-3.13)	0.826
	PCT > 0.25	89 (60%)	6 (60%)	0.973
	CRP	119 (52-224)	181 (37-263)	0.531
	proADM	0.98 (0.67-1.44)	0.86 (0.62-1.21)	0.495
Day 3				
	PCT	0.22 (0.08-1.08)	0.08 (0.04-0.72)	0.358
	PCT ≤ 0.25	71 (56%)	5 (56%)	0.984
	PCT ≤ 0.25 or PCT decline ≥ 80%	82 (65%)	6 (67%)	0.899
	proADM	0.74 (0.53-1.03)	0.74 (0.47-1.02)	0.609

Data presented as median (IQR) or number (%).
CRP at presentation missing: n=41 in short treatment and n=41 in long treatment.

DISCUSSION

In the current study we assessed the ability of the biomarkers PCT, MR-proADM and CRP in predicting treatment failure in a randomized trial of antibiotic treatment duration (i.e. 7 versus 14 days) in patients with febrile urinary tract infection. Overall treatment success was high: 94% of the randomized 165 and 88% of 84 non-randomized patients who all were given an identical follow-up reached the endpoint of clinical cure. Of the biomarkers, PCT and MR-proADM were significantly correlated to clinical parameters such as fever, blood pressure and subjective complaints that represent the acute febrile illness of invasive urinary tract infection. Also, the biomarker signature of PCT and MR-proADM correlated with severity of disease, such as presence of bacteraemia and need for initial administration of antibiotics intravenously rather than orally. Finally, the course of biomarkers over the first three days correlated with signs of clinical recovery, such as time to defervescence, intensity of subjective complaints and length of hospital stay. As opposed to PCT and MR-proADM, the currently popular biomarker CRP did not display any correlation to relevant clinical parameters like course of fever or bacteraemia. We hypothesized that the biomarker signature, as an objective laboratory proxy of physical status in fUTI, would help the physician in guiding the length of antibiotic therapy. In our study, however, neither PCT nor MR-proADM or CRP were distinctive in identifying upfront those patients at risk for a treatment failure, i.e. who might benefit from a more prolonged antibiotic treatment.

The biomarkers we chose are in current use as predictors of morbidity and mortality in a variety of conditions. Procalcitonin is a precursor hormone of calcitonin and is upregulated by cytokines released in response to bacterial infection.[4] MR-proADM has been detected in a variety of tissues, including heart, vessels and the kidneys and has both immune modulating and vasodilating properties.[2] Levels of MR-proADM are elevated in sepsis, contributing to hypotension in these patients. Thus, procalcitonin has shown to be a marker of bacteraemia in patients with febrile UTI,[12-14] whereas mid-regional proadrenomedullin (MR-proADM) is a predictor of a complicated course of disease, the need for ICU admission, and mortality.[6,7]

PCT-based algorithms have been developed to aid decisions on the initiation and/or discontinuation of antibiotics in patients with acute respiratory tract infections and in critically ill patients admitted to the intensive care ward.[15] In these specific patient groups, the use of PCT has been shown to be a powerful tool in antibiotic stewardship.[16] Although in the intensive care studies patients with infections originating from the urinary tract were included, numbers were small (e.g. 7% of patients in the PRORATA trial, 3% of patients in the SAPS trial) and these results cannot directly be extrapolated to patients with community-acquired urinary tract infections.[4,17]

Elevation of these biomarkers is a reflection of the systemic inflammatory response to bacterial invasion. This inflammatory response coincides with acute illness, and often in a measurable deviation of vital signs, such as a raised temperature and decreased blood pressure. In daily clinical practice, severity of the acute illness is assessed on basis of history, comorbidity and on severity of local and vital signs. It has become clear that severity of illness can also be more objectively expressed in biomarkers, which individually are associated with complicated

course of disease, such as bacteraemia, need for ICU admission, time to defervescence and length of hospital stay. Although PCT and proADM were correlated to these clinical outcome parameters in our patients, they did not help predict outcome of the otherwise standardized antibiotic treatment, irrespective of a treatment duration of 7 or 14 days. The most likely reason is that all patients were treated for at least 7 days after which treatment success was high already (i.e., 89%). Thus, the lack of predictive value for treatment guidance is likely explained by the fact that historically, empiric treatment duration is based on the anticipated time to clinical recovery, while taking into account the inter-individual variety in severity of acute illness at the start of treatment (i.e. practically, adding some days of treatment to average recovery time). Thus, in our patients, the median time to defervescence was 2.0 (IQR 1-3) days while randomization for short or prolonged treatment duration did not start until day 7. In other words, one could have predicted that our biomarker approach might have been successful in guiding treatments up to one or a few days after clinical recovery, but it lacked the ability to do so after the minimum 7 days of treatment, by which a strong margin surpasses the time for the biomarker signature to return to normal. Differences between patients in severity of illness at presentation and corresponding biomarker levels are likely to have normalized after 7 days of treatment, and definitely after 14 days.

In the original study we described that although 7 days of treatment was inferior to 14 days in male patients in terms of short term clinical cure, there was no difference in the requirement for antibiotic retreatment for UTI during longer follow up (90 days) in both women and men.[1]

A retrospective analysis of a large database of male veterans also found that treatment duration longer than 7 days was not associated with a reduction of UTI recurrence.[18] In this study, UTI recurrence was independently associated with comorbidities and age. It is likely that a subgroup of patients can be treated with an even shorter course of antibiotics then 7 days. Hypothetically, within the current time frame of treatment, there is a moment when the patient has recovered from the acute illness and the biomarker levels have dropped below a certain cut-off, likely leaving room for further limitation of treatment duration. It stands to reason that the time to this recovery is defined by host related factors, and therefore differs between patient subgroups. Biomarkers have the potential to objectively identify the optimal moment for cessation of therapy, irrespective of patient characteristics. Unfortunately, as reasoned above, our study design did not allow for further discrimination.

The main strength of this trial is its pragmatic nature reflecting daily clinical practice. We randomized patients between the up until then standard treatment duration for fUTI of 14 days and half of that length (7 days), and included enough subjects to demonstrate non-inferiority. We enrolled consecutive patients with fUTI, irrespective of age, gender and underlying medical condition. Different biomarker concentrations were available at the day of presentation and after three days of treatment, each representing different aspects of the physiological condition of the acute illness.

Limitations of our study include the design in randomizing patients between 7 and 14 days, not allowing for analysis of biomarker value in shorter antibiotic treatment. This was unforeseen,

because at the start of the trial 14 days of therapy were standard clinical practice, and reduction to 7 days was already a great improvement. Furthermore, our sample size may have been too small to exclude a type II error, but such a difference will have been limited.

To our knowledge, only one study addressing biomarker-guided antibiotic therapy in community acquired urinary tract infection has been published.[10] In this study, Drozdov et al. randomized both patients with cystitis (N=36) and febrile urinary tract infections (N=84) between PCT-guided or standard treatment. Overall, antibiotic exposure was reduced whereas adverse outcomes (clinical recurrence and rehospitalization rate) were similar in both groups. In the subgroup analysis of patients with fUTI/pyelonephritis, PCT-guided duration of antibiotic therapy was significantly shorter than standard care (7.5 vs. 11.0 days). In bacteraemic patients, recurrence rate (56% vs. 16%) and persistent infection after treatment (13% vs. 6%) were higher in the PCT guided group, although numbers were too small to reach significance. Although this study is limited by the small sample size and heterogenicity of patients, it supports the potential use of PCT-based approach in patients with community acquired fUTI to safely reduce antibiotic consumption.

Future interventional studies should be conducted to examine if PCT-guided duration of antibiotic treatment can indeed aid in distinguishing a subgroup of patients with fUTI who can be treated with antibiotics for even shorter than 7 days, i.e. those with rapid normalization of elevated biomarkers, in order to further decrease antibiotic exposure and limit development of antimicrobial resistance.

In conclusion, although the biomarkers PCT and MR-proADM are correlated to clinical parameters indicating disease severity, they did not predict treatment outcome in patients with community acquired febrile urinary tract infection, who are treated for 7 or 14 days. CRP has no added value in the management of fUTI.

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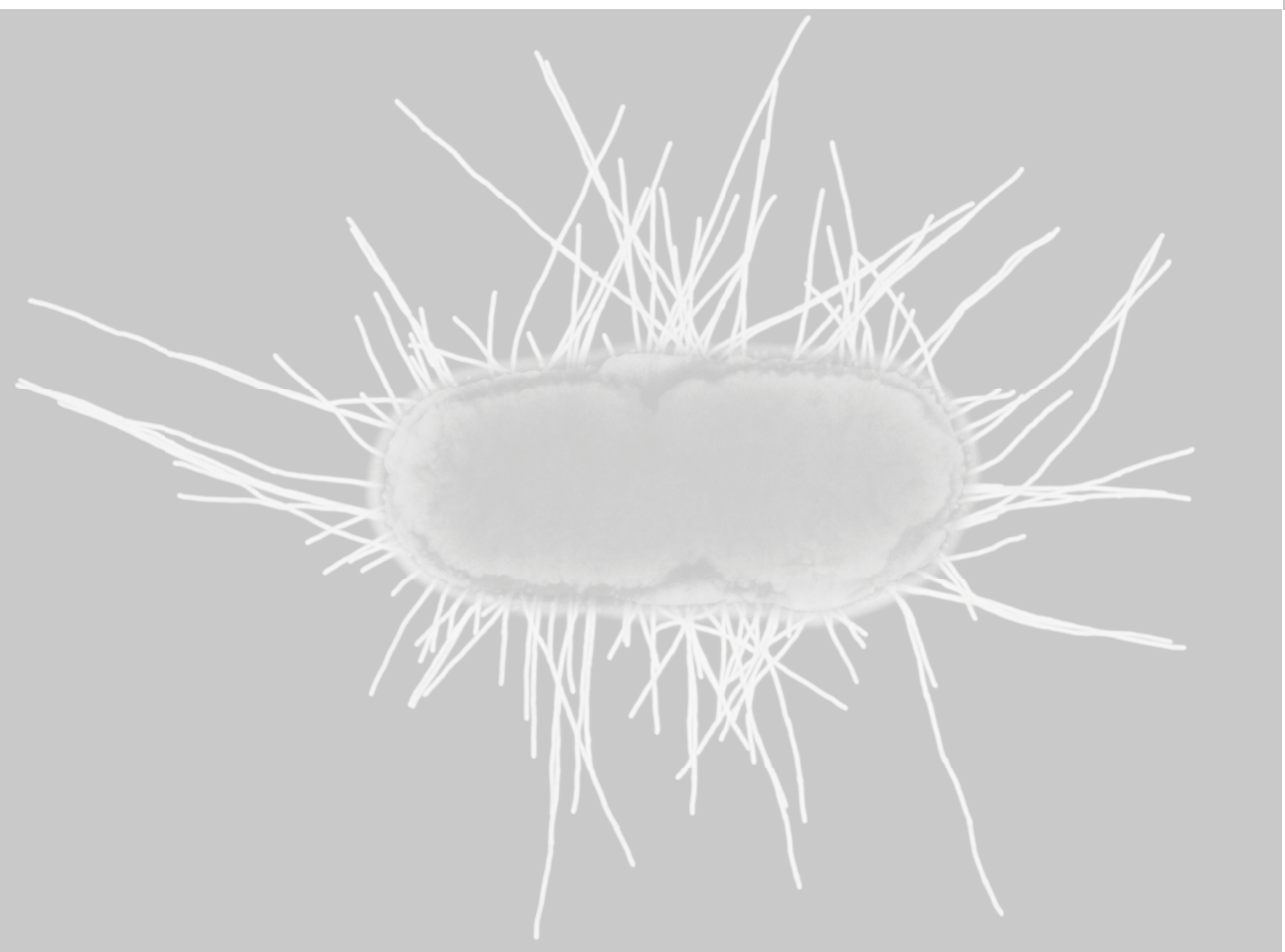

CHAPTER 6

Intravesical gentamicin treatment for recurrent urinary tract infections caused by multidrug-resistant bacteria

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ABSTRACT

Background

Antimicrobial resistance leads to complications in the management of recurrent urinary tract infections (rUTIs). In some rUTI patients with limited treatment options, intravenous therapy with reserve antibiotics is often required.

Objective

To assess the effectiveness, safety, and feasibility of prophylactic treatment with intravesical gentamicin in patients with refractory rUTI caused by multidrug-resistant (MDR) microorganisms.
Design, setting, and participants
Prospective trial of 63 adults with rUTI caused by MDR pathogens, enrolled at one academic and one general hospital in the Netherlands between 2014 and 2017, and a retrospective analysis of 27 adults with rUTI treated with intravesical gentamicin between 2009 and 2014.

Intervention

Overnight intravesical instillations of gentamicin for 6 months. In the retrospective cohort, the frequency and duration varied (predominantly once daily).

Outcome measurements

The primary outcome was the recurrence rate of UTIs compared to that in the preceding 6 months. Secondary objectives included the assessment of the safety of intravesical gentamicin instillation and its influence on the development of antibiotic resistance in uropathogens.

Results and limitations

The mean number of UTIs during the treatment reduced from 4.8 to 1.0 in the prospective trial. The mean number of UTIs in the retrospective cohort was 0.6 during treatment. The resistance rate of the uropathogens dropped from 78% to 24%. No systemic absorption or clinically relevant side-effects were observed. Limitation: lack of a control group.

Conclusions

Intravesical gentamicin instillation reduced the number of UTI episodes and the degree of antimicrobial resistance.

Patient summary

We studied the effect of administration of gentamicin into the bladder after self-catheterisation in patients with frequent urinary tract infections caused by multidrug-resistant bacteria. The treatment reduced the incidence rates of infection and resistance to antibiotics, without relevant side-effects.

INTRODUCTION

The management of patients with recurrent urinary tract infections (rUTIs) is more complex in this era of rising antibiotic resistance.[1] Guidelines on the management of rUTI recommend the consideration of continuous antimicrobial prophylaxis after counselling, and behavioural modification has been attempted in patients without a source of bacterial persistence.[2,3] Continuous antibiotic prophylaxis reduced the number of clinical and microbiological recurrences in healthy, young women with rUTI in trials conducted in the 80s and 90s, when the resistance rates were considerably lower.[4]

Nowadays, patients are often refractory to the above-mentioned measures, as the availability of oral antibiotic agents is either limited or absent due to resistance, allergies or side-effects.[1] The use of systemic antimicrobial prophylaxis augments the emergence of resistant organisms, further limiting the antimicrobial treatment options.[5,6] Particularly, in patients with complex urological histories, such as those with neurogenic bladder disorders necessitating intermittent catheterisation and renal transplant recipients, the spectrum of uropathogens and their sensitivity patterns differ from those of the general population.[5,7] In rUTI patients with limited treatment options, intravenous therapy with reserve antibiotics is often required, which affects health-related costs and the quality of life.

Intravesical treatment with gentamicin may be a reasonable treatment option for rUTIs in some patients.[8] Aminoglycosides have concentration-dependent antimicrobial activity and by direct intravesical instillation, high concentrations can be achieved at the site of infection, well (>10 times) above the minimum inhibitory concentration (MIC) of even uropathogens with lower susceptibilities. Antimicrobial resistance is unlikely to occur in the urinary tract due to high urinary drug concentrations and a lack of selective antibiotic pressure on the commensal flora at other sites of the body including the intestines, perineum and vagina. With the local use of gentamicin, the recovery of this commensal flora may even lead to the clearance of multidrug-resistant (MDR) microorganisms. Concerns regarding systemic uptake and, therefore, the side-effects associated with parenterally administered aminoglycosides (nephrotoxicity and ototoxicity) are minimal, although long-term data are lacking.[8,9]

This prospective study aimed to assess the effectiveness, safety, and feasibility of treatment with intravesical gentamicin in patients with refractory rUTI. Furthermore, we described a retrospective cohort of patients treated with intravesical gentamicin. These data were combined as the treatment and follow-up were almost similar to those in the prospective trial.

MATERIAL AND METHODS

Design and study population

Competent adults with a history of rUTI were recruited from the Leiden University Medical Center (LUMC) and the Haga Teaching Hospital, the Netherlands, after referral by urologists from across the Netherlands, between May 2014 and March 2017. Recurrent UTI was defined as ≥3 episodes of UTI in the last 12 months in women and ≥2 episodes of UTI in the last 12 months in men. UTIs in the preceding year were defined by self-report, but ≥1 episode of UTI had to be documented

by urine culture with the isolation of ≥10^3 CFU/mL of an identified MDR pathogen. Multidrug resistance was defined as acquired non-susceptibility to ≥1 agent in ≥3 antimicrobial classes.[10] Exclusion criteria were a glomerular filtration rate <15 ml/min, abnormalities of the upper urinary tract including the presence of urinary stones, a permanent urinary catheter, complete urinary incontinence, known hypersensitivity to gentamicin, pregnancy or lactation and a positive urinary culture for high-level gentamicin-resistant Enterobacteriaceae or enterococci (MIC >128 mg/L) in the preceding 6 months. Before enrolment, urological evaluation was performed, and modifiable behavioural practices were addressed in all patients.

The original study was designed as a randomized trial (registered at www.trialregister.nl NTR4646), but because of the lack of patients willing to participate in a randomized trial that beheld the risk of being assigned to standard treatment (oral prophylaxis) that had failed them before, the study design was converted to a prospective non-controlled trial. The study protocol, including the conversion to a non-controlled trial, was approved by the local ethical committee (#P13.254). The patients in this study provided written informed consent for the publication of their case details.

The retrospective study included a cohort of adult patients treated with intravesical gentamicin to treat rUTIs, between June 2009 and April 2014, in the infectious diseases outpatient clinic of the LUMC. All data were collected from electronic patient files.

Treatment

Patients were trained on self-catheterisation and the preparation of a gentamicin solution by a specialised nurse during outpatient clinic visits. When patients were unable to perform self-catheterisation, instillations were performed by a home-care nurse. Patients were instructed to self-administer the gentamicin solution (80 mg of gentamicin dissolved in 20 mL 0.9% sodium chloride) following self-catheterisation at bedtime, and to retain the solution in the bladder overnight until the next micturition or catheterisation.

The standard frequency of instillations was daily for 2 weeks, every other day for 10 weeks, and twice weekly for 12 weeks (total of 24 weeks).

Patients with symptomatic UTI were instructed to collect midstream urine for microscopy and culture. In patients receiving intravesical gentamicin twice weekly, the instillation frequency was intensified to once daily. After 1 week of daily instillations, the frequency, according to the regular schedule, was continued. In other cases, empirical treatment based on prior sensitivity patterns was started. Patients with febrile UTI were admitted to receive intravenous antibiotics.

UTI was defined as an episode of ≥1 urinary symptom (dysuria, frequency, urgency, suprapubic or perineal pain) and isolation of ≥10^3 CFU of uropathogens/mL in a urine culture with leukocyturia. Chronic bacterial prostatitis was presumptively diagnosed in men with rUTI with the same uropathogen and susceptibility patterns.

Assessments

Baseline data on demographic variables and clinical characteristics, and serum creatinine samples were collected, cultures of urine and rectal swabs were performed, and audiometric testing was conducted to document pre-existing hearing impairments.

Follow-up visits were scheduled at 2, 12 and 24 weeks after the start of treatment, and at 3 and 6 months after the discontinuation of the instillations. The systemic uptake of gentamicin was evaluated by measurement of serum gentamicin concentration immediately after the bladder was emptied of the instillation solution.

Serum creatinine levels, urine cultures and rectal swabs were repeated during and after treatment. The occurrence of UTIs and adverse events was documented at follow-up visits. Audiograms and ear-nose-throat consultations were ordered for patients with complaints of hearing loss or tinnitus. Follow-up cystoscopy was planned one year after the start of gentamicin instillation.

Cultures were analysed according to standard microbiological methods, as described previously [11]. Susceptibility testing was performed using the VITEK-2 system (bioMérieux, The Netherlands), and the detected gentamicin resistance was confirmed by the Etest (bioMérieux) in the available isolates. Rectal swabs were cultured on selective plates for the detection of Gram-negative bacteria and (multi)drug resistance. Resistance was defined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[12]

Statistical analysis

Descriptive statistics were expressed as counts (percentages), means (standard deviation [SD]) or medians (interquartile range or range), as appropriate. Univariate analysis was performed using the Student's t-test or Mann-Whitney U test for continuous variables and chi-square tests for categorical variables. We modelled the probability of the participants being UTI-free at each time-point during the 6 months of treatment using Kaplan-Meier estimates. P-values <0.05 were considered statistically significant. SPSS software version 24.0 (SPSS Inc. Chicago, IL) was used for the statistical analysis.

RESULTS

Baseline patient characteristics

A total of 90 patients with rUTI were analysed; 63 and 27 patients participated in the prospective and retrospective studies, respectively. The patients' characteristics are outlined in Table 1. In most patients (91%), oral prophylactic antibiotics had previously failed.

A majority of the UTIs before enrolment was caused by MDR bacteria (78%) and the resistance rates for the antibiotics commonly used for UTI–nitrofurantoin (41%), ciprofloxacin (64%) and trimethoprim-sulfamethoxazole (65%)–were much higher than those in the general population (Table S1).[13] Gentamicin resistance was detected in 16% of the 80 available isolates before enrolment (available MICs are provided below Table 1).

Table 1. Patient characteristics

Patient characteristics	Prospective study n = 63	Retrospective cohort n = 27	Total n = 90
Age in years, median (IQR)	61 (53–72)	67 (56–74)	64 (53–72)
Sex – female	51 (81)	16 (59)	67 (74)
Post-menopausal	40/51 (78)	13/16 (81)	53/67 (79)
Allergy for antibiotics	32 (51)	14 (52)	46 (51)
Diabetes mellitus	14 (22)	7 (26)	21 (23)
Immunocompromised	9 (14)	6 (22)	15 (17)
eGFR (CKD-EPI), median (IQR)	81 (69–92)	82 (56–96)	82 (63–92)
Urologic history			
Intermittent catheterisation	33 (52)	20 (74)	53 (59)
Urolithiasis	5 (8)	2 (7)	7 (8)
Malignancy of urinary tract	1 (2)[§]	1 (4)[§§]	2 (2)
History of urologic surgery:			
- Kidney transplant	7 (11)	5 (18)	12 (13)
- Transurethral resection of the prostate	3 (5)	1 (4)	4 (4)
- Tension-free vaginal tape-obturator	5 (8)	0	5 (6)
- Bladder surgery	4 (6)	2 (7)	6 (7)
- Urethrotomy / meatus dilatation	5 (8)	0	5 (6)
- Neobladder	0	7 (26)	7 (8)
- Other	1 (2)[#]	3 (5)[##]	4 (4)
Cause of recurrent UTI			
Dysfunctional voiding / neurogenic bladder	31 (49)	12 (44)	43 (48)
Urethral strictures	0	1 (4)	1 (1)
Vesicoureteral reflux	0	2 (7)	2 (2)
Neobladder	0	4 (15)	4 (4)
Kidney transplant only	3 (5)	1 (4)	4 (4)
Kidney transplant + dysfunctional voiding / neurogenic bladder	3 (5)	0	3 (3)
Kidney transplant + urethral strictures	1 (2)	1 (4)	2 (2)
Kidney transplant + neobladder	0	3 (11)	3 (3)
No anatomical or structural urinary tract abnormalities	25 (40)	3 (11)	28 (31)
Suspected underlying chronic bacterial prostatitis	9/12 (75)*	4/11 (36)**	14/23 (61)
Previous antibiotic treatment			
Urinary tract infections in preceding year, mean (SD)	8.7 (3.0)	unknown	-
Urinary tract infections in preceding 6 months, mean (SD)	4.8 (1.5)	unknown	-
Patients treated with IV antibiotics for UTI in preceding year; courses mean (SD)	23 (36); 2.0 (1.5)	unknown	-
Previous oral antibiotic prophylaxis	55 (87)	27 (100)	82 (91)
Microbiology			
Causative pathogen in preceding UTI:			
- Escherichia coli	38 (60)	17 (63)	55 (61)
- Klebsiella spp.	16 (25)	5 (18)	21 (23)
- Proteus mirabilis	4 (6)	1 (4)	5 (6)
- Other bacteria	5 (8)[$]	4 (15)[$$]	9 (10)
Gentamicin-resistant pathogen in preceding UTI	8/57 (14)^	5/23 (22)^^	13/80 (16)
Multidrug-resistant pathogen in preceding UTI	49 (78)	21 (78)	70 (78)
ESBL-positive pathogen in preceding UTI	15 (24)	8 (30)	23 (26)
NDM-1-positive pathogen in preceding UTI	1 (2)	0	1 (1)
Multidrug-resistant bacteria in rectal swab	9/58 (15)	-	-

Data are presented as n (%) unless stated otherwise.
SD, standard deviation; IQR, interquartile range; UTI, urinary tract infection; eGFR, epidermal growth factor receptor; CKD-EPI, The Chronic Kidney Disease Epidemiology Collaboration; IV, intravenous; EBSL, Extended-spectrum β-lactamases; NDM-1, New Delhi metallo-β-lactamase; MIC, minimum inhibitory concentration.
[§]non-muscle invasive bladder cancer (n=1). [§§]prostate cancer (n=1).
[#]orchiectomy (n=1). [##]nephrectomy (n=1); prostatectomy + pelvic lymph node dissection (n=1); ureteral reimplantation (n=1). *participants with dysfunctional voiding/neurogenic bladder (n=6); urethral strictures (n=1); benign prostatic hyperplasia (n=2). **participants with dysfunctional voiding / neurogenic bladder (n=3); kidney transplant + urethral strictures (n=1). [$]*Enterobacter asburiae* (n=1), *Morganella morganii* (n=1), *Providencia rettgeri* (n=1), *Stenotrophomonas maltophilia* (n=1), *Aerococcus* sp. (n=1). [$$]*Pseudomonas aeruginosa* (n=2), *Enterococcus faecalis* (n=1), *Citrobacter freundii* (n=1). ^MIC 16 mg/L (n=1); MIC 32 mg/L (n=2); MIC 256 mg/L (n=1); resistant: MIC >4 mg/L (n=4). ^^Intermediate: MIC 2-4 mg/L (n=2); resistant: MIC >4 mg/L (n=3).

Details on intravesical treatment

In the prospective study, 57% (n=36) of the patients were treated exactly according to the treatment protocol. In the other patients, circumstances such as the persistence of urinary complaints, treatment success (patients unwilling to stop instillations) and other patient-related factors led to an alternative duration or frequency of administration. In four patients, other aminoglycosides were used due to gentamicin resistance in the baseline cultures. The median duration of the aminoglycoside instillations was 26 weeks in both the prospective study (range 6–65) and retrospective cohort (range 6–280). Details on the treatment are provided in Table 2.

Table 2. Treatment details

Treatment details	Prospective study n = 63	Retrospective cohort n = 27	Total n=90
Self-instillation of aminoglycoside	58 (92)	22 (81)	80 (89)
Aminoglycoside used			
- Gentamicin	60 (95)	26 (96)	86 (96)
- Tobramycin	2 (3)	1 (4)	3 (3)
- Amikacin	1 (2)	0	1 (1)
Duration of aminoglycoside instillation in weeks, median (IQR)	26 (23–29)	36 (15–80)	26 (23–36)
Frequency of aminoglycoside instillations			
- Standard frequency (tapering according to protocol)	55 (87)	7 (26)	62 (69)
- Daily	4 (6)	10 (37)	14 (16)
- 2 or 3 times a week	0	10 (30)	8 (9)
Early termination			
- Due to treatment failure	7 (17)	1 (4)	8 (9)
- Other reasons (surgery, diagnostics, planned pregnancy, wrist fracture)	4 (6)	1 (4)	5 (6)
Prolonged treatment (>28 weeks)	16 (25)	16 (59)	32 (36)
Restart of aminoglycoside instillations during follow-up	20 (32)	2 (7)	22 (24)

Data are presented as n (%) unless stated otherwise. IQR, interquartile range.

Outcome

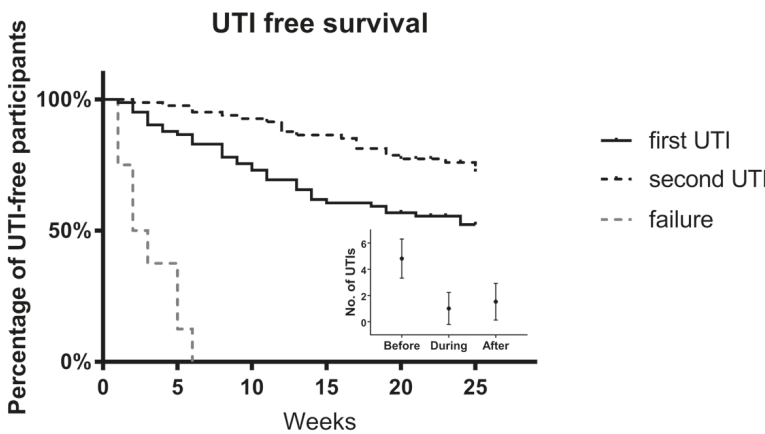
The mean number of UTIs in the prospective trial was significantly reduced to 1.2 (SD 1.3), compared to the 4.8 (SD 1.5) in the 6 months before treatment (Table 3). The mean number of culture-proven UTIs was 1.0 (SD 1.2). Twenty-six (41%) patients in the prospective trial were completely UTI-free during intravesical gentamicin treatment (Table 3). In the remaining patients, breakthrough infections (n=73 in 37 patients) during gentamicin treatment were managed by extra gentamicin instillations in 23% of the UTI episodes, oral antibiotics in 68% and intravenous treatment in 8%.

The mean number of all UTIs remained low (1.5 [SD 1.4]) after the cessation of intravesical gentamicin, and 31% of the patients remained UTI-free during the follow-up. Noteworthily, many patients (63%) continued using intravesical gentamicin off-protocol (continued prophylaxis, or restarted after experiencing one or more UTIs).

The mean number of UTIs in the retrospective cohort was 0.6 (SD 1.0) during the 6 months of gentamicin instillation, and 67% of the patients were UTI-free. Breakthrough UTIs were managed with oral antibiotics in 20/22 (91%) episodes, and intravenous treatment in 2/20 (9%) cases.

In the total population, the mean number of UTIs reduced to 1.0 (SD 1.2) during the 6 months of intravesical gentamicin treatment (Figure 1). No differences were observed between subgroups (e.g. sex, cause of rUTI, immunocompromised state). Data on the UTI-free survival during gentamicin treatment are shown in Figure 1.

Figure 1. Urinary tract infection-free survival.



Time in weeks to the first and second UTIs after the start of gentamicin instillation. Failure: early termination of gentamicin instillation because of failure (n=8).
Inset: Mean number of urinary tract infections (with standard deviation) in the 6 months before, during and after the gentamicin instillations (prospective trial and retrospective cohort combined, n=90, $p < 0.01$).
UTI, urinary tract infection

In eight patients, intravesical treatment was stopped because of clinical failure (Table 3). Three patients experienced bacteriologic failure. One man with suspected prostatitis had persistent asymptomatic bacteriuria. Two women in the retrospective cohort had persistent asymptomatic positive cultures under gentamicin instillation and were diagnosed with urolithiasis. In both cases, the cultures yielded negative results after the treatment of urolithiasis.

Details on the microbiology results are shown in Supplementary Table S1. The rate of multidrug resistance of the causative uropathogens dropped from 78% before gentamicin instillation to 24% afterwards. In the 13 patients who had UTIs caused by gentamicin-resistant bacteria, the mean number of UTIs reduced to 0.7 (SD 0.9), and one patient with chronic bacterial prostatitis (MIC 16 mg/L) experienced treatment failure.

Post-treatment, gentamicin resistance was observed in 13 patients; four patients exhibited the asymptomatic colonisation of gentamicin-resistant bacteria and nine experienced UTIs caused by gentamicin-resistant uropathogens. In three of these patients, gentamicin resistance was also observed before the start of the instillations (Table S2).

Table 3. Patient outcomes

Outcome	Prospective study n = 63	Retrospective cohort n = 27	Total n = 90
Urinary tract infections during the 6 months of instillation	n=63	n=27	n=90
All UTIs*, mean (SD)	1.2 (1.3), range 0–4	0.6 (1.0), range 0–4	1.0 (1.2), range 0–4
UTI according to protocol definition**, mean (SD)	1.0 (1.2), range 0–4	0.6 (1.1), range 0–4	0.8 (1.1), range 0–4
Patients without UTI	26 (41)	18 (67)	44 (49)
Patients without systemic antibiotics for UTI during instillations	32 (51)	18 (67)	50 (56)
UTI-free time in weeks, mean (SD)	15.2 (9.0)	18.4 (9.0)	16.1 (9.1)
Episodes of UTI	n=73	n=22	n=95
- Treated with oral antibiotics	50/73 (68)	20/22 (91)	70/95 (74)
- Treated with iv antibiotics	6/73 (8)	2/22 (9)	8/95 (8)
- Treated with extra aminoglycoside instillations only	17/73 (23)	0	17/95 (18)
Days of oral antibiotic use for UTI, mean (SD)	6.0 (8.4)	-	-
Days of IV antibiotic use for UTI, mean (SD)	0.63 (3.6)	-	-
Treatment failures			
Causes of treatment failure (early termination of instillations):	7 (11)	1 (4)	8 (9)
- Exogenous reinfections (no cause found)	2[#]	1[##]	3
- Chronic bacterial prostatitis with persistent symptoms and positive cultures with Escherichia Coli (MIC of gentamicin 16 mg/L)	2	0	2
- Persistent bacteriuria suspected for endogenous focus in the higher urinary tract (selective sampling of urine was positive in both ureters, suspicions of chronic infections of the upper urinary tract, nephrolithiasis or other causes of endogenous infection were ruled out by computed tomography)	1	0	1
- Alternative diagnosis (IC/BPS with resolution of symptoms after coagulation of Hunner's ulcer)	1	0	1
- Alternative diagnosis (female patient who had no improvement on culture-directed antibiotic treatment and reported resolution of symptoms after start of solifenacin; suggestive of overactive bladder syndrome)	1	0	1

Data are presented as n (%) unless stated otherwise. SD, standard deviation; UTI, urinary tract infection; IC/BPS, interstitial cystitis / bladder pain syndrome; MIC, minimum inhibitory concentration.
*Culture proven and non-culture proven combined. **Culture proven only. [#]Female patients with frequent breakthrough infections with different uropathogens during gentamicin instillation. [##]Male patient with recurrent UTI with different microorganisms (secondary to intermittent self-catheterisation 4–6× daily).

Safety

Systemic uptake of gentamicin did not occur, as evidenced by the undetectable serum titres around the instillations (Table 4). Two patients in the prospective study showed a temporary mild increase in the serum creatinine level, which normalised at the next follow-up visit. Three patients reported experiencing hearing loss; this was unlikely to have been related to gentamicin use, since the concentrations of serum gentamicin were undetectable (Table 4).

Follow-up cystoscopy was performed in 49% of the patients. Ten patients showed signs of cystitis/trigonitis, one patient had papillary urothelial carcinoma, and three patients had alternative diagnoses (Table 4).

Table 4. Side-effects

Side-effects	Prospective study n = 63	Retrospective cohort n = 27
Laboratory results		
Undetectable serum gentamicin levels	62/62 (100)	25/25 (100)
Increase in serum creatinine >25%	2 (3)	0
Reported side-effects		
Abdominal discomfort	3 (5)	1 (4)
Reported hearing loss	2 (3)*	1 (4)**
Reported vaginal discomfort or discharge	10/51 (20)	-
Other[#]	3 (5)	-
Patients' satisfaction with instillations		
Mean (SD) satisfaction grade after 2 weeks between 0 – 10, response rate 90%	7.5 (1.4)	-
Mean (SD) satisfaction grade after 12 weeks between 0 – 10, response rate 76%	7.9 (1.3)	-
Mean (SD) satisfaction grade after 24 weeks between 0 – 10, response rate 76%	8.0 (1.2)	-
Cystoscopy 6 months after instillations		
Cystoscopy performed	30 (48)[##]	14 (52)
Abnormalities at cystoscopy	10/21 (48)	3/14 (21)
- Hunner's ulcer	2	0
- Trigonitis	1	1
- Suspected cystitis (mucosal erythema)	5	0
- Cystitis cystica	2	1
- Low-grade papillary urothelial carcinoma	0	1

Data are presented as n (%) unless stated otherwise. SD, standard deviation.
*Patient with otitis media with effusion (n=1), patient with unchanged audiogram (n=1).
**Patient with repeatedly undetectable tobramycin levels who was also treated with intravenous vancomycin and declined a follow-up audiogram (n=1). [#]painful catheterisation (n=1); cold sensation after instillation (n=1); mild headache during first day of instillation (n=1). [##]Cystoscopy results pending (n=9), refused by patient (n=16), not performed due to the early termination of instillation or loss to follow-up (n=9).

DISCUSSION

Our study demonstrates that intravesical gentamicin is a practical, safe, and feasible prophylactic treatment option in patients with rUTIs refractory to conventional measures. The mean UTI frequency reduced by 79% during the 6 months of treatment and the use of systemic antibiotics was avoided completely in 56% of the patients during gentamicin instillation.

Treatment with gentamicin instillations had a positive effect on the antimicrobial susceptibility of the uropathogens causing breakthrough UTIs, since the percentage of MDR pathogens dropped from 78% to 24%. No increase in the number of gentamicin-resistant uropathogens was observed. The observed decrease in the antibiotic resistance in the breakthrough UTIs after the start of gentamicin instillations may be attributed to the decrease in the overall oral antibiotic use. Breakthrough infections were managed by extra gentamicin instillations in nearly a quarter of the UTI episodes, and because of lower antimicrobial resistance, the majority of UTI episodes were successfully managed with oral antibiotics.

Positive effects of intravesical gentamicin were also observed in patients with gentamicin-resistant uropathogens before treatment (16%), possibly because the high concentration of gentamicin instilled directly in the bladder (80 mg gentamicin in 20 mL of saline=4000 mg/L) was well above the MIC of uropathogens with lower susceptibility (MIC breakpoint according to EUCAST >4 mg/L, based on reachable serum concentrations).[14] In the case of high-level resistance, gentamicin is no longer active, irrespective of concentration. This enzymatic degradation was avoided by using an alternative aminoglycoside, such as tobramycin (n=3) or amikacin (n=1).

No systemic absorption was observed and no ototoxicity or other relevant side-effects were noted. Bladder instillations were also well-accepted by patients inexperienced in self-catheterisation (41%). The number of UTIs in the prospective study was higher than in the retrospective cohort. This difference could be attributed to the higher number of patients in the retrospective cohort using daily instillations, compared to those in the prospective study who used a tapering protocol.

Five out of eight patients with clinical failure and all those with microbiological failure had alternative diagnoses of urinary complaints, or cause of persistent bacteriuria (e.g. chronic bacterial prostatitis or infected kidney stones).

Intravesical gentamicin can also be used for diagnostic purposes to localise the site of bacterial persistence. In patients with persistent gentamicin-sensitive bacteriuria between instillations, a source in the upper urinary tract is likely, and further analysis should be performed, including imaging of the urinary tract and urine cultures obtained by selective sampling of both ureters. There are some concerns regarding local toxic effects after the intravesical instillation of gentamicin. One patient was diagnosed with low-grade papillary urothelial carcinoma during the follow-up cystoscopy; a causative relationship with gentamicin instillation is unlikely. Aminoglycosides do not provoke inflammation, and instillation into the pleural space, abdominal cavity or cerebrospinal fluid causes no irritation.[15]

After intravenous administration, gentamicin is excreted by glomerular filtration, almost entirely in the active form. The urine concentration varies inversely with urinary volume and in oliguric patients, high concentrations (500–1000 mg/L) have been observed.[16]

Direct intravesical administration of aminoglycosides has been described before [8]. Most of those previous clinical trials did not differentiate between UTI and asymptomatic bacteriuria. However, three studies performed with intravesical gentamicin showed a significant reduction in the prevalence of bacteriuria and UTIs among selected patients.[17-19] Although systemic absorption was not detected in some studies,[18,19] caution is still warranted as ototoxicity has been reported following bladder irrigation with neomycin through indwelling catheters.[20,21] In patients with end-stage renal disease, serum neomycin levels were not monitored. We excluded patients with end-stage renal failure.

Two recently published retrospective case series on prophylactic gentamicin bladder instillations reported a reduction in the frequency of UTI occurrence, with few side-effects.[9,22] Although Cox et al. used once-daily instillations with a lower gentamicin concentration, their results are comparable to those of our study. Additionally, a reduced rate of drug resistance (58% to 47%) of uropathogens was observed, without an increase in gentamicin resistance in the urine cultures.

Ours is the first prospective study, and the largest cohort of patients treated with gentamicin instillations aimed at preventing rUTIs. A limitation of our study is that, due to the lack of control participants we compared the prevalence of UTI after treatment with the self-reported UTI frequency before; this may have led to overestimation. Another limitation is that not all the UTIs were culture-confirmed.

Before starting gentamicin instillation, the rectal swabs of 15% of the patients showed MDR bacteria at levels that were about 3× higher than those in the general Dutch population, as expected in individuals frequently exposed to antibiotics.[23] The intestinal carriage of MDR Enterobacteriaceae remained the same (16%, Table S2) after the instillations, but as the resistance rate of uropathogens causing breakthrough infections dropped, there might have been a quantitative reduction that remained below the detection level.

CONCLUSIONS

The emergence of antimicrobial resistance and the increase in the number of rUTI patients without oral treatment options underline the urgent need for alternative treatment in daily patient care. In patients with UTIs caused by MDR microorganisms, with limited or no oral treatment options, intravesical gentamicin instillation may be valuable.

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SUPPLEMENTARY MATERIAL

Table S1. Resistance of the causative uropathogen before gentamicin instillation

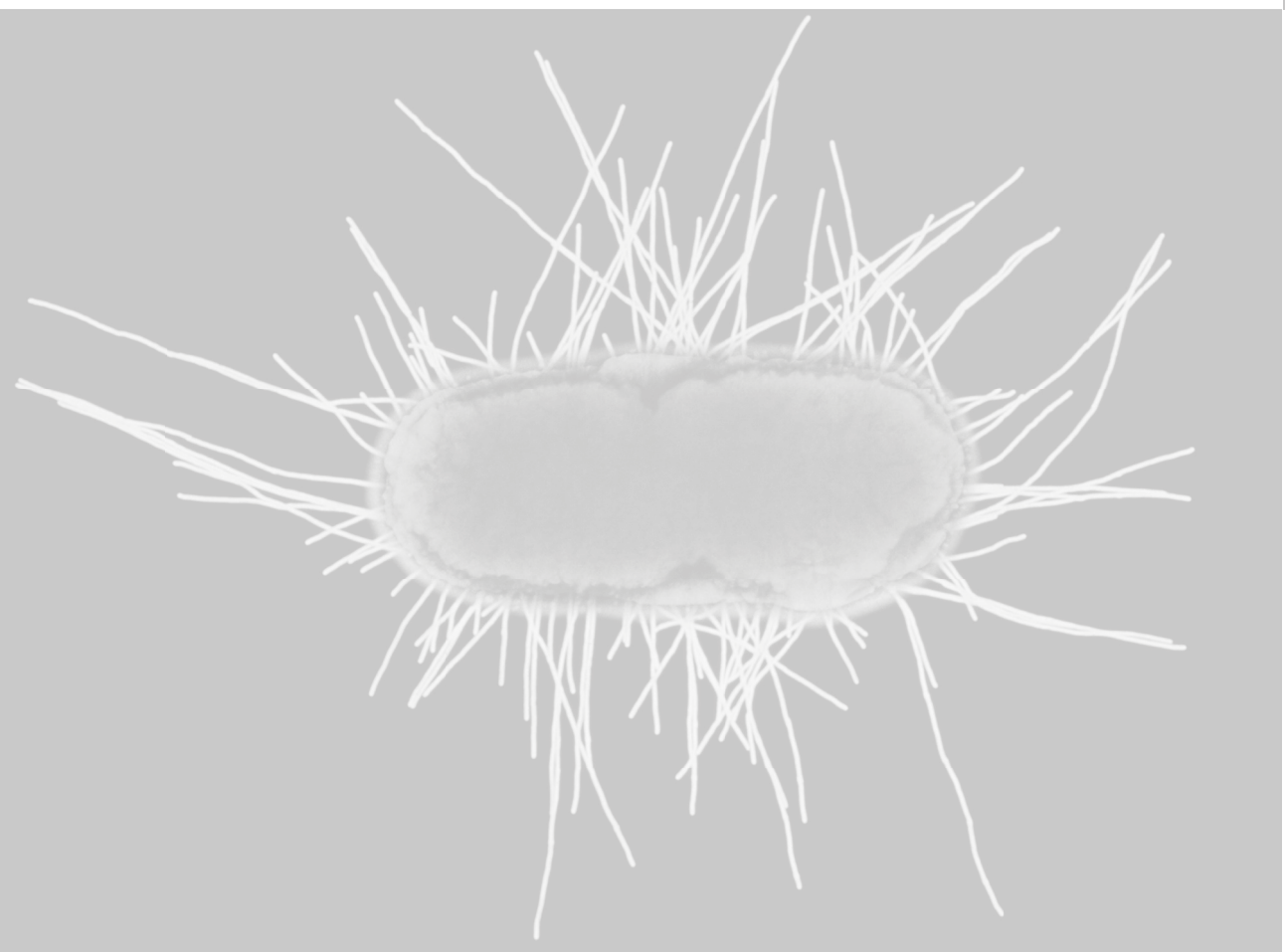
Causative pathogens in the preceding UTI resistant to	Prospective study n = 63	Retrospective cohort n = 27	Total n = 90	p
Amoxicillin	47/56 (84)	24/26 (92)	71/82 (87)	ns
Amoxicillin-clavulanic acid	30/55 (54)	21/26 (81)	51/81 (63)	0.03
Ciprofloxacin	38/62 (61)	18/26 (69)	56/88 (64)	ns
Trimethoprim-sulfamethoxazole	36/61 (59)	21/26 (81)	57/87 (65)	ns
Nitrofurantoin	24/60 (40)	11/25 (44)	35/85 (41)	ns
Fosfomycin	18/56 (32)	4/15 (27)	22/71 (31)	ns
Cefuroxim	26/61 (42)	15/26 (58)	41/87 (47)	ns
Ceftazidim	15/37 (40)	8/21 (38)	23/58 (40)	ns
Gentamicin	8/57 (14)^	5/23 (22)^^	13/80 (16)	ns
ESBL positive	15 (24)	8 (30)	23 (26)	ns
NDM-1 positive	1 (2)	0	1 (1)	ns

Data are presented as n (%) unless stated otherwise. UTI, urinary tract infection; ESBL, Extended-spectrum beta-lactamase; NDM-1, New Delhi Metallo-beta-lactamase; MIC, minimum inhibitory concentration.
^MIC 16 µg/ml (n=1); MIC 32 µg/ml (n=2); MIC 256 µg/ml (n=1); resistant: MIC >4 mg/L (n=4). ^^Intermediate: MIC 2-4 mg/L (n=2); resistant: MIC >4 mg/L (n=3).

Table S2. Microbiology after the start of gentamicin instillation

Microbiology after the start of instillation	Prospective study n=63	Retrospective cohort n=27	Total n=90
Causative uropathogens during 6 months of instillation	**n=60**	**n=19**	**n=79**
Escherichia coli	38 (63)	13 (68)	51 (65)
Enterococcus faecalis	8 (13)	4 (21)	12 (15)
Klebsiella pneumoniae	7 (12)	1 (5)	8 (10)
Candida albicans	0	1 (5)	1 (1)
Other Gram-negatives	3 (5)*	0	3 (4)*
Other Gram-positives	4 (7)**	0	4 (5)**
Antimicrobial resistance			
Multidrug resistance of uropathogens during instillations	14/60 (23)	5/19 (26)	19 (24)
Patients with gentamicin resistance after start instillations	9 (14)	4 (15)	13 (14)
- symptomatic UTI with gentamicin-resistant uropathogen	6 (9)	3 (11)	9 (10)^
- colonization with gentamicin-resistant bacteria without symptoms	3 (5)	1 (4)	4 (4)
Multidrug-resistant pathogen in rectal swab after 6 months of instillations	7/44 (16)	-	-
Multidrug-resistant pathogen in rectal swab after 6 months of follow-up	5/21 (24)	-	-

Data are presented as n (%) unless stated otherwise. UTI, urinary tract infection; MIC, minimum inhibitory concentration
*Enterobacter aerogenes (n=1); *Pseudomonas aeruginosa* (n=1); *Proteus mirabilis* (n=1). **Staphylococcus saprophyticus* (n=1); B-haemolytic streptococcus (n=2); *Aerococcus urinae* (n=1).
^Three of these patients had a gentamicin-resistant microorganism before the start of treatment in the rectal swab (n=1, MIC >4) or urine (n=2, MIC 16 and 256, this last patient was treated with amikacin).



CHAPTER 7

Fecal microbiota transfer for multidrug resistant Gram-negatives; a clinical success combined with microbiological failure

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ABSTRACT

Combined fecal microbiota transfer (FMT) and antibiotic treatment prevented recurrences of urinary tract infections with multidrug resistant (MDR) *P. aeruginosa*, but failed to eradicate intestinal colonization with MDR *E. coli*. Based on microbiota analysis, failure was not associated with distinct diminished microbiota diversity.

INTRODUCTION

Multidrug resistance (MDR) of *Enterobacteriaceae* is an increasing worldwide problem that challenges the treatment of common bacterial infections. MDR has been declared one of the greatest challenges to global public health today, and innovative strategies for decolonization of MDR bacteria are urgently needed to reduce the use of reserve antibiotics and prevent transmission.[1] A few reports mention success with fecal microbiota transfer (FMT) to eliminate extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae*. Failures have not been reported. We present a 34-year old patient on peritoneal dialysis, treated with FMT to eradicate a Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *Pseudomonas aeruginosa* causing recurrent urinary tract infections, which hampered planned kidney-pancreas transplantation. Microbiome analysis was performed prior to and after infusion of fecal microbiota.

Case description

A 34-year old male with type 1 diabetes mellitus was referred to our tertiary hospital because of diabetic nephropathy. Screening for combined kidney pancreas transplant started. Two months after starting hemodialysis, he was admitted because of bacteremia and catheter related thrombophlebitis of the brachiocephalic vein by *Staphylococcus aureus*, which was treated with flucloxacillin for 6 weeks. Because the extensive thrombosis prohibited shunt or catheter placement, he was converted to peritoneal dialysis (PD). During admission, a transurethral catheter was placed because of neurogenic bladder dysfunction. Shortly after discharge he returned to our hospital with a febrile catheter-related urinary tract infection (UTI) and was treated empirically with ceftazidim. Urinary cultures were positive with a bla_{VIM} carbapenemase producing *P. aeruginosa*, resistant to carbapenems, cephalosporins, quinolones, aminoglycosides and fosfomycin, only susceptible to colistin with a MIC of 4mg/L. The same *P. aeruginosa* was isolated from a rectal swab and the PD-catheter exit site. The patient received colistin intravenously (IV) for 2 weeks and the urinary and PD catheter were replaced. In the following months, the patient suffered from recurrent febrile UTIs due to the MDR *P. aeruginosa* (details on antibiotic use shown in Figure 1). Because of the high likelihood of recurrence of UTI caused by this MDR organism for which the only antibiotic was nephrotoxic, kidney transplantation was considered contraindicated and the patient was removed from the waiting list. During colistin treatment of the third episode, a plan for decolonization was developed.

The transurethral catheter was removed and intermittent catheterization with twice weekly prophylactic intravesical high dose gentamicin instilments was started. Repeated negative cultures of urine, PD catheter-skin interface, skin, ears and throat excluded chronic prostatitis or colonization at other sites than the gut. No oral selective digestive decontamination was given. After consultation with our ethics committee, informed consent was obtained from the patient for treatment with fecal microbiota. Six weeks after the last IV course of colistin, the infusion of FMT was performed.

MATERIAL & METHODS

Donor feces infusion was performed using the support of the National Donor Feces Bank (http://www.ndfb.nl/) according to the FECAL trial protocol with minor modifications.[2] In summary, donor feces was obtained from an unrelated healthy volunteer. Donor serum and feces were extensively screened for fecal and blood transmitted diseases including MDR bacteria. 75 gram of feces was homogenized with saline, and sieved (300µm mesh) to remove undigested food fragments. Within 8 hours after defecation of the donor, 300ml fecal suspension was infused in the duodenum of the patient through a nasoduodenal tube, after full colon lavage. Stool samples were collected prior to infusion, after 1 week, 2 weeks, 1 month, 2 months and 3 months and screened for MDR presence using selective enrichment media, as described previously.[3] A portion of the feces was stored within 4 hours after delivery at -80°C for microbiome research. To assess the relatedness of bacterial strains, Amplified Fragment Length Polymorphism (AFLP) technique was performed as described previously.[4]

Microbiota analysis

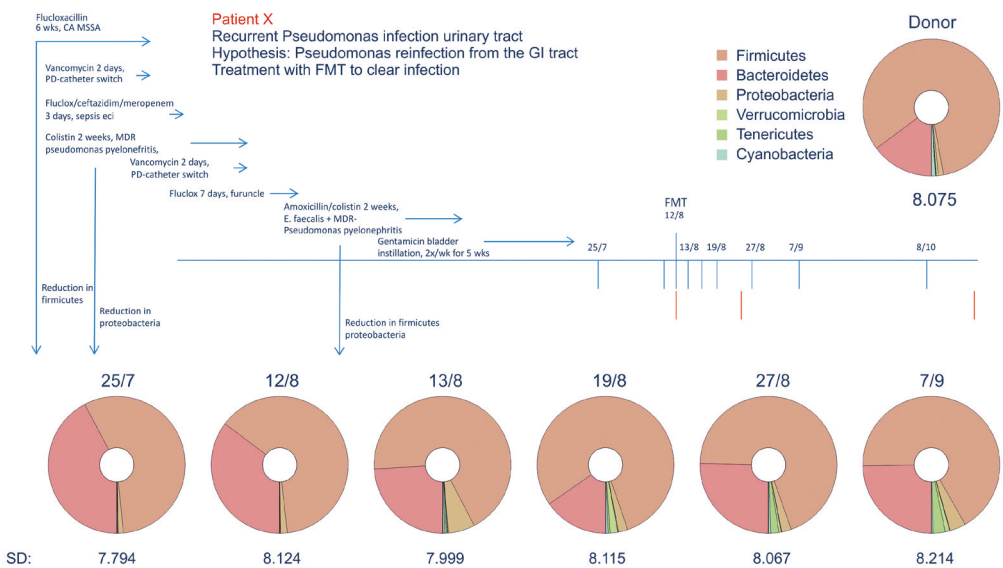
Bacterial DNA was isolated from the fecal samples using the ZR Fecal DNA MiniPrep kit (Zymo Research). Library preparation and amplification of the V4 hyper variable region 16S rRNA gene was performed using NEXTflex 16S V4 Ampliconseq kit v2.0. High-throughput sequencing was executed at ServiceXS (Leiden, the Netherlands) on the Illumina Hiseq 2500 platform (Illumina, San Diego, USA) in rapid run mode paired-end 250 base pairs read length. Raw sequences were processed and analysed using the open-source bioinformatics pipeline QIIME 1.9.1 (http://qiime.org/), the Operational Taxonomic Unit were picked using the open-reference protocol. Subsequently, microbiota profiles were reported at phylum level and visualized using the visualization tool Krona.[5]

RESULTS

No adverse event occurred during or after the infusion of microbiota, other than loose stools for 3 days. The stool culture taken prior to FMT was negative for the MDR *P. aeruginosa*, but did contain an ESBL producing *Escherichia coli*, susceptible to carbapenems, gentamicin, piperacillin/tazobactam and colistin. Subsequently 5 stool cultures up to 3 months of follow up remained negative for *P. aeruginosa*, however persisted in containing the ESBL producing *E. coli*. The *E. coli* post-FMT was identical to the *E. coli* found prior FMT, using AFLP. No infectious complications caused by *P. aeruginosa* were noted during 18 months of follow up. However, the patient was treated once with trimethoprim-sulfamethoxazole for cystitis caused by an ESBL positive *E. coli* 8 months after FMT. Unfortunately, this strain was not available for AFLP analysis.

16S analysis of the patient's stool 19 and 1 days prior FMT revealed a diverse microbiota composition, i.e., high Shannon diversity index of 7.8 and 8.1 respectively. No significant changes in microbiota diversity of the recipient were observed following the FMT (figure 1). At phyla level a high similarity of donor and recipient microbiota was observed with respect to the *Firmicutes* and *Bacteroidetes* as the expected main phyla of the microbiota (figure 1).

Figure 1. Timeline of recurrent infections, antibiotic use, and microbiota diversity prior to and after fecal microbiota transfer.



CA MSSA: catheter related bacteremia with methicillin sensitive *Staphylococcus Areus*. MDR: multidrug resistant. PD: peritoneal dialysis. FMT: fecal microbiotica transfer.

DISCUSSION

A 34-year old patient on peritoneal dialysis and recurrent urinary tract infections with a VIM-positive *P. aeruginosa* was treated with infusion of fecal microbiota to eradicate *P. aeruginosa* from the intestinal tract. A clinical success was observed, since at a follow-up period of 18 months no recurrent infections by *P. aeruginosa* were diagnosed. FMT may have contributed to clinical success but it cannot be excluded that MDR *P. aeruginosa* was already eradicated from the gut before FMT, as the *P. aeruginosa* could not be cultured the day before FMT.

A remarkable observation is the persistence of an ESBL positive *E. coli* after FMT. The *E. coli* was presumably acquired after eradication treatment for *P. aeruginosa,* since it had not been detected in earlier cultures. It is possible that the incomplete eradication of the MDR *E. coli* is the result of coexistence of donor and patient *E. coli* strains after FMT. A recent study showed this coexistence of donor and recipient strains, which persisted for at least 3 months after FMT for treatment of patients with metabolic syndrome.[6] This suggests that novel strains, acquired via FMT, can colonize the gut without replacing the indigenous strain population of the recipient.

In contrast to the diminished microbiota of recurrent CDI patients, our patient had an intact microbiota diversity and composition at phylum level prior to FMT. Previous antibiotic treatment (Figure 1) had not resulted in a distinct disturbance of the intestinal flora. Only minor changes of the microbiota composition were observed after FMT with a slight increase of cyanobacteria and tenericutes. We suggest that diminished diversity appears not to play a role in MDR carriership as opposed to recurrent CDI.[7] Therefore, one might question the efficacy of fecal transplantation in patients with a normal microbiota diversity. The disturbed microbiota and its recovery after FMT might explain the positive results of MDR eradication in patients with recurrent CDI.[8,9] Interestingly, a recent paper showed that infusion of fecal microbiota in patients with recurrent CDI decreased the number and diversity of antimicrobial resistance genes, particularly by restoring dysbiosis and reducing the number of *Proteobacteria*.[10] Furthermore, beneficial effect of microbiota transfer has been shown in mice colonized with vancomycin resistant *Enterococcus* (VRE) [11]. Clearly, more research on FMT for eradication of colonization of different MDR bacterial species is required.

A total of only eight case reports have been published, showing FMT resulted in intestinal decolonization of ESBL- and carbapenemase-producing Enterobacteriaceae, VRE, or methicillin-resistant *Staphylococcus aureus*.[12-15] Unfortunately, no information has been provided on microbiota composition before and after transplantation. Five trials are currently underway regarding the use of FMT for MDR bacterial decolonization which should provide more insight on the role of the microbiota on colonisation with specific microorganisms.[12]

A limitation of our analysis is that the microbiota was determined by 16S analysis. Although very useful in bacterial taxonomic classification, it lacks the required resolution to track transmission of bacterial strains in the microbiota using single-nucleotide variants in metagenomes.[6] Therefore, it was not possible to compare the composition of the microbiota at strain level, allowing a comparison between the donor and patient *P. aeruginosa* strains. However, no VIM-gene was

detected by PCR on DNA from three feces samples after FMT.

In conclusion, combined FMT and antibiotic treatment prevented recurrence of UTI with MDR *P. aeruginosa*. Intestinal colonization with ESBL producing *E. coli* persisted in the presence of a microbiota with intact diversity, suggesting that eradication of *E. coli* requires perhaps other specific strain(s) of microbes. More detailed analysis such as metagenomics, could identify specific strains that add to decolonization and should be applied in current studies on FMT for intestinal eradication of different MDR bacterial species.

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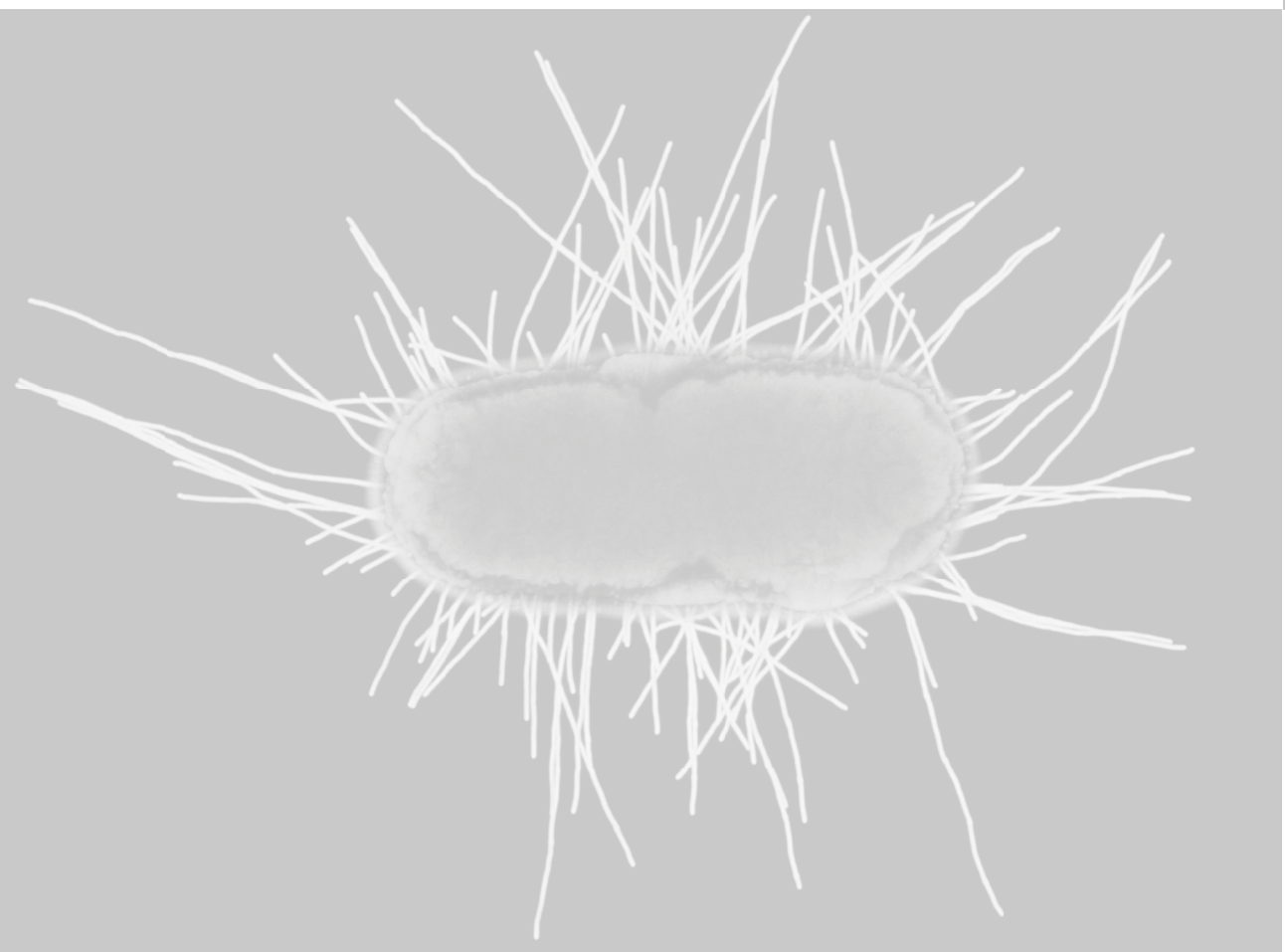

CHAPTER 8

Comparative virulotyping of extended-spectrum cephalosporin-resistant *E. coli* isolated from broilers, humans on broiler farms and in the general population and UTI patients

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ABSTRACT

During the last decade extended-spectrum cephalosporin (ESC)-resistant *Escherichia coli* from food producing animals, especially from broilers, have become a major public health concern because of the potential transmission of these resistant bacteria or their plasmid-encoded resistance genes to humans.

The objective of this study was to compare ESC-resistant *E. coli* isolates from broilers (n = 149), humans in contact with these broilers (n = 44), humans in the general population (n = 63), and patients with a urinary tract infection (UTI) (n = 10) with respect to virulence determinants, phylogenetic groups and extended-spectrum b-lactamase (ESBL)/plasmidic-AmpC (pAmpC) genes. The most prevalent ESBL/pAmpC genes among isolates from broilers and individuals on broiler farms were $bla_{\text{CTX-M-1}}$, $bla_{\text{CMY-2}}$ and $bla_{\text{SHV-12}}$. In isolates from humans in the general population $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ were found most frequently, whereas in UTI isolates blaCTX-M-15 predominated. The marker for enteroaggregative *E. coli*, *aggR*, was only identified in a broiler and human isolates from the general population. The extraintestinal virulence genes *afa* and *hlyD* were exclusively present in human isolates in the general population and UTI isolates. Multivariate analysis, based on ESBL/pAmpC resistance genes, virulence profiles and phylogenetic groups, revealed that most UTI isolates formed a clearly distinct group. Isolates from broilers and humans associated with broiler farms clustered together. In contrast, isolates from the general population showed some overlap with the former two groups but primarily formed a separate group. These results indicate than transmission occurs between broilers and humans on broiler farms, but also indicate that the role of broilers as a source of foodborne transmission of ESC-resistant *E. coli* to the general population and subsequently causative agents of human urinary tract infections is likely relatively small.

INTRODUCTION

Escherichia coli is generally considered a beneficial commensal of the gastrointestinal tract of humans and animals. Certain *E. coli* strains, however, can cause community-acquired infections, including urinary tract infections (UTIs).[1]

Acute, uncomplicated UTI is one of the most common bacterial infections seen in general practice with an incidence of 70 per 1000 women each year in the Netherlands [1]. Between 70–95% of UTIs are caused by uropathogenic *E. coli* (UPEC) [2]. UPEC belong to the broader group of extraintestinal pathogenic *E. coli* (ExPEC), which possess specific virulence traits allowing them to colonize environments other than the gastrointestinal tract, such as the urogenital tract.[3] Some ExPEC isolates from humans have similar virulence factors as *E. coli* isolated from food-producing animals and therefore it has been postulated that a proportion of human UTI is caused by ExPEC strains originating from these animals. Recent studies have suggested that animals and food may be a source of antimicrobial-resistant extraintestinal pathogenic *E. coli* isolates.[4-7]

The prevalence of extended-spectrum b-lactamase (ESBL)- and pAmpC b-lactamase-producing *E. coli* among Dutch broilers is high and broilers may therefore form a reservoir from where spread of these resistant bacteria or ESBL/pAmpC encoding resistance genes to humans may occur.[8] [9, 10] Transmission of extended-spectrum cephalosporin (ESC)-resistant *E. coli* from broilers to humans through the food chain has been proposed [11] [12, 13]. It has been demonstrated that close contact between humans and broilers on broiler farms increased the risk of carrying ESC-resistant *E. coli*.[9]

Transmission of ESC-resistant *E. coli* or their resistance genes between animals and humans may form a direct public health threat when dealing with pathogenic types. The aim of this study was to compare ESBL/pAmpC resistance genes, virulence determinants and phylogenetic groups from ESC- resistant E. coli isolates from broilers, humans working or living on broiler farms, humans in the general Dutch population and patients with urinary tract infection (UTI) in order to investigate potential public health implications.

2. MATERIAL AND METHODS

2.1. Isolate origin

The ESC-resistant broiler isolates (n = 149) originated from two studies on conventional (n = 98) and organic (n = 51) broiler farms in The Netherlands.[9, 10] Broiler isolates were included when ESC-resistant *E. coli* from individuals living and/or working on these farms were also available. Multiple broiler isolates from one farm were selected based on as many different resistance genotype and phylogenetic group as possible with a maximum of three isolates with an identical ESBL/pAmpC gene and phylogenetic group per farm. ESC-resistant isolates from individuals living and/or working on these farms (n = 44) originating from conventional (n = 35) or organic (n = 9) broiler farms were also included.[9, 10] In addition, ESC-resistant isolates from individuals in the general

population living in areas with either a high and low broiler density (n = 63) were investigated.[14] Isolates from febrile UTIs (n = 10) were obtained from two hospitals in the Netherlands.

2.2. Molecular characterization

The ESBL/pAmpC genes and phylogenetic groups of all isolates from broilers and humans, except for those from febrile UTIs, had been determined in previous studies.[9, 10, 14] The phylogenetic groups of the UTI isolates were investigated according to Doumith et al. and they were subgrouped as described by Escobar-Páramo et al.[28, 15] The ESBL or pAmpC encoding genes of the UTI isolates were characterized as described by van Hoek et al. (2015).[14] Multi locus sequence typing (MLST) had been conducted for 130 isolates, while the ESC-resistance plasmids had been identified for 115.[9, 10, 14] MLST of the UTI isolates was performed according to Wirth et al.[16]

Analysis of the virulence factors was performed by PCR targeting the aggregative virulence regulator (*aggR*) of enteroaggregative *E. coli* (EAEC) as described by Boisen et al.[17] In addition, several markers were selected to identify ExPEC; i.e. afimbrial adhesion (*afa*), type 1 fimbriae (*fimH*), F1C fimbriae (*focG*), cytolytic protein toxin (*hlyD*), increased serum survival (*iss*), iron acquisition system (*iutA*), group 2 polysaccharide capsule (*kpsMII*), P fimbriae (*papA*) and S fimbriae (*sfaS*). The PCR primers and protocols applied to identify these ExPEC genes were described by Franz et al.,[18] with the exception of *fimH* and *iss*. The *fimH* PCR originated from Dias et al.,[19] while the following two primers were used to amplify a nearly complete *iss* gene; iss_39F: 50-cgctctggcaatgcttattac-30 and iss_285R: 50-ttccagcggagtataaatgcc-30. Both primers were also used to sequence this virulence determinant by BaseClear B.V. (Leiden, the Netherlands). The obtained alleles were typed according to Johnson et al.[4]

2.3. Statistical analysis

Differences in frequencies of ESC-genotypes, phylogenetic groups and virulence markers among groups was evaluated using chi-squared tests (x2) on contingency tables with a significance level of P = 0.05. Univariate analysis of variance was performed for inference on differences in average numbers of virulence markers between ESBL-genotypes and phylogenetic groups. Analyses were performed in IBM SPSS Statistics version 19. Principal component analysis and construction of a scatterplot was calculated from the ESBL-genotypes, the presence/absence of the different virulence genes, and the origin of the isolate in SAS studio 3.4.

Table 1 ESC genotypes of all isolates studied.

Resistance genotype	Broiler isolates (n = 149 (%))[c]	Human isolates, broiler farm (n = 44 (%))	Human isolates, general population (n = 63 (%))	UTI isolates (n = 10 (%))	Total (n = 266 (%))
CTX-M-1 62	62 (42)	12 (27)	16 (25)	1 (10)	91 (34)
CMY-2	55 (37)	18 (41)	3 (4.8)		76 (29)
SHV-12	17 (11)	7 (16)	2 (3.2)		26 (9.8)
CTX-M-15			13 (21)	6 (60)	19 (7.1)
TEM-52	12 (8.1)	2 (4.5)	1 (1.6)		15 (5.6)
Promoter mutation		4 (9.1)	9 (14)		13 (4.9)
CTX-M-14	1 (0.7)		9 (14)	1 (10)	11 (4.1)
CTX-M-2	2 (1.3)	1 (2.3)	3 (4.8)		6 (2.3)
CTX-M-3			2 (3.2)		2 (0.8)
CTX-M-24			2 (3.2)		2 (0.8)
CTX-M-32	1 (0.7)		1 (1.6)		2 (0.8)
CTX-M group 8[a]				1 (10)	1 (0.4)
CTX-M-9	1 (0.7)				1 (0.4)
CTX-M-27			1 (1.6)		1 (0.4)
DHA-1			1 (1.6)		1 (0.4)
Unknown[b]				1 (10)	1 (0.4)
Total	**151**	**44**	**63**	**10**	**266**

Note: [a]Unfortunately the sequencing reaction failed to identify the allele. [b]This UTI isolate, displaying an AmpC phenotype had an unknown resistance genotype because the CMY and DHA PCR screening was negative. Presumably this isolate has a promoter mutation, but this was not further investigated. [c]Two broiler isolates harboured two ESBL genes, i.e. $bla_{CTX\text{-}M\text{-}1}$ & $bla_{CTX\text{-}M\text{-}9}$ and $bla_{CTX\text{-}M\text{-}1}$ & $bla_{SHV\text{-}12}$, consequently, the total number of genes is larger than the number of isolates.

3. RESULTS AND DISCUSSION

3.1. Distribution of ESBL/pAmpC genes, phylogenetic groups and virulence genes

Fourteen different ESBL/pAmpC genes were identified among the 266 *E. coli* investigated (Table 1). The most prevalent ESBL/ pAmpC genes among isolates from broilers were $bla_{CTX\text{-}M\text{-}1}$ (42%), $bla_{CMY\text{-}2}$ (37%) and $bla_{SHV\text{-}12}$ (11%). The ESBL/pAmpC gene distribution among *E. coli* isolates from humans living/working on broiler farms resembled those from the broilers; the most common genes were $bla_{CMY\text{-}2}$ (41%), $bla_{CTX\text{-}M\text{-}1}$ (27%) and $bla_{SHV\text{-}12}$ (16%). In contrast, the gene distribution among isolates from individuals in the general population was different: $bla_{CTX\text{-}M\text{-}1}$ (25%), $bla_{CTX\text{-}M\text{-}15}$ (21%) and $bla_{CTX\text{-}M\text{-}14}$ (14%) were the predominant genotypes. The diversity in ESBL/pAmpC genes among human isolates from the general population was larger than among those from humans associated with broiler farms and broilers. Interestingly, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}14}$ were only observed among UTI isolates (60% and 10%, respectively) and isolates obtained from the

general population (21% and 14%, respectively), but not in isolates from humans associated with broiler farms. In addition, $bla_{CTX\text{-}M\text{-}15}$ was also absent among broiler isolates, while $bla_{CTX\text{-}M\text{-}14}$ was found in only one broiler isolate (0.7%) (Table 1).

All phylogenetic groups and subgroups were found among isolates from broilers, humans on broiler farms and individuals in the general population (Table 2). Notable was the predominance of phylogenetic group B2 among UTI isolates, however, this might partly be due to the small number investigated here. The fact that all phylogenetic groups and subgroups were found among isolates from broilers, humans on broiler farms and individuals in the general population indicates that ESC-resistance is acquired by *E. coli* from all phylogenetic groups. Consequently, this complicates source attribution of ESC-resistance as horizontal transfer of ESBL/ pAmpC genes among *E. coli* seems to be a common event.

Screening for the presence of ten virulence genes revealed that the F1C and S fimbriae determinants *focG* and *sfaS*, respectively, were not identified in any of the investigated isolates, while the type 1 fimbrial gene *fimH* was shown to be present in nearly all isolates (92%, Table 3). The extraintestinal virulence markers *iss, iutA*, *kpsMII*, and *papA* were found in all groups of isolates investigated, whereas *afa* and *hlyD* were exclusively observed among human isolates from the general population and UTI isolates (Table 3). The aggregative virulence regulator *aggR* was also demonstrated in only two groups of isolates studied, i.e. in four human isolates from the general population and one broiler isolate. Isolates were classified as potential ExPECs based on the presence of two or more of the ExPEC-defining markers *afa*, *focG* and/or *sfaS*, *iutA*, *kpsMII* and *papA*.[20]

Table 2 Phylogenetic (sub)group distribution of the ESC-resistant *E. coli* isolates.

Phylogenetic (sub)group[a]	Broiler isolates (n = 149 (%))	Human isolates, broiler farm (n = 44 (%))	Human isolates, general population (n = 63 (%))	UTI isolates (n = 10 (%))	Total (n = 266 (%))
A	**46 (31)**	**17 (39)**	**20 (32)**	**1 (10)**	**84 (32)**
A_0	29 (20)	9 (21)	5 (7.9)	-	43 (16)
A_1	17 (11)	8 (18)	15 (24)	1 (10)	41 (15)
B1	**33 (22)**	**6 (14)**	**16 (25)**	**-**	**55 (21)**
B2	**19 (13)**	**9 (21)**	**10 (16)**	**7 (70)**	**45 (17)**
$B2_2$	1 (0.7)	3 (6.8)	2 (3.2)	1 (10)	7 (2.6)
$B2_3$	18 (12)	6 (14)	8 (13)	6 (60)	38 (14)
D	**51 (34)**	**12 (27)**	**17 (27)**	**2 (20)**	**82 (31)**
D_1	10 (6.7)	4 (9.1)	6 (9.5)	-	20 (7.5)
D_2	41 (28)	8 (18)	11 (18)	2 (20)	62 (23)
Total	**149**	**44**	**63**	**10**	**266**

Note: [a]In bold letters phylogenetic groups are indicated, in normal font the subgroups.

Table 3 Prevalence of the virulence genes studied.

Gene	Broiler isolates (n = 149 (%))	Human isolates, broiler farm (n = 44 (%))	Human isolates, general population (n = 63 (%))	UTI isolates (n = 10 (%))	Total (n = 266 (%))
afa	-	-	9 (14)	1 (10)	10 (3.8)
aggR	1 (0.7)	-	4 (6.3)	-	5 (1.9)
fimH	134 (90)	44 (100)	56 (90)	10 (100)	244 (92)
focG	-	-	-	-	-
hlyD	-	-	2 (3.2)	6 (60)	8 (3.0)
iss	121 (81)	35 (80)	34 (54)	8 (80)	198 (74)
iutA	110 (74)	24 (55)	37 (59)	10 (100)	181 (68)
kpsMII	48 (32)	13 (30)	23 (37)	8 (80)	92 (35)
papA	2 (1.3)	2 (4.5)	9 (14)	7 (70)	20 (7.5)
sfaS	-	-	-	-	-
Total	**149**	**44**	**63**	**10**	**266**

Note: A minus sign indicates that a gene was not identified in any of the isolates tested.

Table 4 Matrix of the significant differences in the frequencies of ESBL/pAmpC-genes, phylogenetic groups and virulence markers between the investigated groups of isolates.

	Broiler	Human_broiler farm	Human_general population	UTI
Broiler	-	**Pmut (x2 = 14.5, P = 0.002)**	**CTX-M-14 (x2 = 17.9, P < 0.001)** **CTX-M-15 (x2 = 32.2, P < 0.001)** **Pmut (x2 = 21.9, P < 0.001)** **afa(x2 = 21.9, P < 0.001)** **aggR(x2 = 6.1, P = 0.029)** **papA(x2 = 14.8, P < 0.001)** CMY-2 (x2 = 23.5, P < 0.001) iss (x2 = 18.8, P < 0.001) iutA (x2 = 5.4, P = 0.024)	**B2 (x2 = 22.5, P < 0.001)** **CTX-M-15 (x2 = 92.9, P < 0.001)** **hlyD(x2 = 92.9, P < 0.001)** **kpsMII(x2 = 9.4, P = 0.004)** **papA(x2 = 82.7, P < 0.001)** CMY-2 (x2 = 5.6, P = 0.016)
Human_ broiler farm	NA	-	**CTX-M-14 (x2 = 6.5, P = 0.011)** **CTX-M-15 (x2 = 9.7, P = 0.001)** **afa(x2 = 6.5, P = 0.011)** CMY-2 (x2 = 23.3, P < 0.001) SHV-12 (x2 = 6.0, P = 0.027) iss (x2 = 10.2, P = 0.002)	**B2 (x2 = 8.9, P = 0.006)** **CTX-M-15 (x2 = 28.5, P < 0.001)** **hlyD(x2 = 28.5, P < 0.001)** **iutA(x2 = 6.6, P = 0.01)** **kpsMII(x2 = 8.1, P = 0.009)** **papA(x2 = 24.0, P < 0.001)** CMY-2 (x2 = 6.6, P = 0.01)
Human_ general population	NA	NA	-	**B2 (x2 = 14.45, P = 0.001)** **CTX-M-15 (x2 = 7.1, P = 0.015) hlyD(x2 = 29.0, P < 0.001) iutA(x2 = 6.6, P = 0.001) kpsMII(x2 = 6.9, P = 0.014) papA(x2 = 16.0, P = 0.001)**
UTI	NA	NA	NA	-

Note: Pmut indicates a promoter mutation. The bold versus normal letters display in which particular group of isolates the genetic characteristic occurs at a significant higher frequency.

Potential ExPECs were identified in 33% of the broiler isolates, 27% of the isolates from humans associated with broiler farms, 44% of isolates from humans in the general population, and 90% of the UTI isolates. These percentages indicate that a considerable fraction of these isolates combine pathogenic potential with ESC-resistance.

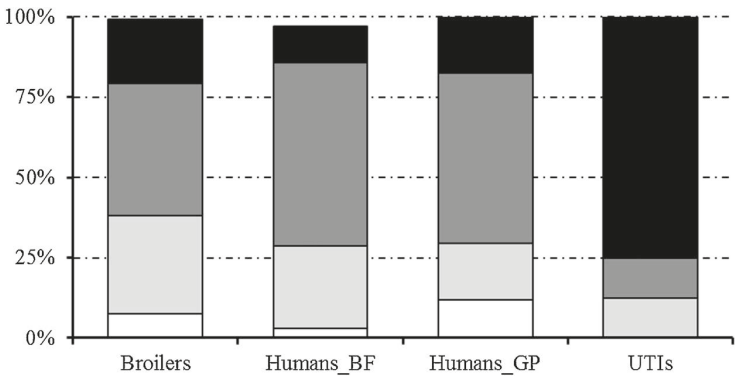
Based on the detection of the *aggR* gene, five ESC-resistant *E. coli* isolates (1 broiler and 4 human isolates) could be classified as EAEC. The general consensus is that humans are the only natural reservoir of EAEC but transmission to other hosts can occur via contaminated food and/or water.[29] Therefore, the EAEC broiler isolate most likely is a transient event and not indicative for a reservoir. Recently, seven out of 170 ESC-resistant *E. coli* from wastewater (2/88) and surface water (5/82) were identified to be positive for *aggR*.[18] Whether these isolates originated from humans or other sources like broilers remains unknown. EAEC are known to cause diarrhoea but have also been associated with UTIs, possibly due to a combination of EAEC (*aggR*) and ExPEC virulence factors like *iutA*.[30, 31] Two EAEC from this study (one broiler and one human general population isolates) were also classified as potential ExPECs.[20]

The increased serum survival gene iss has been recognized for its role in ExPEC virulence due to its association with an increase in complement resistance.[4] In the current study, the prevalence of *iss* was significantly higher among broiler isolates (81%) than among human isolates (45%). In addition, the percentage of *iss*-positive isolates obtained from humans living and/or working on a broiler farm (80%) was significantly higher ($x2 = 10.2$, $P = 0.002$) compared to human isolates from the general population (54%) (see Table 3 and 4). Sequence analysis of the iss amplicons revealed a different distribution of the iss types in broiler and healthy human isolates versus UTI isolates (Fig.1). Most genes could be differentiated in the three known iss allele types, but in 14 isolates the bacteriophage I gene bor was found. This determinant encodes for an outer-membrane lipoprotein involved in serum resistance, which is believed to be the precursor of the iss alleles.[4] Broiler and human (non UTI) isolates predominantly contained iss type 1 and 2, with the latter allele found most often, whereas nearly all iss positive UTI isolates harboured type 3 (Fig. 1). This corresponds with the findings of Johnson et al. (2008) where type 3 occurred frequently among ExPEC isolates. These results imply that different ESC-resistant *E. coli* populations reside in broilers compared to people with an UTI, and therefore clonal transmission of ESC-resistant *E. coli* from broilers to humans does not seem to play a significant role in the epidemiology of human UTIs.

Statistical analysis of the molecular characteristics revealed that $bla_{CTX\text{-}M\text{-}15}$ was strongly associated with the presence of virulence genes; *aggR* ($x2 = 21.5$, $P = 0.003$), *afa* ($x2 = 16.9$, $P = 0.003$), *hlyD* ($x2 = 22.8$, $P = 0.001$), *kpsMII* ($x2 = 10.4$, $P = 0.002$) and *papA* ($x2 = 10.4$, $P = 0.008$). The predominance of $bla_{CTX\text{-}M\text{-}15}$ among ESC-resistant *E. coli* with certain virulence factors in the general population and the occurrence of isolates with this CTX-M allelic variant together with similar virulence factors among clinical human isolates in the Netherlands as well as globally suggests that healthy humans can be a source of these bacteria for vulnerable persons.[13, 21, 22] The occurrence of $bla_{CTX\text{-}M\text{-}15}$ among ESBL-producing *E. coli* is partially associated with the spread of the pathogenic ST131 clone, causing urinary tract and bloodstream infections worldwide.[23-25] In this study 18 *E. coli* ST131 were found, however, only five of them carried $bla_{CTX\text{-}M\text{-}15}$, one human

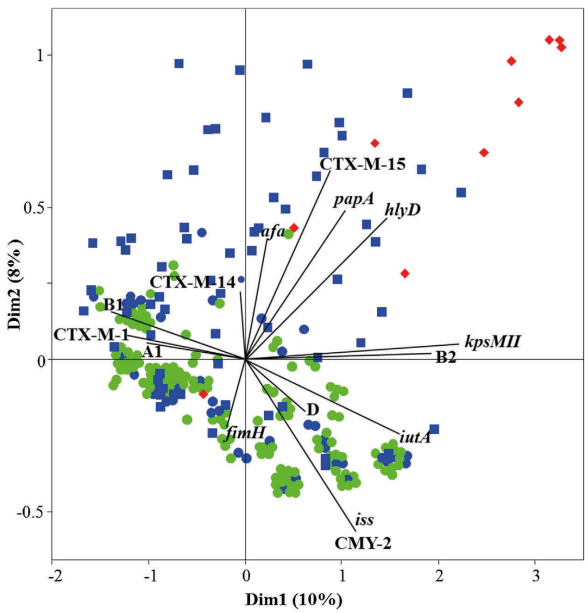
isolate from the general population and four among the UTI isolates tested. The other ST131 isolates obtained from broilers and healthy humans predominantly had different resistance genes, i.e. bla_{CMY-2} and $bla_{CTX-M-1}$ which is indicative of another clonal line.

Fig. 1. Prevalence of the different iss alleles among iss-positive isolates.



Humans_BF represent isolates from humans living and/or working on a broiler farm, Humans_GP indicate isolates from humans in the general population. Because some sequences failed not all prevalences add up to 100% (see Supplementary file). White bars represent bor, light grey iss type 1, dark grey iss type 2, and black iss type 3.

Fig. 2. Principal component analysis scatterplot calculated from the ESBL-genotypes, the presence/absence of the different virulence genes, and the origin of the isolate.



The analysis successfully separated out different clusters primarily based on isolate origin. The length and direction of the lines represent those components which show a contribution to the separation of the first two dimensions (i.e., the explained variance). Green circles represent broiler isolates and blue circles human isolates from individuals living/working on those broiler farms.[9, 10] Blue squares indicate human isolates from the general population[14] and red diamonds the UTI isolates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Multivariate analysis of isolates from different sources

Table 4 shows the ESBL/pAmpC genes, virulence markers and phylogenetic groups that differ significantly in frequency between isolates from broilers, humans living and/or working on a broiler farm and humans from the general population. Isolates from humans in the general population varied from broiler isolates with respect to the frequency of ESBL/pAmpC genes *bla*$_{CMY-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$ and virulence determinants *afa, aggR, iss, iutA* and *papA* (Table 1 and 4). The prevalence of certain genes, i.e. *afa*, *bla*$_{CMY-2}$, *bla*$_{CTX-M-14}$, *bla*$_{CTX-M-15}$, *bla*$_{SHV-12}$, and *iss*, differed significantly between the isolates from humans on broiler farms and those from the general population. The ESBL gene *bla*$_{CTX-M-15}$, virulence markers *hlyD, kpsMII, papA*, and phylogenetic group B2 were strongly associated with UTI isolates and the frequency of occurrence among UIT isolates was different compared to the non-UTI groups (Table 4).

Principal component analysis based on the ESBL/pAmpC genes, virulence determinants and phylogenetic groups revealed that most UTI isolates were distinct from the other three groups investigated (Fig. 2). Isolates from broilers and humans living and/ or working on a broiler farm clustered together. In contrast, isolates from humans in the general population showed some overlap with the former two groups but also formed a separate cluster in the same dimension as the UTI isolates. The separation of UTI isolates and those of humans in the general population from the broiler and broiler farm related human ones was mainly due to the higher frequency of *afa*, *bla*$_{CTX-M-15}$, *hlyD, kpsMII*, *papA*, and phylogenetic group B2 (Fig. 2). Within the groups of broiler isolates and isolates from humans living and/or working on a broiler farm two clusters could be identified. One group was primarily defined by *bla*$_{CTX-M-1}$ or *bla*$_{CTX-M-14}$ and phylogenetic (sub)group B1 or A1, whereas the second one was characterized by ESC-resistance gene *bla*$_{CMY-2}$, phylogenetic group D and virulence determinant *iss* or *iutA*.

Currently, much effort is directed towards determining the contribution of various livestock reservoirs to human colonization and infection with ESC-resistant bacteria. Recent studies demonstrated a high prevalence of ESC-resistance among *E.coli* isolates from broilers[8-10] and high similarity of the b-lactamase encoding genes and *E. coli* genotypes in humans, poultry, and retail poultry products,[11-13] suggesting that broilers are an important source of these bacteria or resistance genes for humans. Poultry meat has been postulated to be a source for human ExPEC based on a certain similarity in phylogenetic backgrounds and virulence genes.[4, 26] The current results showed a more nuanced view. Multivariate analysis displayed a high level of similarity between ESC-resistant *E. coli* from broilers and from humans living and/or working on a broiler farm, confirming that contact with broilers is a risk factor for carriage of these bacteria.[9] In contrast, isolates from humans in the general population and clinical (UTI) isolates differed considerable from broiler isolates with respect to the frequency of ESC-resistance genes as well as virulence genes. Although there is some overlap between broiler isolates and isolates causing UTI, the results challenge the opinion of some researchers that poultry is an important reservoir for human infections by indicating a less strong link between the broiler reservoir and the human general population and clinical cases of urinary tract infection. This is best illustrated

by the relatively high frequency of the ESC-resistance gene $bla_{CTX\text{-}M\text{-}15}$ and virulence genes *afa* and *hlyD* and their absence among broiler isolates.

In order to make a valid estimation of the relative importance of poultry with respect to transmission of ESC-resistance and ExPEC to humans, some major hurdles have to be resolved. First, the level of genetic similarity between isolates and their plasmids from different hosts needs to be studied at a much higher resolution, which can be achieved by employing whole-genome-sequencing (WGS) including plasmid analysis. We propose a multilevel genotyping approach where comparative high resolution typing of strains, plasmids and genes is combined in order to obtain a more complete picture of the complex ESBL attribution problem. Second, conceptual frameworks are necessary to provide means to quantify the frequency and directionality of transmission. Third, improvements are essential in experimental design of studies aiming at source attribution of ESC-resistance and ExPEC.[2] Key is that poultry should not a priori be considered as the main reservoir. *E. coli* with the same resistance genes and virulence factors have been found in several potential reservoirs like other food-producing animals, but also horses and companion animals,[5, 22, 27] therefore, these should not be neglected. In addition, the human general population itself should not be underestimated as a major reservoir.

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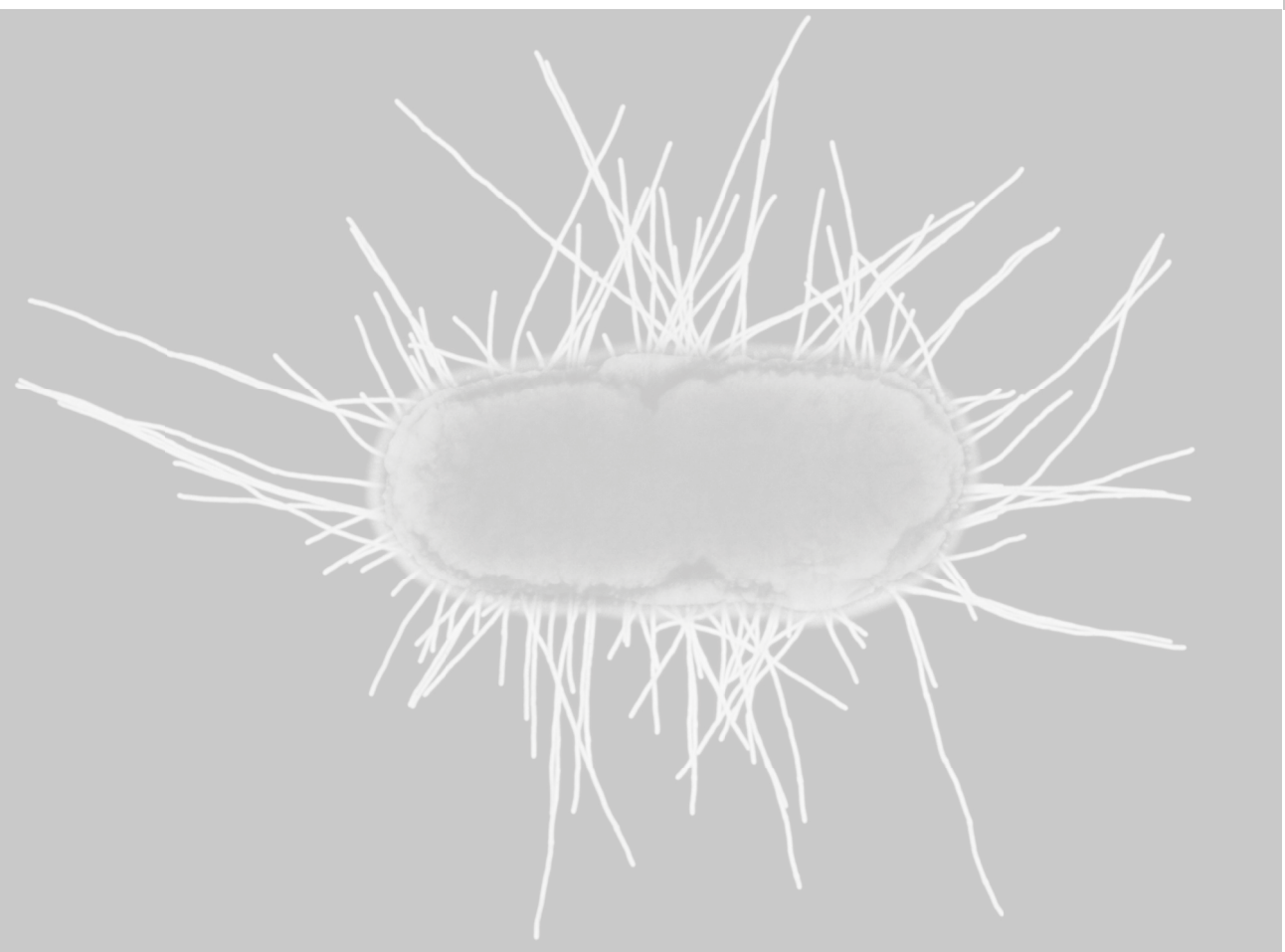

CHAPTER 9

The use of automated urine microscopy analysis in the clinical diagnosis of urinary tract infection; defining an optimal diagnostic score in an academic medical center population

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ABSTRACT

A retrospective case record study was conducted that established a scoring tool based on clinical and iQ200 parameters, able to predict or rule out the clinical diagnosis of UTI in the majority of adult patients in an academic hospital.

Automated standardized quantitative urine analysis, such as iQ200 analysis, is on the rise because of its high accuracy and efficiency compared to those of traditional urine analysis. Previous research on automated urinalysis focused mainly on predicting culture results but not on the clinical diagnosis of urinary tract infection (UTI). A retrospective analysis was conducted of consecutive urine samples sent in for culture because of suspected UTI. UTI was defined by expert opinion, based on reported symptoms, conventional urine sediment analysis, and urine cultures. Parameters of iQ200 analysis and clinical symptoms and signs were compared between cases and controls. Optimal cut-off values were determined for iQ200 parameters, and multivariate logistic regression analysis was used to identify the set of variables that best predicts the clinical diagnosis of UTI for development of a scoring tool. A total of 382 patients were included. Optimal cut-off values of iQ200 analysis were 74 white blood cells (WBC)/µl, 6,250 "all small particles" (ASP)/µl, and a bacterial score of 2 on an ordinal scale of 0 to 5. The scoring tool attributed 1 point for frequent micturition or increased urge, 2 points for dysuria, 1 point for a bacterial score of ≥2, 2 points for WBC/µl of ≥50, and an additional point for WBC/µl of ≥150. This score had a sensitivity of 86% and a specificity of 92% when using a threshold of <4 points. The combination of iQ200 analysis and a simple survey could predict or rule out UTIs in a majority of patients in an academic medical center.

INTRODUCTION

Urinary tract infection (UTI) is among the most frequently occurring infections and is the second most frequent clinical indication for empirical antibiotic treatment in primary and secondary care.[1,2] The gold standard for diagnosis is detection of a pathogen in the urine in the presence of clinical symptoms. Because the result of a traditional urine culture is not readily available, presumptive diagnosis of UTI is based on diagnostic tests such as dipstick or urinary sediment analysis.[3]

In some populations, the diagnosis of UTI is not as straightforward and should be distinguished from asymptomatic bacteriuria or inflammatory conditions, such as interstitial cystitis.[2] This is especially the case in a tertiary hospital, where relatively many patients have complex urinary tract problems or kidney transplants or are treated with immunosuppressive medication.

In the past few years, automated, standardized, quantitative urine analysis has been introduced in clinical practice and has shown high efficiency and accuracy compared to traditional sediment analysis.[4] One of these systems is the IRIS Diagnostics iQ200 Elite (iQ200), currently marketed by Beckman Coulter Inc., which analyzes urinary samples using flow imaging technology and auto particle recognition. The iQ200 classifies and quantifies particles, including bacteria, yeasts, white blood cells (WBC), and squamous epithelial cells, and correlates well with traditional urinary sediment examination with manual cell counts.[5]

Our group and other research groups have so far focused mainly on the use of automated urinalysis as a screening tool to predict negative urine cultures and thus to reduce the culture workload in the laboratory.[6-9] For this purpose, a positive culture was used as the "laboratory" gold standard of UTI without taking clinical symptoms into account, therefore predicting the presence of bacteriuria, but not of symptomatic UTI.[10,11] This distinction is important because it is currently thought that there is no role for treatment of patients with asymptomatic bacteriuria other than for pregnant women and patients undergoing urologic procedures.[12]

The test results of automated urine analysis are, however, subject to different clinical interpretations. This is partly because of unfamiliarity with quantitative results, instead of the semi-quantitative test results that clinicians used before, and the lack of optimal cutoff values for the clinical diagnosis of UTI.

The goal of the current study was to establish cutoff values for parameters of iQ200 analysis, to be used in diagnosing symptomatic UTI in a tertiary hospital population. Subsequently, we aimed to develop a scoring model to predict the clinical diagnosis of urinary tract infection, based on both symptoms and these cutoff values.

MATERIALS AND METHODS

Setting and patient population.

A retrospective study was performed at Leiden University Medical Center, which is an academic tertiary hospital in Leiden, the Netherlands. It has approximately 400 hospital beds and focuses on transplant medicine (solid organ transplants and stem cell transplants), resulting in a large proportion of immunocompromised patients. Samples from inpatients and outpatients of the hospital constitute the majority of samples sent to the clinical chemistry and microbiological laboratories (6). The study was approved by the Ethics Committee.

Urine samples.

Upon receipt at the Department of Medical Microbiology, all urine samples submitted for bacterial culture during a 12-week period from 25 February to 17 May 2013 were divided into two portions under sterile conditions if they had sufficient volume (at least 2 ml for culture and Gram stain and 3 ml for the iQ200 screening). One portion was analyzed by the iQ200 system in the clinical chemistry laboratory within 2 h after receipt from the microbiological laboratory. Results were not reported to the clinician because the iQ200 was still under validation. The other portion was analyzed by the microbiological laboratory. For more detailed information regarding procedures we refer to Russcher et al.[6]

For the purpose of this study, urine samples from children, pregnant women, and patients with an indwelling urinary catheter for more than 24 h, a nephrostomy, or a urostomy were excluded because the diagnosis of UTI is defined differently within these groups. Urine samples from patients without clinical data or clinical suspicion for UTI (e.g., preoperative routine urine controls) were excluded as well. Only the first sample of each patient was included.

Microbiological analysis.

Urine samples were analyzed using local standard microbiological methods for Gram stain and culture.[6] The bacterial load was assessed and scored from <100 CFU/ml (no growth) to $\geq 10^5$ CFU/ml. The relevance of the urine sample was assessed according to our standard protocol for urine cultures, taking a quality score (the Q score) based on white blood cell (WBC) and squamous epithelial cell (SEC) counts in the Gram stain into account, as previously described (6). In urine samples with a high Q score (≥1, corresponding with a high WBC and low SEC count), all growth was identified to the species level. Colonies in samples with a Q score of zero were only identified to the species level if a monoculture with a bacterial load of $\geq 10^5$ CFU/ml was present. Samples with Q scores of ≤0 were generally classified as mixed flora.

A positive culture was defined as having $\geq 10^3$ CFU/ml of not more than two different usual uropathogens or as having $\geq 10^5$ CFU/ml of a single unusual urinary pathogen. Common and uncommon pathogens and non-pathogens that were cultured are listed in Table 1.

Table 1. Pathogens isolated from urine cultures of 381 patients with and without UTIs.

Pathogen group or pathogen	No. with UTI (*n* = 59)	No. without UTI (*n* = 322)
Usual urinary pathogens		
Escherichia coli	31	26
Klebsiella spp.	4	6
Enterococcus spp.	2	5
Pseudomonas aeruginosa	2	4
Aerococcus urinae	2	2
Proteus mirabilis	0	3
Unusual urinary pathogens		
Other Enterobacteriaceae	2	4
β-hemolytic streptococci	2	3
Staphylococcus aureus	2	0
Haemophilus parainfluenzae	1	0
Candida spp.	0	2
Nonurinary pathogens		
Staphylococcus haemolyticus	2	0
Other staphylococci	0	3
Gardnerella vaginalis	0	1
Remaining		
Mixed flora	6	186
No growth	3	77

Automated urine microscopic analysis.

All samples derived from the Department of Medical Microbiology were tested by the iQ200® Elite analyzer (Iris Diagnostics, Chatsworth, CA), which is an automated urine microscopy analyzer that uses flow cytometry and digital photography. Automatic particle recognition software categorizes urine particles into 12 groups, including leukocytes, erythrocytes, bacteria, and "all small particles" (ASP). The ASP group consists of unclassified particles of <3 µm, such as cocci, which are not recognized well by the iQ200, some other bacteria, crystals, and other formed elements.[4,6,10] All elements other than bacteria were quantitatively reported (per microliter), and bacteria were reported semi-quantitatively (on a scale from 0 to 5). After automatic classification, a trained technician reviewed all images. Misplaced or unclassified images were placed in the correct categories, and bacterial counts were adjusted in cases when cocci were present.

Conventional urine analysis.

In a vast majority of patients from whom a urine sample was sent in for culture, a different sample was sent to the clinical chemistry for dipstick analysis. If the dipstick tested positive for leukocytes or erythrocytes, sediment analysis was performed using local standard protocol.

The positively tested urine was centrifuged for 5 min at 2,000 rpm. Subsequently, urine was poured off until 0.5 ml supernatant remained. This was shaken, and one drop was analyzed on a slide under a microscope. Observed elements were quantified as the number per high power field and reported qualitatively in the medical record.

Clinical assessment.

Clinical data and characteristics of included patients were obtained from the electronic medical records. Patients were retrospectively classified as either cases having a UTI or controls who did not have a UTI by two infectious diseases specialists using medical chart review. The expert reviewers used data on symptoms, signs, antibiotic (pre)treatment, and outcome, as documented in the electronic patient files. They used data on culture results and conventional urine analysis, which consisted of dipstick and sediment analysis. They also considered whether another diagnosis was more likely or could be the cause of complaints and/or fever. They were blinded to the iQ200 results, which were not reported in the medical records. If they differed in opinion, they reached consensus by means of discussion.

Statistical analysis

Baseline characteristics were compared with $\chi 2$-tests for dichotomous variables and an unpaired t-test for age. Symptoms and signs were compared with $\chi 2$-tests. Parameters from the iQ200 analysis were compared using unpaired t-tests and receiver operating characteristic (ROC) curves were plotted. Cutoff values were determined based on the optimal tradeoff between sensitivity and specificity. These cutoffs correspond with coordinates on the ROC curves which are closest to 0.1 (the upper left corner).[13] Distances for all coordinates on the ROC curves to 0.1 were calculated by the formula: $\sqrt{d} = (1\text{-sensitivity})^2 + (1\text{-Specificity})^2$.

A logistic regression model was established using symptoms and parameters from the iQ200. In the case of information on a specific sign or symptom not being documented in the electronic patient file, that patient was excluded for this specific analysis. Backward selection excluded parameters based on likelihood ratios without significantly changing the fit of the model. The final model retained all variables significantly associated with the presence of UTI at a $p<0.05$ level. A numerical scoring tool was developed using the model by simplifying β-coefficients of all independent predictor variables. We calculated the area under the receiver operating characteristic curve (AUC) with 95% confidence interval (CI) to assess the scoring tool's discriminatory power to predict or rule out UTI. ROC curves were also plotted for the separate iQ200 and clinical variables derived from the model.

Cutoff values were considered based on sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively). All analyses were performed using SPSS 21.0 (SPPS Inc, Chicago, IL, USA).

RESULTS

Population characteristics

During the study period 1442 urine samples from 1084 unique patients were submitted. The following samples were excluded: 641 samples from patients not suspected of having a UTI, 152 samples from patients with an indwelling urinary catheter >24 hours, 76 samples from pregnant patients, 62 samples of children below the age of 18, 33 samples from a nephrostomy or urostomy drain, 91 subsequent samples of patients already included, and 13 samples lacking data in the corresponding electronic patient files. After exclusion, 381 unique patients and urine samples remained. A total of 29 of 381 urine samples submitted were obtained by one-time catheterization and the rest by midstream clean catch. The prevalence of UTI among the 381 patients according to the expert review was 59. The expert reviewers initially differed in opinion in 30 of 381 patients (7.8%) but reached consensus by means of discussion. Table 2 shows demographic and clinical characteristics of the two patient groups (with and without urinary tract infections). Patients who had a UTI were significantly older (p = 0.041) than those who did not. None of the other characteristics differed significantly between both groups.

Table 2. Baseline characteristics of 381 patients with and without UTIs.

Characteristic	With UTI (*n* = 59)	Without UTI (*n* = 322)	*P* value
Age in yrs (mean [SD])[a]	61.1 (17.7)	55.8 (18.2)	0.04
Male (no. [%])	33 (56)	152 (47)	0.22
Hospitalized (no. [%])	23 (39)	151 (47)	0.26
Indwelling catheter removed <7 days prior to culture (no. [%])	4 (7)	27 (8)	0.70
Immunosuppressive medication <3 months prior to culture (no. [%])	16 (28)	113 (35)	0.26
Neutropenia (no. [%])	0 (0)	15 (5)	0.09
Antibiotics <48 hours prior to culture (no. [%])	16 (29)	98 (31)	0.74
Renal transplant (no. [%])	6 (10)	39 (12)	0.70
Pancreatic transplant (no. [%])	0 (0)	8 (3)	0.23
Hematopoietic stem cell transplant (no. [%])	2 (3)	14 (4)	0.75
Fever (no. [%])[b]	14 (25)	100 (31)	0.31

[a] $P < 0.05$.
[b] Fever was defined as a temperature higher than 38.1 ^{0}C.

Culture results

Table 1 shows culture results of cases and controls. *Escherichia coli* was the most prevalent pathogen (n = 57). 192 Cultures displayed mixed flora and 80 cultures showed no growth. Patients who were assessed as cases with a UTI while their culture showed no growth were all treated with antibiotics in the 48 hours prior to culture (n = 3).

Signs and symptoms

The prevalence of signs and symptoms among both patient groups are listed in Table 3. Dysuria, recognition of symptoms from a previous UTI, frequent micturition, and cloudy urine were most strongly associated with UTI. Subgroup analysis was conducted for aggravated lower urinary tract symptoms (LUTS) in male patients, increased cognitive impairment in patients older than 59 years of age and vaginal irritation or changed discharge in women. None of the three symptoms was significantly associated with UTI in their respective subgroups (not shown). The concentration of C-reactive protein in serum and leukocyte count in blood did not differ significantly between patients with and without UTIs (p values were 0.95 and 0.69 respectively). The same applied to the proportion of patients with positive blood cultures when comparing both groups (p = 0.31).

Table 3. Signs and symptoms of 381 patients with and without UTIs.

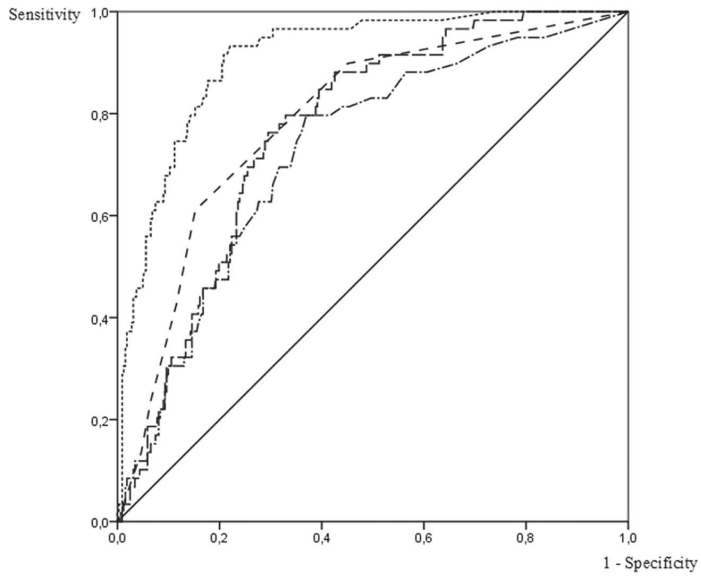
Sign/symptom[a]	With UTI (no. [%])	Without UTI (no. [%])	Odds ratio (CI)	*p* value[c]
Frequent micturition/increased urgency[b]	27 (54)	56 (19)	5.0 (2.7 – 9.3)	0.00
Dysuria	36 (69)	55 (18)	10.1 (5.2 – 19.5)	0.00
Aggravated LUTS	5 (10)	23 (8)	1.3 (0.5 – 3.6)	0.59
Suprapubic pain[b]	7 (13)	15 (5)	2.9 (1.1 – 7.4)	0.02
Recognition of symptoms from a previous UTI[b]	14 (27)	15 (5)	7.0 (3.1 – 15.6)	0.00
Increased incontinence	7 (13)	21 (7)	2.1 (0.8 – 5.3)	0.10
Macroscopic haematuria	3 (6)	16 (5)	1.1 (0.3 – 4.0)	0.87
Cloudy urine[b]	12 (24)	18 (6)	4.9 (2.2 – 11.0)	0.00
Foul smelling urine[b]	10 (20)	21 (7)	3.3 (1.4 – 7.5)	0.00
Increased cognitive impairment	6 (10)	30 (9)	1.1 (0.4 – 2.8)	0.82
Suprapubic tenderness	5 (18)	16 (9)	2.2 (0.7 – 6.5)	0.16
Costovertebral angle tenderness	3 (19)	9 (16)	1.3 (0.3 – 5.3)	0.76
All signs/symptoms	59 (15)	322 (85)		

[a]All symptoms and signs as reported in the patient file. LUTS, lower urinary tract symptoms.
[b]$P < 0.05$.
[c]CI, confidence interval.

iQ200 parameters

The difference in distribution of white blood cells in urine between cases and controls was obvious. Most cases had a count of > 20 leukocytes/µl (97%) while most controls had a count ≤ 20 leukocytes/µl (69%), p < 0.01. A somewhat similar result was found for the concentration of bacteria. The iQ200 reported a bacterial value of 2 or more for 61% of the cases and < 2 for 85% of the controls. ROC curves were plotted for iQ200 parameters and are shown in figure 1. The count of white blood cells per microliter (WBC/µL) had the largest area under curve (AUC, 0.91 CI 0.87 – 0.94) and the highest discriminative value when compared to the other parameters. Optimal cutoff values were calculated for WBC/µL, Bacteria and ASP/µL and are shown in table 4.

Figure 1. Receiver operating characteristic curves of different iQ200 parameters predicting UTI.



····· WBC/µL (AUC 0.91, CI 0.87-0.94)
– – Bacteria (AUC 0.79, CI 0.73-0.85)
—— ASP/µL (AUC 0.77, CI 0.71-0.82)
—·—· Red blood cells (RBC)/µL (AUC 0.72, CI 0.66-0.79)
— Reference line
On the Y-axis sensitivity, on the X-axis 1-specificity. AUC, area under curve, CI, 95% confidence interval.

Because of the high discriminative value of WBC/µL, this parameters was subsequently divided in 3 categories using the cutoffs of 50 and 150. The first cutoff of 50 was selected by prioritizing sensitivity over specificity while maintaining a good tradeoff between both of them (sensitivity 91%, specificity 79%) . The second cutoff of 74 was the optimal calculated cutoff (sensitivity 86%, specificity 82%), and the third cutoff of 150 was selected by approximately reducing the amount of false negatives by half (sensitivity 69%, specificity 89%). Cutoffs were rounded to increase clinical applicability.

Table 4. Cut-off values of iQ200 parameters and corresponding sensitivity and specificity.

	Cutoff value	Sensitivity (%)	Specificity (%)
WBC/µL (optimal calculated)	<74	86	82
WBC/µL (selected for categorization)	<50	91	79
	<150	69	89
Bacteria	<2	61	84
ASP/µL[a]	<6,250	76	70

[a] ASP, all small particles.

Table 5. Variables retained after logistic regression analysis of factors independently associated with UTI and attribution of points based on β coefficients.

Variable	AOR (CI)[a]	β coefficient	Points attributed
Frequent micturition/ increased urge	2.8 (1.1 – 7.3)	1.0	1
Bacterial score, ≥2	3.7 (1.3 – 10.2)	1.3	1
Dysuria	12.1 (4.5 – 32.5)	2.5	2
WBC/μL, 50 - 149	15.6 (4.1 – 59.8)	2.8	2
WBC/μL, ≥ 150	44.5 (12.1 – 164.1)	3.8	3

[a] AOR, adjusted odds ratio; CI, confidence interval.

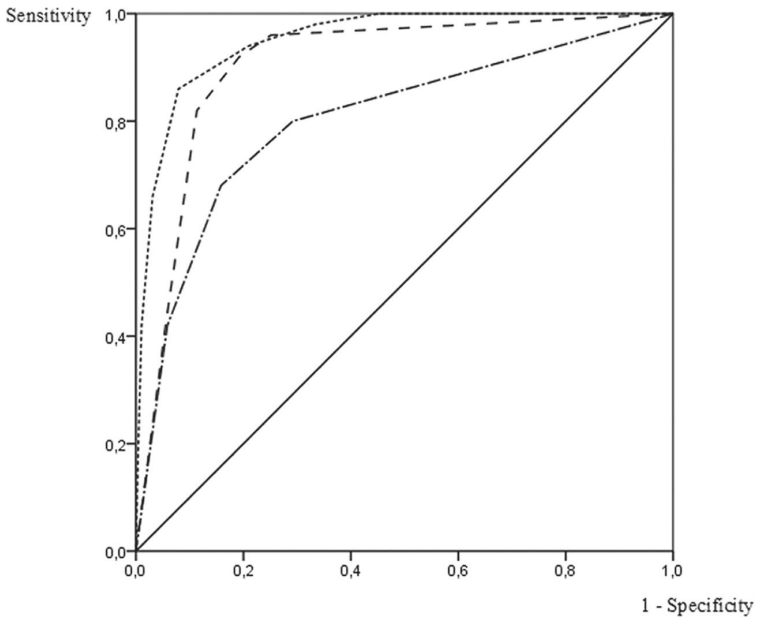
Establishment of a scoring tool

Logistic regression analysis was performed. Symptoms significantly associated with UTI were entered into the model together with the categorized concentration of WBC/μL (using the two selected cutoff values from table 4) and the iQ200 parameter 'bacteria'. We did not use ASP/μL in our model because we aimed to establish a clinically applicable model and this parameter is nonspecific for measurement of bacteria. A scoring tool was developed to confirm or rule out the diagnosis of UTI (table 5). Points were attributed based on β coefficients with 1 point being given to the parameter with the smallest coefficient.[14,15] The maximum possible score was 7.

ROC curves of the scoring tool (AUC 0.95; CI 0.93-0.98) and of its separate components, iQ200 (AUC 0.90, CI 0.86 – 0.93) and clinical (AUC 0.80, CI 0.73-0.88) variables, are shown in figure 2. As expected, the scoring tool had a higher sensitivity and specificity in predicting UTI as compared to the separate iQ200 and clinical parameters as derived from the model, as well as the single iQ200 parameters (figure 1).

Different cutoff scores and corresponding characteristics of the scoring tool are displayed in table 6. When using a single threshold, a score of <4 has the optimal tradeoff between sensitivity (86%) and specificity (92%) which are both remarkably high given the complexity of the study population. Using this cutoff 7 patients would incorrectly be scored as negative (14% of cases) while 23 patients would incorrectly be scored as positive (8% of controls). If two cutoffs were to be used, three categories are formed: UTI likely, UTI possible or UTI unlikely. By using one threshold of below 3 and one of 5 or more, both false negatives and false positives are reduced by more than half compared to using a single threshold of 4. However, 19% of all patients would be classified as possibly having a UTI.

Figure 2. Receiver operating characteristic curves of the scoring tool and its separate components (iQ200 and clinical variables) predicting UTI.



On the Y-axis sensitivity, on the X-axis 1-specificity. AUC, area under curve; CI, 95% confidence interval.

·····	Combined score (AUC 0.95, CI 0.93-0.98)
– –	WBC/µL and bacteria (AUC 0.90, CI 0.86-0.93)
—·—	Dysuria and frequent micturion/increased urge (AUC 0.80, CI 0.73-0.88)
—	Reference line

DISCUSSION

Our study defined cutoff values for parameters as measured by an automated urine analysis system, the IRIS iQ200, for prediction of the clinical diagnosis of urinary tract infection in a heterogeneous, academic population of adult patients. In contrast to previous research on automated urine analysis by the iQ200,[6,7,10] we did not solely use a positive urine culture as the 'gold' standard, but focused on clinical symptoms and course of disease in combination with culture results. This clinical assessment allowed exclusion of false-positive urine cultures of patients without urinary symptoms and with a diagnosis other than UTI, limiting unnecessary treatment of UTI. Prudent use of antibiotics has become increasingly relevant because of the problem of antibiotic resistance, which currently has become one of the most serious and growing threats to public health.[16]

We found that urinary white blood cell count had the highest discriminative value for UTI (AUC 0.91) compared to the other individual parameters, bacterial score and ASP/µL. The

calculated optimal cutoff for WBC/µL was 74, with a sensitivity of 86% and specificity of 82%. For development of the scoring tool we choose to use a lower cutoff of 50 WBC/µL with a higher sensitivity of 91% and acceptable specificity of 79% (AUC 0.85), to reduce the amount of false negative results.

The finding that only 3% of the cases had a concentration of ≤20 WBC/µL in urine corresponds with previous research using conventional urine analysis.[17,18] The role of the count of ASP/µL in UTI diagnosis by iQ200 remains to be determined. One study reported that ASP/µL has a better test performance than bacteria at certain cutoffs,[8] but our findings confirm the observation of Parta et al. who did not find ASP count to contribute in ruling out UTIs.[8,10]

Table 6. Possible thresholds for the scoring tool and corresponding characteristics.

Score threshold	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
< 2	98	67	34	100
< 3[a]	94	79	44	99
< 4	86	92	65	98
< 5[b]	66	97	79	94
< 6	42	99	88	91

[a]Threshold selected to rule out UTI.
[b]Threshold selected to confirm UTI.

The optimal scoring tool for diagnosis of UTI obtained by multivariable analysis included the iQ200 parameters 'WBC/µL' and 'bacteria' and the clinical symptoms 'dysuria' and 'frequent micturition/ increased urge'. The test characteristics of the scoring tool depend on the chosen threshold(s). Through the selection of different cutoff criteria, the score can be adapted to different clinical situations depending on the relative benefits of maximizing sensitivity or specificity.
While a high sensitivity is important to minimize the number of false negatives, specificity might be of equal importance to minimize the number of false positives and limiting inappropriate antibiotic use.

Obviously, the selection of the best cutoff depends on the setting, the clinical condition and individual characteristics of the patient, and the risk of delaying antibiotic treatment. In case of a febrile patient with suspected invasive UTI, a threshold of <2 seems appropriate to rule out UTI and search for an alternative diagnosis, whereas in case of suspected cystitis the threshold of 4 could be used to withhold antibiotics. Therefore, the use of three categories (UTI likely (≥5), UTI possible (3 to 4) or UTI unlikely (<3)) is probably most useful for application in patient care, leaving room for interpretation and risk analysis by the clinician.

Table 7. Number of patients with and without UTI in each score group and predictive values using two cut-offs.

Score	With UTI (*n* = 50)	Without UTI (*n* = 291)	Positive predictive value (%)	Negative predictive value (%)
<3	3	230	1	99
3-4	14	52	21	79
>4	33	9	79	21

Previous research on automated urine analysis showed that its findings correspond well with conventional urine sedimentation.[5] Most research on analysis by the IRIS iQ200 aimed to predict a positive urine culture in order to reduce laboratory work load and associated costs.[6,8,10] One of these articles also took clinical data into account, which lead to a reduction of cases considered to be false positive using urine culture only as the gold standard.[10] Since the purpose of this study by Parta was to evaluate the iQ200 as a screening tool to decrease unnecessary urine cultures, a low cutoff for WBC (≥6/µL) was chosen to achieve high sensitivity, resulting in a poor specificity of 67-70%.

Luciano developed a risk score combining both dipstick and iQ200 sediment reading results with age that improved UTI diagnosis in a pediatric population.[19]

Similar studies were performed on different commercial systems using flow cytometry, e.g. the Sysmex UF-1000i (Sysmex Japan) to define optimal cutoff points for WBC or bacteria for ruling out bacterial UTI, but these data cannot be extrapolated directly to the iQ200 because both systems work differently. The UF-1000i is laser based and uses fluorescent dye which the iQ200 does not.[7,9,20,21]

Strengths of our study are the particular academic population and its reflection of a real life situation which includes a very heterogeneous group of both in- and outpatients of whom some had renal transplants, had a fever, were already treated with antibiotics, had contaminated urine samples or were difficult to classify as either having a UTI or not.

The present study has its limitations. First, data from electronic patient files was obtained retrospectively and might not always have been complete. Second, the entire available dataset was used for the prediction score, which as a result could not be validated in a different patient set. Validation is therefore required before the score could be implemented in clinical use. Third, the diagnosis of UTI lacks a gold standard. However, we feel that assessment by two independent blinded experts who take clinical data, conventional sediment analysis as well as culture results into account is the best reference test currently available. Finally, the study population was too small to distinguish between uncomplicated cystitis and invasive UTIs and to determine if cutoff values of iQ200 parameters would be different for certain subgroups, such as patients with neutropenia or renal transplants.[2,22]

Further research should prospectively validate the scoring tool for diagnosis of UTI on a new set of data and in different subgroups of patients and demonstrate potential benefits, such as reduction in the unnecessary use of antibiotics.

In conclusion, although the diagnosis of UTI can be challenging in an adult academic patient population, the combination of a simple survey and the results of the iQ200 could rule out infection in the majority of patients and therefore improve antibiotic stewardship in suspected UTI cases.

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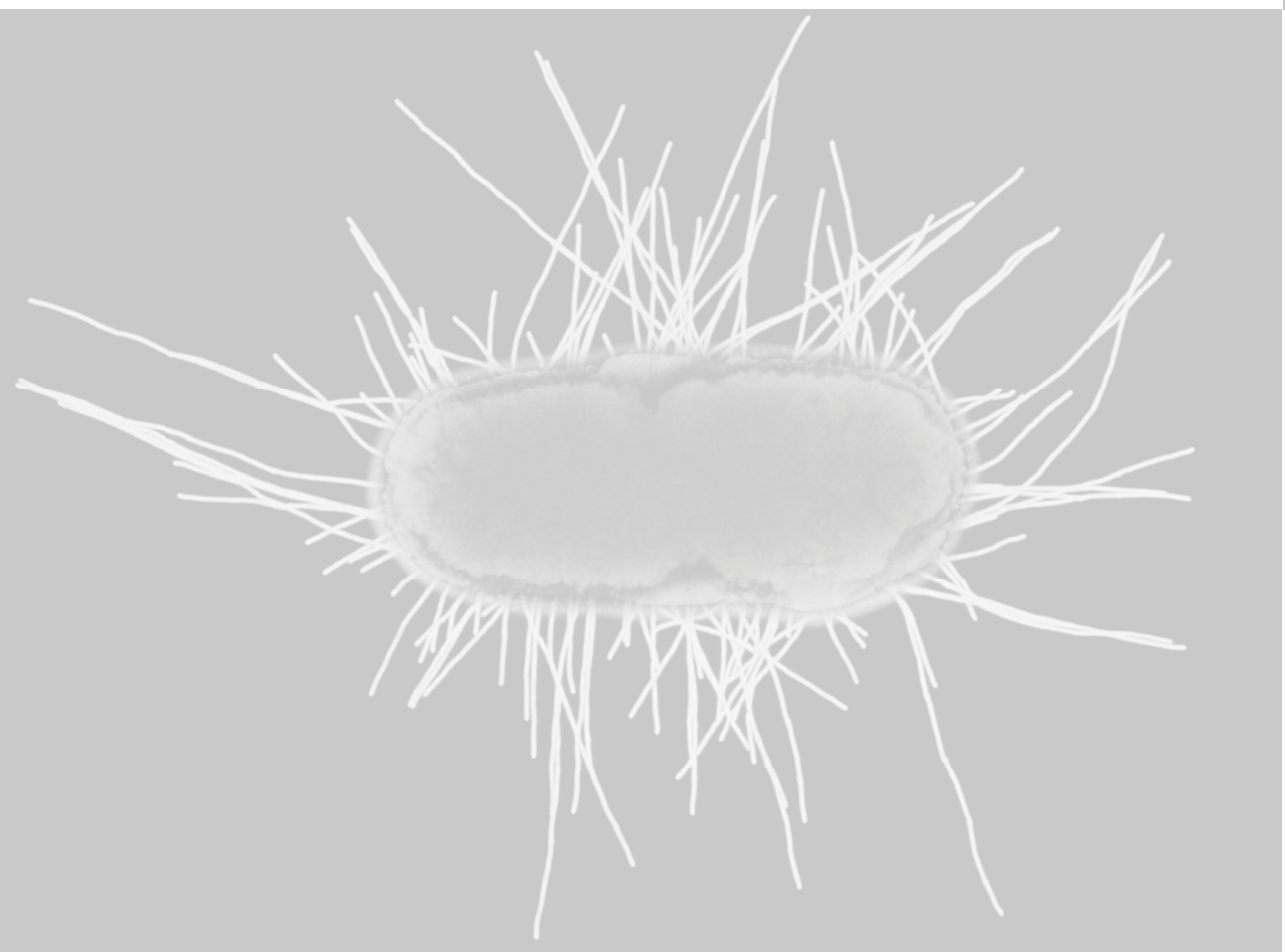


CHAPTER 10

Summary and general discussion

OPTIMIZING TRIAGE AND TREATMENT STRATEGIES IN URINARY TRACT INFECTION

Urinary tract infections (UTI) are among the most frequently encountered reasons for medical consultation in infectious diseases. The majority of UTI are confined to the bladder (i.e. acute cystitis) and belong to the routine daily practice of primary care. In otherwise healthy patients, acute cystitis can be self-limiting and in case antimicrobial treatment is indicated, a short course of antibiotics is usually sufficient.[1-3] In patients with underlying urologic abnormalities or recurrent cystitis, management can be more complex, especially of infections caused by drug-resistant uropathogens.[4]

Acute cystitis should be distinguished from infections extending beyond the bladder, such as acute pyelonephritis and prostatitis, which are tissue-invasive forms of urinary infection characterized by fever or other systemic symptoms.[5] Fever is a sign with little specificity and may indicate the mere presence of local kidney infection or of impending urosepsis. Prompt recognition and start of adequate antibiotic treatment of the latter condition is essential, to prevent progression to septic shock and death. However, the majority of patients with febrile UTI present with mild illness and respond favorably to antibiotic treatment.

The first part (**Chapters 2 to 5**) of this thesis focuses on research questions concerning the management of patients with febrile urinary tract infections (fUTI). In the second part, urinary tract infections complicated by multidrug-resistance (**Chapters 6 to 8**), and diagnosis of UTI (**Chapter 9**) are addressed.

Optimal triage strategy: when should a patient with community- acquired fUTI be hospitalized?

Although patients who present to the emergency department with fUTI generally have a low risk of a complicated course, many are admitted to the hospital for observation. Apparently, because of a small chance of life-threatening complications that cannot reliably be predicted, physicians tend to apply low thresholds for hospitalization. This suggests that many admissions may be avoidable, and that this practice leads to over-treatment of low severity patients and increased healthcare costs.[6,7] Accurate assessments of initial disease severity and the likelihood of disease progression are, therefore, crucial to provide a more personalized treatment strategy in the most appropriate setting.

Outpatient management is recommended for mildly ill patients who have minimal nausea, no vomiting, and stable coexisting medical conditions,[3,8] but literature to support this recommendation is limited as the majority of studies on outpatient management were restricted to healthy young to middle-aged women.[9-11] For suspected respiratory tract infection there are validated clinical tools, such as the *Pneumonia Severity Index* (PSI), to calculate the risk of clinical deterioration or death, that help the clinician decide whether hospital admission is indicated.[12] Such a clinical tool to identify those who require hospital admission is not available for fUTI. The predicting factors for mortality in the PSI, such as age, co-morbidity, and physical or laboratory

signs of sepsis are not specific for pneumonia. This risk assessment tool may, therefore, also be suitable for community-acquired infections other than pneumonia.

In **Chapter 2** we designed and validated a clinical severity assessment tool, called the '*Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden*' (PRACTICE).[13] In a cohort of 787 consecutive fUTI patients, the PRACTICE identified those at very low risk for 30-day mortality and ICU admission. These risks were very low (<2.5%) in patients with a PRACTICE score below 100, yet 60% of patients in this group had been hospitalized. The use of the PRACTICE in guiding admission policy was subsequently evaluated in a stepped wedge cluster randomized trial, enrolling patients presenting to the emergency departments (ED) of seven hospitals. All participating centers started with a control period in which routine clinical practice with regard to hospitalization policy was applied. At the start of the intervention period, that was introduced at all participating centers in random order, the ED physicians were instructed to calculate the PRACTICE. Based on the patients' scores, recommendations were as follows: hospitalization for high risk (>100 points) and discharge to home for low-risk patients (<75 points). Preferably admission policy was done according to the PRACTICE, however, the attending physician was responsible for the final decision on treatment location.

Our hypothesis that the use of this prediction rule would reduce the hospitalization rate was confirmed in this study, as shown by a 20% absolute reduction. Unfortunately, the trial was stopped after inclusion of 370 patients due to safety concerns, because the percentage of patients who needed to be hospitalized for presumptive fUTI after initial home-based treatment (the secondary admission rate) of 27% exceeded the predefined stopping criterion.

Looking at the total of all patients included in the validation and intervention study, risks for mortality and ICU admission are still very low (Table 1a), much lower than the risks found in pneumonia patients in the initial validation study of the *Pneumonia Severity Index* (Table 1b).[12]

Table 1a. Clinical outcome of febrile urinary tract infection according to PRACTICE score risk class.

PRACTICE score in fUTI	Low risk 0-75	Intermediate risk 76-100	High risk >100	n
No. of patients	634	330	196	1157
Outpatient, No (%)	241 (38)	69 (21)	19 (10)	329
Inpatient, No (%)	393 (62)	261 (79)	177 (90)	831
30-day mortality, %	0.15	0.18	9.7	26
ICU admission, %	1.1	2.7	11.2	38

Table 1b. Clinical outcome of pneumonia according to PSI score risk class, adapted from Fine et al.[14]

PSI score in pneumonia	Low risk 0-70	Intermediate risk 71-90	High risk >90	n
No. of patients	1249	326	712	2287
Outpatient, No (%)	831 (66)	72 (22)	41 (6)	944
Inpatient, No (%)	418 (33)	254 (78)	671 (94)	1343
30-day mortality, %	0.32	0.92	14.9	113
ICU admission, %	4.3	5.9	13.2	167

Why did the implementation of the PRACTICE rule lead to more secondary hospitalizations in fUTI as compared to the PSI in pneumonia, which has been successfully introduced in routine patient care?

First of all, the course of disease and the pathway leading to the failure of home treatment is probably different in these two infections. Whereas respiratory distress is probably the main cause of secondary hospitalization of pneumonia patients; inability to take oral medication and the need for volume resuscitation is more important for fUTI patients. These factors may have been underrepresented in the outcome of complicated course of fUTI as predicted by the PRACTICE in the validation cohort since the majority of low-risk patients were traditionally hospitalized and treated with intravenous fluids and antibiotics. Secondly, two re-admissions because of *E. coli* bacteremia might have been avoided. These two patients were contacted by the treating physician after receiving the blood culture results and asked to return to the hospital for intravenous treatment. Ciprofloxacin has however been shown to be equally effective orally as intravenously in bacteraemic UTI[15] so that hospitalization would not have been warranted. Last but not least, nearly half of secondary admissions (4 out of 10) were not related to deterioration of the course of the fUTI, but due to diagnostic errors at the ED. Patients with primary bacteremia caused by salmonella, staphylococci, and streptococci, presenting with aspecific symptoms, such as fever and back pain, were mistaken for pyelonephritis and sent home. Apparently, these patients were 'misdiagnosed' at first consultation as having fUTI, and subsequently were treated for other diagnoses at secondary admission. Our real-world study underlines the importance of the validation of clinical prediction rules in a new cohort to verify its predictive value and usefulness in a clinical setting.

Evidently, the diagnosis of fUTI is not as straightforward as the diagnosis of pneumonia, where the presence of an infiltrate on a chest X-ray is both definitive and confirmative. It is of importance to be aware that other infectious diseases can mimic the general symptoms of fUTI and to realize that the presence of leukocyturia or bacteriuria, a common condition especially in elderly patients, can distract attention from the correct diagnosis. Improved diagnosis of fUTI is needed, not only to optimize fUTI treatment and to ensure safe implementation of clinical prediction rules but also to improve antibiotic stewardship. Differentiation between asymptomatic bacteriuria (ASB) and symptomatic UTI is important because for ASB antibiotic

treatment is not indicated, except for pregnant women and patients undergoing urological procedures.[16] Antimicrobial treatment of ASB outside these settings leads to unnecessary side-effects, potential drug interactions, unnecessary costs, and, importantly, adds to the development and spread of antibiotic resistance.[17] Inappropriate treatment of ASB is substantial in the emergency care setting and ranges from 20% in patients aged >12 years, up to 43% in older adults.[18-20] Clearly, there is a considerable potential for reduction of antibiotic use in these patients.

How can the prediction rule for admission of febrile urinary tract infection patients be optimized?

Prognosis of the patient presenting with severe febrile illness depends on two factors. First, the severity of the acute host response to the infection and the ensuing inflammatory cascade eventually leading to shock and multi-organ failure which causes hyperacute mortality. Secondly, the patient´s general health condition, mainly defined by age and comorbidity, determines the 30-day mortality in patients who survive the first days of illness. The severity of the acute host response is underrepresented in the PRACTICE, because it was based on the 30-day mortality in the validation cohort. In **Chapter 3**, we hypothesized that the addition of an objective blood biomarker reflecting the severity of sepsis, such as procalcitonin (PCT), midregional pro-adrenomedullin (MR-proADM) or C-reactive protein (CRP),[21] might improve the decision rule in identifying patients who benefit from hospital-based treatment in the acute phase without compromising safety. Therefore, we conducted a secondary analysis of the study presented in **Chapter 2**.[22] In this study, only patients with blood samples collected upon ED presentation available for biomarker analysis were included (n=313).

MR-proADM exhibited the strongest predictive value for a severe course of fUTI, defined as a composite of all-cause 30-day mortality, ICU admission, and extended hospitalization (> 10 days). Combinations of MR-proADM, PCT or PRACTICE did not significantly increase predictive ability over the use of MR-proADM alone. Concentrations of MR-proADM and PCT were both significantly higher in patients who were hospitalized as compared to those who were treated as outpatients. In the subgroup of patients that were initially treated as outpatients but that required secondary admission, MR-proADM was significantly elevated as compared to those who completed outpatient treatment at home. PCT concentrations were similar in these two groups. CRP did not have any added value in any of the groups for clinical guidance, although CRP has found its way into clinical practice and is routinely measured in all patients with fUTI.

Since the ability of MR-proADM to identify patients at risk for secondary admission was not improved by combination with the PRACTICE score, we further assessed the sole use of MR-proADM in a virtual biomarker-guided treatment allocation study. Using a cut-off of 0.80 nmol/L, MR-proADM guided triage could decrease hospital admissions by 24% and would allow a higher proportion of patients to be safely treated as outpatients. The use of this cut-off would have resulted in only 2% of outpatient re-presentations to the ED, as well as no mortalities within 30 days, and no requirement for ICU admission. All of the patients with primary bacteremia who needed secondary admission in the primary study would have been hospitalized upon

first ED presentation if the MR-proADM cut-off was set at 0.80 nmol/L. We, therefore, consider MR-proADM to be the optimal biomarker for UTI triage, and 0.80 nmol/L the optimal cut-off concerning patient safety. However, though these results are promising, our virtual biomarker-guided triage can only be considered as hypothesis generating. The next step is to verify the use of MR-proADM to identify those who benefit from hospital admission in a clinical trial. Furthermore, any decision based on a biomarker or triage algorithm should be critically appraised for the use in an individual patient. Factors such as comorbidity, compliance, lack of family support, or risk of an antimicrobial resistant pathogen should all be considered in the final decision.

Optimal treatment duration: how long should a patient with community-acquired fUTI be treated?

Although the benefits of using antibiotics are indisputable, its excessive use leads to resistance of pathogens. Antimicrobial resistance is a serious and growing public health threat, and thus it is essential to develop strategies to maintain the effectiveness of the available antimicrobials. The determination of the optimal duration of treatment is a simple and effective approach to antibiotic stewardship. Therefore, the general approach to treatment duration of common infections should be that "shorter is better". With respect to febrile UTI or acute pyelonephritis, trials for testing treatment duration have usually focused on otherwise healthy young women and have addressed optimal treatment duration by comparing the same drug for various durations of therapy or compared various treatment durations of several antimicrobial agents.[23] As such, recommendations about optimal treatment duration of UTIs in men, the elderly, hospitalized patients, and patients with comorbidities or bacteremia, remain unclear.

We 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 were similar to those in a 14-day ciprofloxacin course in an unselected population of both men and women.[24] Patients with community-acquired fUTI were recruited at regional hospitals and primary care centers, and clinical and microbiological cure rates were assessed. Clinical cure in this study was defined as being alive, free of fever and UTI symptoms, and without additional antimicrobial therapy (for relapse of UTI).

The results of this study are discussed in **Chapter 4**. We found that community-acquired fUTI can be safely and efficaciously treated with a 7-day instead of a 14-day course of oral ciprofloxacin in women, including the elderly with severe comorbidities, and irrespective of the 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. In men, however, 7-day treatment did not reach non-inferiority with a 14-day course of treatment, as shown by an increase in the 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. Surprisingly, clinical cure rates assessed after longer follow-up (70–84 days post-treatment) were similar between 7 and 14 days of treatment in both women *and* men. In other words, the need for additional antibiotic UTI treatment during

longer follow-up is similar, irrespective of whether the initial treatment of fUTI was 7 or 14 days. Our findings suggest that fUTI in men likely involves the prostate, as involvement of the prostate is a known cause for recurrence of UTI even after appropriate antimicrobial treatment.[25]

These results extend the findings of a previous highly similar Swedish study performed in women with acute pyelonephritis, showing non-inferiority of 7- and 14-day antimicrobial treatment.[26] Compared to our study, their patient group was younger, had fewer comorbidities, and fewer of their patients had complicated UTI. In a recent study regarding the duration of antimicrobial therapy for Gram-negative bacteremia, a subgroup analysis in 282 patients with a urinary source of bacteremia confirmed that a different treatment duration is indicated for men and women.[27] After adjustments for confounders, there was no significant difference in the risk of treatment failure between short and long duration of therapy in women, but a 7- to 10-day course of therapy for men was associated with a significant increase in the risk of treatment failure compared to >10 days of therapy.

Unfortunately, due to the limited number of patients enrolled, our study lacks statistical power to draw confident conclusions on the various subgroups. Overall, we can conclude that in women - including postmenopausal women and those with significant comorbidities- febrile UTI can be treated successfully 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. Additional studies to confirm optimal treatment duration in subgroups, and to determine optimal treatment duration with other classes of antimicrobial agents are needed. These studies should include outcome measures set at three months or even longer instead of the traditional 2-6 weeks.

Can biomarkers in blood provide guidance on optimal duration of antibiotic treatment for fUTI?

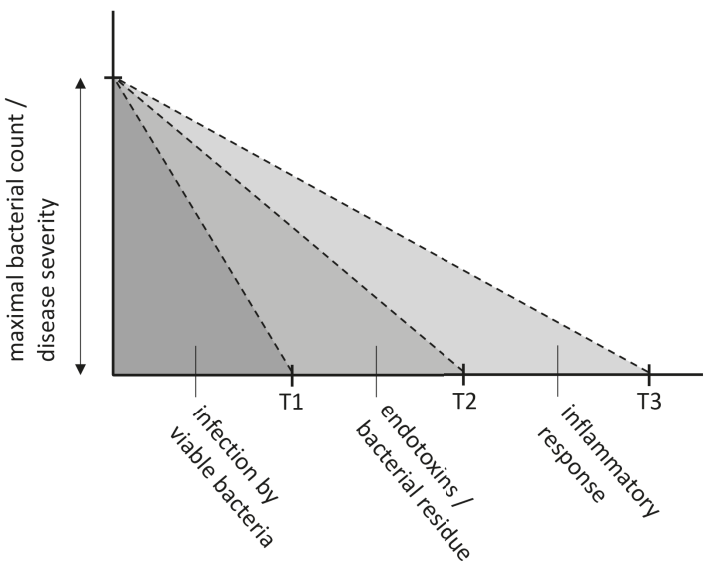
Procalcitonin (PCT) has been shown to provide useful guidance for duration of antibiotic treatment in patients with respiratory tract infections and sepsis.[28-30] PCT has also been shown to be a biomarker of bacteremia in patients with febrile UTI,[31-33] but little is known about its value as a biomarker for required treatment duration of fUTI. MR-proADM is a predictor of a complicated course of disease in fUTI, the need for ICU admission, and mortality,[21,34] but its use has not been tested for guidance of antibiotic treatment duration.

In **Chapter 5**, we assessed whether PCT measurement on days 0 and 3 could more accurately identify patients at risk of treatment failure and in need of a prolonged course of antibiotics compared to either MR-proADM or C-reactive protein. We found that the biomarker signatures of both PCT and MR-proADM correlated significantly with parameters that reflect the severity of invasive urinary tract infection, such as temperature, presence of bacteremia, shaking chills, and the need for initial administration of antibiotics intravenously rather than orally. Also, the course of PCT and MR-proADM correlated with signs of clinical recovery, such as time to defervescence and length of hospital stay. As opposed to PCT and MR-proADM, the popular biomarker CRP did not show any correlation with relevant clinical parameters. Although these findings seem

promising for the potential use of PCT and MR-proADM to aid the clinician with determining the length of antibiotic therapy, neither of these biomarkers could identify patients at risk for treatment failure in our study. This is likely due to the fact that all patients in our study were treated with antibiotics for at least 7 days, after which treatment success was already high (89% overall).

Historically, empiric treatment was based on the anticipated time to clinical recovery, while taking into account the interindividual variability of the severity of disease at the start of treatment by adding a 'safety margin' of some days to the average recovery time. Traditionally, the standard duration of antimicrobial treatment for acute pyelonephritis was 6 weeks until in 1987 a 2-week regimen was shown to be equally effective.[35] Only recently, additional studies including ours have provided evidence for a shorter therapy,[23,24,26] and this has already led to the introduction of a 7-day treatment regimen for all female patients with fUTI as the standard of care.[8] Some studies lend additional support for an even shorter fluoroquinolone regimen for mild to moderate pyelonephritis.[36,37] Our study design, unfortunately, did not allow for further assessment whether a (probably female) subgroup of patients could have been treated shorter than 7 days.

Figure 1. Hypothetic model of febrile urinary tract infection.



T0 medical consultation and start of antimicrobial treatment, T1 killing of all viable bacteria, T2 resolution of endotoxin / bacterial residue, T3 resolution of inflammatory response.

In Figure 1 a hypothetical model of febrile urinary tract infection is presented. Severity of the acute febrile UTI is on the Y-axis, which is a sum of the bacterial load and the intensity of the host response to the bacterial infection. On the X-axis, is the time necessary for bacterial killing

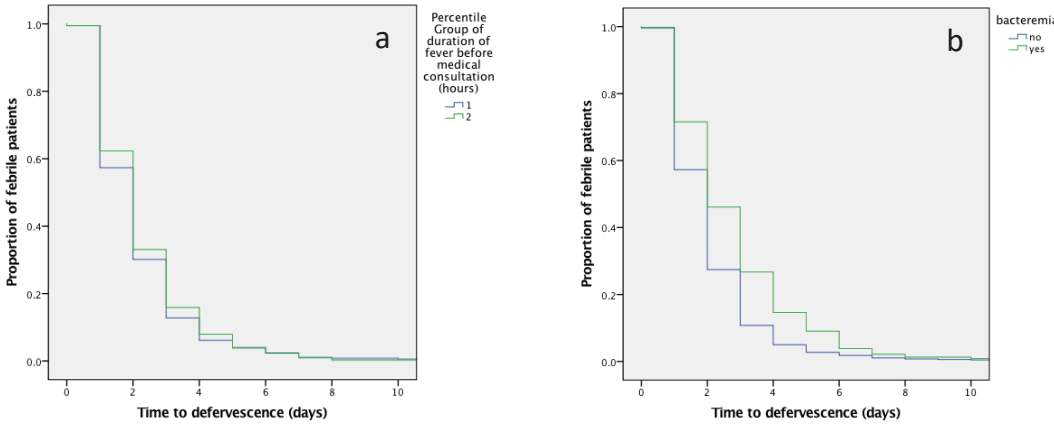
(T1), resolution of bacterial endotoxins and residue (T2) and for recovery from the symptoms of the infection (T3). After the start of antimicrobial treatment, the killing of bacteria results in the release of bacterial components that continue to trigger the host proinflammatory response. Since there is a considerable interindividual variability in disease severity, the starting point on the Y-axis differs substantially between patients. However, the rate of reduction of the bacterial load in response to the provided antimicrobial as represented by the gradient of the line will probably be quite similar in different patients. After all, all patients were treated either with an intravenous β-lactam antibiotic with or without aminoglycoside or with oral ciprofloxacin. These antibiotics have an excellent bioavailability and tissue concentrations well above the minimum inhibitory concentrations of the causative uropathogen have been reached, except in patients with pyonephrosis or renal abscess.

If the hypothetical model is correct, then the duration of symptoms of the infection will depend on the initial disease severity and may be assessed at first presentation. One could have predicted that our biomarker approach might have been successful in guiding treatments up to one or a few days after clinical recovery. The biomarker signature, however, lacked the ability to do so after the 7 days of treatment, when a strong margin surpasses the time for the biomarker signature to return to normal. Differences between patients in severity of illness at presentation and corresponding biomarker levels are likely to have normalized after 7 days of treatment, and definitely after 14 days.

If we look at all patients who participated in the PRACTICE study (validation and interventional cohort) and the FUTIRST trial combined (N = 1485),[13,24] the mean time to defervescence after the start of treatment was 2.3 days (SD 1.8). Once on antibiotic treatment, 40%, 68%, and 85% of the patients become afebrile within 1, 2, and 3 days, respectively.

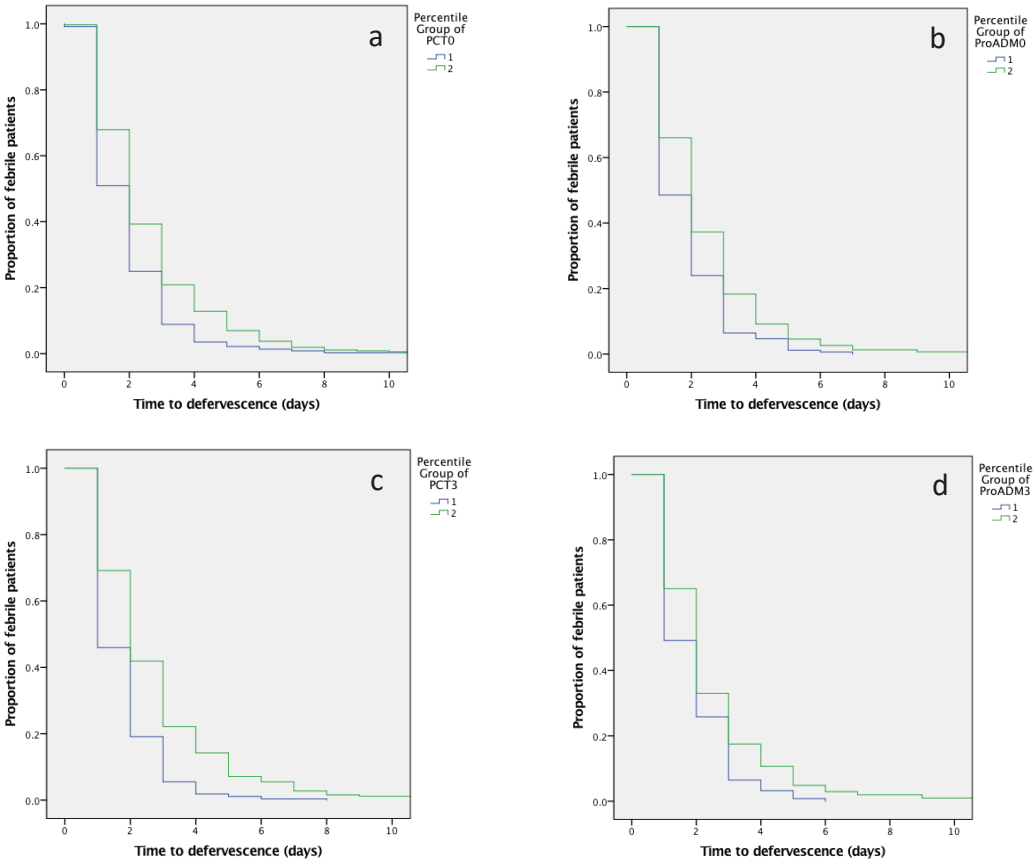
The time to defervescence is not correlated to duration of fever before presentation, as illustrated by the survival plot for two percentile groups in Figure 2a. Apparently, the bacterial load and the intensity of the provoked systemic inflammatory response before patients seek medical consultation, is not directly proportional to the duration of the infection. Patients with bacteremia and probably a higher bacterial load, needed more time to become afebrile after the start of treatment (mean fever duration 2.8 (SD 2.2) days in patients with bacteremia vs. 2.1 (SD 1.5) days in patients without bacteremia, $p < 0.01$; Figure 2b).

Figure 2. Survival function for the different levels of percentile group of a. duration of fever (hours) before medical consultation (N = 765, median 26, IQR 12 - 60, P = 0.24) and b. patients with (N = 232, 26%) and without (N = 660, 74%) bacteremia (P < 0.01).



As described in **Chapter 5**, the biomarkers PCT and MR-proADM, assessed at presentation and after three days of treatment, were also positively correlated with the time to defervescence in this larger study population. The strongest relationships were seen between PCT and time to defervescence (PCT at presentation t = 0.17, P < 0.01; PCT assessed at day 3 t = 0.22, P < 0.01). This is illustrated by the survival plots in Figure 3.

Figure 3. Survival function for the different levels of percentile group of a. PCT concentrations measured at presentation (N = 747, median 0.43, IQR 0.13 - 1.84, P < 0.01); b. MR-proADM concentrations measured at presentation (N = 324, median 0.95, IQR 0.68 - 1.47, P < 0.01); c. PCT concentrations measured at day 3 (N = 525, median 0.23, IQR 0.08 - 1.04, P < 0.01); d. MR-proADM concentrations measured at day 3 (N = 227, median 0.69, IQR 0.52 – 0.98, P < 0.01).



Finding the optimal time point between T1 and T3 (as close to T1 as possible) in our model in Figure 1, is key to minimize antibiotic treatment duration. It is likely that after the start of antimicrobial treatment, when the bacterial count decreases to below the threshold at which a fever response is provoked, the patient becomes afebrile while there are still viable bacteria present. If treatment duration is too short, these bacteria may grow back above the threshold level of symptoms and cause prompt relapse of disease. Measurement of bacterial compounds such as lipopolysaccharide (LPS) or bacterial DNA will not differentiate between viable bacteria that can cause relapse, or residue of killed bacteria after exposure to antibiotics. Therefore, it is advisable to use a biomarker that reflects both bacterial load and host response for guidance on treatment duration.

Based on our data, as well as previous experience in patients with acute respiratory tract infections and critically ill patients admitted to the ICU ward,[38,39] PCT appears to be the most

promising marker to help minimize antibiotic treatment duration in fUTI. Obviously, a prospective clinical trial including sufficient study subjects with community-acquired febrile urinary tract infection would be the next step forward in this matter.

Complex and multidrug-resistant urinary tract infections

The management of patients with recurrent urinary tract infections is challenging, even more so in the era of rising antimicrobial resistance. Multidrug-resistance is leading to an increased need for intravenous treatment of UTIs with last-resort antibiotics and subsequent hospitalizations. Prophylaxis with low dose oral antibiotics, as recommended by current guidelines, is often limited by multidrug-resistance of uropathogens, and may even extend the development of resistance. In patients with recurrent UTI due to multi-drug resistant uropathogens, intravesical gentamicin instillation is a valuable treatment option for either the suppression or prevention of UTI. Locally administered aminoglycosides circumvent systemic toxicity while development of antimicrobial resistance is unlikely because of high levels of the antibiotic in the urine and lack of antibiotic pressure on commensal gut flora. In **Chapter 6** the effectiveness, safety, and feasibility of prophylactic treatment with intravesical gentamicin after self-catheterization are described in patients with refractory recurrent urinary tract infections caused by multidrug-resistant (MDR) microorganisms.

We found that overnight intravesical gentamicin instillation reduced the number of UTI episodes in these patients, was well accepted, feasible, and safe. Systemic uptake of gentamicin was not detected, and no relevant side effects were reported. Although the number of infections was significantly reduced, there were nonetheless patients who had 'breakthrough infections'. The mean number of UTI episodes during six months of treatment was 1.0 (SD 1.2), which was a reduction of 79%. A total of eight patients stopped the prophylactic treatment because of clinical failure due to various causes. In two patients refractory to gentamicin instilments, other causes for their urinary symptoms were diagnosed. Three patients experienced recurrent exogenous reinfections (different uropathogens) with microorganisms less sensitive or resistant to gentamicin, such as *Enterococcus faecalis* and *Candida albicans*. The remaining three patients had recurrent or persistent symptoms and positive cultures with the same gentamicin-sensitive micro-organism consistent with an endogenous focus: two male patients with suspected chronic bacterial prostatitis, and one female patient with a suspected endogenous focus in the upper urinary tract (outside the reach of the antibiotic agent). Two other patients did not have clinical failure; yet, they had persistent asymptomatic positive cultures under gentamicin instillation and were found to have infected kidney stones. In all of these patients, failure of gentamicin instilments became apparent within six weeks. Therefore, a trial period of six weeks of gentamicin prophylaxis seems reasonable. In case of persistent urinary symptoms, further diagnostic evaluation is warranted. Apparently, intravesical gentamicin can also be used for diagnostic purposes to localize the site of bacterial persistence. In patients with persistent gentamicin-sensitive bacteriuria between instillations, a source in the upper urinary tract is likely, and further analysis should be performed, including imaging of the urinary tract and urine cultures obtained by selective sampling of both ureters.

The incidental breakthrough infections in the other patients may have been due to our treatment protocol, in which the frequency of instilments was reduced after two weeks from daily to every other day, and later to twice a week. Apparently, the short interval of one or two days between instillations is enough for the colonization with new bacteria that are not eradicated by the next antibiotic instilment. This finding could not be explained by resistance to gentamicin, and often the symptoms of UTI cleared up after seven days of consecutive daily instilments.

Treatment with overnight gentamicin instilments also had a positive effect on antimicrobial resistance. The percentage of MDR pathogens dropped from 79% to 24% after start of gentamicin prophylaxis, without an increase in resistance to gentamicin. The observed decrease in the antibiotic resistance in the breakthrough UTIs may be attributed to the decrease in the overall oral antibiotic use. More than half (56%) of the patients did not need to use any systemic antibiotics during the prophylactic treatment period and, due to the more favorable susceptibility profile, the majority of patients that needed systemic antimicrobial treatment, could be treated with first-line oral antibiotics.

Unfortunately, our attempt to perform a randomized, controlled trial failed because of the lack of patients willing to participate in a randomized trial that held the risk of being assigned to standard treatment (oral prophylaxis) that had failed them before. The study design was therefore changed to a prospective non-controlled trial. Obviously, a randomized controlled trial would have provided more insight into the efficacy of the treatment. It should be noted that the study participants represent a very specific population, with a high rate of complex urologic comorbidities, such as patients with renal transplant or neobladder, and that 59% of patients were on intermittent catheterization. In the majority of patients, treatment was complicated by multidrug-resistance or allergy to several oral antibiotics. Our data provide evidence that overnight intravesical gentamicin instilments are an effective and safe treatment option for complex and refractory cases, but not until after the standard management as advised by the guidelines has been attempted.[40]

Recurrent UTI by the same strain can arise from re-infection by bacteria that derive from the patients intestinal flora. In case of recurrent MDR infections, targeting the primary (intestinal) niche by decolonization may be a valuable approach to prevent relapsing infections. In **Chapter 7** a case of a 34-year old diabetes patient on peritoneal dialysis is described. This patient had recurrent urinary tract infections caused by a Verona Integron-encoded Metallo-β-lactamase (VIM)-positive *Pseudomonas aeruginosa*.[41] Because the only antibiotic available for systemic use (colistin, MIC 4 mg/ml) is nephrotoxic, a planned kidney-pancreas transplantation was considered contraindicated due to high infection risk and the patient was removed from the transplantation waiting list.

After chronic prostatitis or colonization at other sites than the gut were ruled out, he was treated with overnight intravesical gentamicin instilments and fecal microbiota transfer (FMT) in an attempt to eradicate MDR *Pseudomonas aeruginosa* from the intestinal tract. This treatment prevented recurrences of urinary tract infections with multidrug-resistant (MDR) *P. aeruginosa*,

but failed to eradicate intestinal colonization with MDR *E. coli*. Our findings contradict previous positive reports on FMT for decolonization of drug-resistant enterobacteriaceae.[42]

Microbiota analysis showed that our patient had intact microbiota diversity and composition at phylum level prior to FMT, in contrast to the diminished microbiota seen in patients with recurrent *Clostridium difficile* infection (CDI). Previous antibiotic treatment had not resulted in a distinct disturbance of the intestinal flora. Only minor changes of the microbiota composition were observed after FMT with a slight increase of cyanobacteria and tenericutes. We hypothesize that diminished diversity appears not to play a role in MDR carriership as opposed to recurrent CDI. Therefore, one might question the efficacy of fecal transplantation in patients with a normal microbiota diversity.

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are a major concern worldwide and reported prevalence in clinical isolates is increasing. Urinary tract infections are the most common clinical manifestations of ESBL-producing *Escherichia coli*. The risk among healthy asymptomatic carriers of ESBL-producing *E. coli* to develop clinical infections due to colonizing strains is not yet well known. **Chapter 8** describes extended spectrum cephalosporin-resistant *E. coli* isolates from patients with urinary tract infection, broilers (meat chickens), individuals living and/or working on broiler farms, and individuals in the general population.[43] Multivariate analysis, based on ESBL/plasmidic-AmpC resistance genes, virulence profiles, and phylogenetic groups, revealed that most UTI isolates formed a clearly distinct group. The results show that transmission occurs between broilers and individuals on broiler farms, but also indicate that the role of broilers as a source of foodborne transmission of ESC-resistant *E. coli* to the general population and as cause of urinary tract infections is likely relatively small.

In the past few years, automated, standardized, quantitative urine analysis has been introduced in clinical practice and has shown high efficiency and accuracy compared to traditional sediment analysis. In **Chapter 9**, a retrospective case record study is presented that established a diagnostic scoring tool based on the combination of one of these relatively new automated urine analysis (IRIS Diagnostics iQ200 Elite) and clinical signs.[44] This scoring tool could rule out urinary tract infection in the majority of patients in the derivation cohort, and therefore could potentially improve antibiotic stewardship in suspected UTI cases. Validation in a new cohort of patients is required before the score can be implemented in clinical practice.

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NEDERLANDSE SAMENVATTING

Urineweginfecties zijn een van de meest voorkomende reden voor medische consultatie binnen de infectieziekten. De meerderheid van de urineweginfecties beperkt zich tot de blaas (blaasontsteking, ofwel cystitis) en behoort tot de dagelijkse praktijk van de eerstelijnszorg. Blaasontsteking bij verder gezonde patiënten is vaak 'self-limiting', en in het geval dat antimicrobiële behandeling nodig is, is een korte kuur meestal voldoende.[1-3] Bij patiënten met onderliggende urologische afwijkingen of terugkerende cystitis, is de behandeling vaak meer complex, zeker indien de infecties veroorzaakt worden door bacterien die verminderd gevoelig zijn voor antibiotica.[4]

Acute cystitis moet worden onderscheiden van infecties met uitbreiding buiten de blaas, zoals acute nierbekkenontsteking (pyelonefritis) en prostatitis. Bij deze vormen van urineweginfectie is er sprake van weefselinvasie, hetgeen wordt gekenmerkt door koorts of andere systemische symptomen.[5] Koorts is weinig specifiek, en kan zowel een aanwijzing zijn voor lokale weefselontsteking bij pyelonefritis, als van een dreigende urosepsis. Snelle herkenning en start van adequate antimicrobiële behandeling van deze laatste aandoening is essentieel om progressie naar septische shock met mogelijk fatale afloop te voorkomen. Echter, de meerderheid van patiënten met een febriele urineweginfectie presenteert zich met een milde ziekte die goed reageert op behandeling met antibiotica.

Het eerste deel (**Hoofdstuk 2 tot 5**) van dit proefschrift richt zich op onderzoeksvragen over de behandeling van patiënten met een febriele urineweginfectie (fUWI). Het tweede deel gaat over urineweginfecties door bijzonder resistente micro-organismen (BRMO's) (**Hoofdstuk 6 tot 8**), en over de diagnostiek van urineweginfecties (**Hoofdstuk 9**).

Optimale triage strategie: wanneer moet een patiënt met een febriele urineweginfectie worden opgenomen in het ziekenhuis?

Hoewel patiënten die zich op de Spoedeisende Hulp (SEH) presenteren met een fUWI over het algemeen een laag risico hebben op een gecompliceerd beloop, worden het merendeel opgenomen in het ziekenhuis voor observatie. Kennelijk is de drempel om patiënten in het ziekenhuis op te nemen laag, vanwege de kleine kans op levensbedreigende complicaties die niet betrouwbaar kunnen worden voorspeld. Dit suggereert dat veel van deze opnames mogelijk vermeden kunnen worden, en dat deze praktijk leidt tot overbehandeling van laagrisicopatiënten en daarmee tot hogere gezondheidszorgkosten.[6,7] Betrouwbare beoordeling van de ziekte-ernst bij presentatie en inschatting van de kans op ziekteprogressie zijn cruciaal voor het instellen van een gepersonaliseerde behandeling op de meest geschikte locatie (thuis of in het ziekenhuis).

Thuisbehandeling wordt aanbevolen voor mild zieke patiënten zonder misselijkheid of braken, en zonder andere actuele medische problematiek waarvoor opname geïndiceerd zou kunnen zijn.[3,8] Wetenschappelijk onderzoek om deze aanbeveling te ondersteunen is beperkt, en richt zich veelal slechts op thuisbehandeling van verder gezonde vrouwen van

jonge tot middelbare leeftijd met fUWI.[9-11] Voor luchtweginfecties zijn er gevalideerde klinische hulpmiddelen, zoals de '*Pneumonia Severity Index*' (PSI), om het risico op klinische achteruitgang of overlijden te berekenen, om de arts te ondersteunen in de beslissing of een patiënt wel of niet in het ziekenhuis moet worden opgenomen.[12] Een dergelijk klinisch hulpmiddel om de hoog-risico-patiënten te identificeren die baat hebben bij een ziekenhuisopname is niet beschikbaar voor fUWI. De voorspellende factoren voor sterfte in de PSI, zoals leeftijd, co-morbiditeit, en fysische of laboratorium tekenen van sepsis zijn niet specifiek voor longontsteking. Deze predictiescore zou daarom ook geschikt kunnen zijn voor andere infectieziekten opgelopen buiten het ziekenhuis, anders dan longontsteking.

In **Hoofdstuk 2** wordt de ontwikkeling en validatie beschreven van de klinische beslisregel '*Prediction Rule for Admission policy in Complicated urinary Tract InfeCtion LEiden*' (PRACTICE)', die werd afgeleid van de PSI.[13] Deze beslisregel werd uitgerekend voor een cohort van 787 opeenvolgende patiënten met fUWI, en bleek in staat patiënten te identificeren met een zeer laag risico voor opname op de 'Intensive Care Unit' (ICU) of voor overlijden binnen 30 dagen. Dit risico was zeer laag (< 2,5%) bij patiënten met een PRACTICE-score onder de 100, maar toch werd 60% van de patiënten in deze groep opgenomen in het ziekenhuis.

Vervolgens werd de toepassing van de PRACTICE op het opname beleid onderzocht in een nieuwe groep patiënten op zeven SEHs in de Leids-Haagse regio. Dit gerandomiseerde onderzoek had een 'stepped wedge cluster'-design, waarbij alle deelnemende ziekenhuizen begonnen met een controleperiode, waarin het standaardbeleid met betrekking tot de opnamebeslissing van patiënten met fUWI werd uitgevoerd. Bij de start van de interventieperiode, die in alle deelnemende centra op willekeurige volgorde werd ingevoerd, werden de SEH-artsen geïnstrueerd om de PRACTICE-score uit te rekenen voor elke geïncludeerde patiënt. Gebaseerd op de score van de patiënt, werd de volgende aanbeveling gedaan: ziekenhuisopname voor hoog-risicopatiënten (> 100 punten) en ontslag naar huis voor laag-risicopatiënten (< 75 punten). Bij voorkeur werd het opnamebeleid gestuurd door de PRACTICE, maar uiteindelijk was de behandelend arts verantwoordelijk voor de beslissing op te nemen of thuis te behandelen, en was het toegestaan af te wijken van de PRACTICE-aanbevelingen.

Onze hypothese dat het gebruik van deze beslisregel zou leiden tot een lager opnamepercentage werd bevestigd in deze studie, zoals blijkt uit de 20% absolute afname van het aantal opnames. Maar, tot onze verrassing werden 10 van de patiënten (27%) die initieel thuis werden behandeld voor hun vermoedelijke fUWI, later alsnog in het ziekenhuis opgenomen. Alle patiënten met een 'secundaire opname', werden na een korte en ongecompliceerde ziekenhuisopname weer naar huis ontslagen. Dit secundaire opnamepercentage overschreed het de vooraf vastgelegde veiligheidsmarge, en daarom werd besloten op advies van de 'Data Safety Monitoring Board' de studie na inclusie van 370 patiënten te staken.

Vier van de tien heropgenomen patiënten (11% van de thuis behandelde groep) kwamen op eigen initiatief terug naar het ziekenhuis, vanwege toename van ziekte, misselijkheid of ontregelde bloedsuikers. De 6 anderen werden één dag na presentatie door de behandeld arts opgeroepen

voor opname vanwege een positieve bloedkweek: 2 patiënten met een goed gevoelige *E coli*, hoewel deze opnames wellicht vermeden had kunnen worden omdat de orale behandeling met ciprofloxacin niet onder doet voor intraveneuze behandeling.[14] Vier andere patiënten werden teruggeroepen naar het ziekenhuis omdat bij het positief worden van de bloedkweek (met *S parathyphi*, *S aureus* of Streptococcen, n=2) bleek dat er bij het bezoek aan de SEH een verkeerde diagnose was gesteld. Hoewel deze patiënten bij eerste presentatie door de SEH-arts werden verdacht van een pyelonefritis en leken te voldoen aan de opgestelde inclusiecriteria, was er sprake van een bacteriemie vanuit een andere bron, waarvoor een andere behandeling geïndiceerd was.

Dergelijke diagnostische dwaling behoort tot de dagelijkse praktijk, en onze studie onderstreept het belang van de validatie van klinische voorspellingsregels in een nieuw patiëntencohort om de waarde van toepassing in de klinische praktijk te testen.

Blijkbaar is het stellen van de diagnose fUWI niet zo eenvoudig. Andere koortsende ziekten kunnen overeenkomstige algemene symptomen geven, en het is van belang te realiseren dat de aanwezigheid van leukocyturie of bacteriurie, hetgeen met name bij de oudere patiënt zeer frequent asymptomatisch voorkomt, de aandacht kunnen afleiden van de juiste diagnose. Achteraf bezien is het initiële cohort van 787 patiënten met fUWI waarin de PRACTICE regel werd berekend geen werkelijke afspiegeling van de dagelijkse praktijk geweest, omdat patienten die een dag later toch een andere diagnose bleken te hebben werden geexcludeerd.

Verbetering van de diagnose urineweginfectie is dus nodig, niet alleen om de behandeling van pyelonefritis te optimaliseren en op veilige wijze de implementatie van klinische beslisregels te kunnen implementeren, maar ook vanuit het oogpunt van 'antimicrobial stewardship'. Het is van belang onderscheid te maken tussen asymptomatische bacteriurie (ASB) en een (per definitie symptomatische) urineweginfectie, omdat antibiotische behandeling van ASB niet geïndiceerd is, behalve voor zwangere vrouwen en patiënten die urologische procedures ondergaan.[15] In alle andere gevallen leidt de antimicrobiële behandeling van ASB slechts tot onnodige bijwerkingen, potentiële geneesmiddelinteracties, onnodige kosten, en, belangrijker, tot de ontwikkeling en verspreiding van antibioticaresistentie.[16] Onnodige behandeling van ASB komt frequent voor in de spoedeisende zorg en varieert van 20% bij patiënten in de leeftijd > 12 jaar, tot 43% bij ouderen.[17-19] Op dit gebied valt duidelijk veel winst te behalen in vermindering van het antibioticumgebruik.

Hoe kan de beslisregel PRACTICE voor het opnamebeleid worden geoptimaliseerd?

De prognose van de patiënt die zich presenteert met een ernstige infectieziekte is afhankelijk van twee factoren. In de eerste plaats is de ernst van de acute afweerreactie op de infectie van belang, en de daaruit voortvloeiende ontstekingscascade die uiteindelijk kan leiden tot shock en multi-orgaanfalen, met potentieel fatale afloop in de eerste fase. Bij de patienten die de eerste dagen van de ziekte overleven, speelt de algemene gezondheidstoestand van de patiënt (voornamelijk bepaald door leeftijd en comorbiditeit) een grotere rol bij de kans op sterfte binnen 30 dagen.

Aangezien de PRACTICE was gebaseerd op de 30-dagen sterfte in het validatiecohort, is het mogelijk dat de acute afweerreactie onvoldoende gerepresenteerd wordt in deze beslisregel. Juist in deze eerste acute fase van de infectie, bestaat de behandeling bij ziekenhuisopname naast het starten met antibiotica, uit toediening van infuusvloeistoffen om de bloeddruk op peil te houden.

Daarom onderzochten wij in **Hoofdstuk 3** de hypothese dat de PRACTICE verbeterd kan worden door de toevoeging van een objectieve bloedtest die de ernst van de sepsis representeert, zoals procalcitonine (PCT), midregional pro-adrenomedulline (proADM) of C-reactief proteïne (CRP).[20] De combinatie van de PRACTICE met een dergelijke biomarker, is mogelijk beter in het identificeren van die patiënten die in de acute fase van een ziekenhuisbehandeling profiteren, en leidt daarom potentieel tot minder heropnames. Om deze vraag te beantwoorden hebben wij een secundaire analyse uitgevoerd van het onderzoek dat beschreven werd in **Hoofdstuk 2**.[21] In deze analyse werden patiënten geïncludeerd waarvan een bloedmonster beschikbaar was voor biomarkerbepaling, afgenomen bij presentatie op de SEH (N = 313).

Hierbij bleek proADM van alle biomarkers de sterkste voorspellende waarde te hebben voor gecompliceerd beloop van febriele urineweginfectie. Combinaties van proADM met de PRACTICE, of met andere biomarkers wisten deze voorspellende waarde niet significant te verbeteren.

Concentraties van zowel proADM als PCT waren hoger bij patiënten die werden opgenomen in het ziekenhuis in vergelijking met degenen die in poliklinische setting werden behandeld. Ook in de subgroep van patiënten die aanvankelijk thuis werden behandeld maar later alsnog in het ziekenhuis moesten worden opgenomen, was proADM was significant verhoogd ten opzichte van degenen die de gehele behandeling thuis afmaakten. PCT-concentraties waren hierbij niet onderscheidend.

De biomarker CRP, die in de huidige klinische praktijk bij alle patiënten met fUWI routinematig bepaald wordt, had geen enkele voorspellende waarde in deze studie.

Omdat de combinatie van de PRACTICE en de bepaling van proADM niet beter was in het identificeren van patiënten die baat hebben bij ziekenhuisopname dan proADM alleen, hebben wij vervolgens het effect van gebruik van proADM bij de opnamebeslissing onderzocht in een virtuele triage analyse.

Bij een door proADM gestuurde triage met een afkappunt van 0,80 nmol/L, had het aantal ziekenhuisopnames met 24% verlaagd kunnen worden, en zou een groter deel van de patiënten op veilige wijze thuis behandeld kunnen worden. Gebruik van deze afkapwaarde zou laag-risicopatiënten hebben geïdentificeerd, waarin geen sprake was van 30-dagen sterfte, geen ICU-opname en slechts 2% heropnames van de thuis behandelde patiënten. Ook alle patiënten met een bacteriemie vanuit een andere bron dan fUWI, zoals beschreven in **Hoofdstuk 2**, zouden hierbij zijn geselecteerd voor ziekenhuisopname.

Hieruit concluderen wij dat proADM met een afkapwaarde van 0,8 nmol/L een goede kandidaat is om de triage van fUWI patiënten te sturen, maar uiteraard dient deze interventie prospectief te worden onderzocht in een gecontroleerde klinische studie. Bovendien moet elke beslissing op basis van een biomarker of triage algoritme kritisch worden afgestemd op de

individuele patiënt. Factoren zoals comorbiditeit, therapietrouw, gebrek aan mantelzorg, of kans op antimicrobiële resistentie moeten worden overwogen bij het uiteindelijke besluit.

Optimale behandelingsduur: hoe lang moet een patiënt met een febriele urineweginfectie worden behandeld?

Antibiotica zijn onmisbaar in de huidige gezondheidszorg, maar het overmatig gebruik leidt tot verminderde gevoeligheid van bacteriën. Deze antimicrobiële resistentie is een toenemende en serieuze bedreiging voor de volksgezondheid, en daarom is het essentieel zo efficient mogelijk om te gaan met de beschikbare antimicrobiële middelen. De bepaling van de optimale duur van antibiotische behandeling is een eenvoudige en effectieve strategie om dit probleem aan te pakken, waarbij het adagium "hoe korter, hoe beter" geldt. De duur van de behandeling van febriele UWI of acute pyelonefritis, is gebaseerd op eerder onderzoek dat met name gericht was op jonge, anderszins gezonde vrouwen.[22] Daardoor is er weinig goede wetenschappelijke onderbouwing voor de optimale behandelingsduur van UWI bij mannen, ouderen, ziekenhuispatiënten, en patiënten met comorbiditeit of bacteriëmie. Om deze reden hebben wij een gerandomiseerde placebo-gecontroleerde multicenter onderzoek uitgevoerd (**Hoofdstuk 4)** om te bepalen of een 7-daagse kuur ciprofloxacin qua werkzaamheid en veiligheid gelijkwaardig is aan een 14-daagse kuur in een niet-geselecteerde populatie van zowel mannen als vrouwen.[23] Voor dit onderzoek werden op-een-volgende patiënten met een febriele urineweginfectie (ontstaan in de thuissituatie), geïncludeerd in regionale ziekenhuizen en huisartsencentra. Deze studie toonde dat vrouwen met een fUWI veilig en effectief behandeld kunnen worden met 7 dagen ciprofloxacin in plaats van 14 dagen, met inbegrip van ouderen met ernstige comorbiditeit, en ongeacht de ziekte-ernst bij presentatie. Bij mannen echter, was een 7-daagse behandeling inferieur aan een 14-daagse kuur, te zien aan een hoger percentage van klinisch (14% vs. 2%) en bacteriologisch falen, gemeten 2-3 weken na het einde van de behandeling. Opvallend genoeg, was het verschil in klinisch falen niet meer aanwezig bij een langere follow-upduur (bij controle 70-84 dagen na behandeling). Met andere woorden, noodzaak voor aanvullende behandeling met antibiotica was gelijk bij langere follow-upduur, onafhankelijk van de duur van de initiële kuur (7 of 14 dagen). Deze bevinding is mogelijk te verklaren doordat bij een febriele urineweginfectie bij een man er vaak ook sprake is van prostatitis, hetgeen een bekende oorzaak is van recidieven van UWI ondanks adequate antibiotische behandeling.[24]

Kunnen biomarkers gebruikt worden om de optimale duur van de antibiotische behandeling bij febriele urineweginfecties te bepalen?

Uit onderzoek blijkt dat de biomarker procalcitonine behulpzaam is bij de bepaling van de duur van de antibiotische behandeling bij infecties van de luchtwegen en sepsis.[27-29] Ook is aangetoond dat PCT een voorspeller is van bacteriëmie bij patiënten met een fUWI,[30-32] maar er is weinig bekend over de waarde van PCT bij het sturen van de behandelduur bij fUWI. De biomarker MR-proADM is een voorspeller van een gecompliceerd ziekteverloop bij fUWI, IC-opname en mortaliteit,[20,33] maar de toepassing hiervan is nog nooit onderzocht bij bepaling van de behandelduur.

In **Hoofdstuk 5** onderzochten wij of PCT, gemeten op dag 0 en 3, beter in staat is patiënten te identificeren met een risico op falen van de antimicrobiële behandeling, in vergelijking met proADM en CRP. Falen van de behandeling was gedefinieerd als het persisteren van klachten (koorts of symptomen van UWI) of een nieuwe antibiotische behandeling voor UWI binnen de follow-up van 30 dagen na start van de behandeling. Uit dit onderzoek bleek dat de biomarkers PCT en proADM een significante correlatie vertoonden met parameters die de ziekte-ernst van fUWI weerspiegelen, zoals de temperatuur, de aanwezigheid van aanwezigheid van bacteriëmie, koude rillingen, en de noodzaak voor de initiële toediening van intraveneuze antibiotica (in plaats van oraal). Ook was het verloop van PCT en proADM van dag 0 tot 3 gecorreleerd aan tekenen van klinisch herstel, zoals tijd tot het verdwijnen van de koorts, en de opnameduur in het ziekenhuis. In tegenstelling tot PCT en proADM, vertoonde de populaire biomarker CRP geen enkele correlatie met relevante klinische parameters.

Hoewel deze bevindingen veelbelovend lijken voor het potentiële gebruik van PCT en proADM om de clinicus te ondersteunen bij het bepalen van de duur van antibiotische therapie, waren geen van beiden in onze studie in staat de patiënten te identificeren waarbij de behandeling faalde. Dit is waarschijnlijk te wijten aan het feit dat alle patiënten in onze studie gedurende ten minste 7 dagen werden behandeld met antibiotica en het succes van de behandeling al zeer hoog was (gemiddeld 89% van de patiënten herstelden na behandeling).

De empirische behandelduur is in het verleden gebaseerd op de verwachte tijd tot klinisch herstel, rekening houdend met de interindividuele variabiliteit van de ziekte-ernst bij het begin van de behandeling door een 'veiligheidsmarge' van enkele dagen aan de gemiddelde hersteltijd toe te voegen. Acute pyelonefritis werd traditioneel gedurende 6 weken met antibiotica behandeld, totdat een onderzoek in 1987 aantoonde dat een 2-weekse kuur even effectief was.[34] Pas recent hebben aanvullende studies, waaronder de onze beschreven in **Hoofdstuk 4**, bewijs geleverd voor een kortere behandeling,[22,23,25] en dit heeft al geleid tot de invoering van een 7-daags-behandelschema in de dagelijkse praktijk voor alle vrouwelijke patiënten met fUWI. [8] Er zijn in enkele studies zelfs aanwijzingen voor een nog kortere behandeling met fluoroquinolonen voor milde tot matige pyelonephritis.[35,36] Helaas kunnen wij door onze studie-opzet de vraag of een subgroep van (waarschijnlijk vrouwelijke) patiënten korter dan 7 dagen had kunnen worden behandeld, niet beantwoorden. Mogelijk zou een biomarkergestuurde aanpak succesvol kunnen zijn bij het bepalen van behandelduur tot één of enkele dagen na klinisch herstel. Het biomarkerbeloop is kennelijk niet voorspellend wanneer de behandeling al 7 dagen duurt, aangezien de verschillen tussen patiënten in ziekte-ernst bij presentatie en de daaraan gerelateerde biomarker concentraties waarschijnlijk al zijn genormaliseerd na 7 dagen behandeling, en zeker na 14 dagen.

Op basis van onze gegevens, in overeenstemming met eerdere bevindingen bij patiënten met luchtweginfecties en sepsis,[37,38] is PCT de meest veelbelovende marker om de antibioticumduur bij fUWI te verkorten. Uiteraard moet de werkelijke waarde van PCT op dit gebied in de dagelijkse praktijk in toekomstige prospectieve klinische studies nader worden onderzocht.

Complexe urineweginfecties en infecties veroorzaakt door multiresistente verwekkers

De behandeling van patiënten met wederkerende urineweginfecties is een uitdaging, met name in het huidige tijdperk van toenemende antimicrobiële resistentie. Door multidrug-resistentie is het vaker noodzakelijk om patiënten met een urineweginfectie (zelfs met een simpele blaasontsteking!) op te nemen in het ziekenhuis voor intraveneuze behandeling met antibiotica (frequent uit een reserveklasse). De huidige richtlijnen adviseren om bij recidiverende urineweginfecties (UWIs), wanneer levensstijladviezen en niet-antibiotische maatregelen onvoldoende effect hebben, een dagelijkse preventieve lage dosis orale antibiotica te geven. Deze keuzemogelijkheden voor deze antibiotische profylaxe worden beperkt door de reeds aanwezige antimicrobiële resistentie, en het gebruik van deze orale antibiotica draagt door de antibiotische druk op de darmbacteriën zelfs bij aan verder toenemende resistentie.

Gentamicine-blaasspoelingen zijn een waardevolle behandeloptie bij patiënten met terugkerende UWIs veroorzaakt door multiresistente bacteriën. Door deze aminoglycoside-antibiotica lokaal in de blaas (intravesicaal) toe te dienen, worden de schadelijke bijwerkingen (schade aan gehoor en nierfunctie) die optreden bij de intraveneuze toediening omzeild. Daarnaast is het ontstaan van antimicrobiële resistentie onwaarschijnlijk door de hoge concentratie van het antibioticum in de urine, en het ontbreken van antimicrobiële druk op de darmflora. In **Hoofdstuk 6** wordt de effectiviteit, veiligheid en haalbaarheid beschreven van profylactische behandeling met intravesicale gentamicine na zelf-katheterisatie bij patiënten met hardnekkige wederkerende UWIs veroorzaakt door multiresistente (MDR) micro-organismen.

Behandeling met gentamicine-blaasspoelingen verminderde het aantal UWIs, was veilig, haalbaar en werd door de patiënten als goed beoordeeld. Er was geen sprake van opname van gentamicine in de bloedbaan, en er werden geen relevante bijwerkingen gerapporteerd. Hoewel het aantal infecties significant werd verminderd, waren er wel patiënten die 'doorbraakinfecties' hadden. Het gemiddelde aantal UWI gedurende 6 maanden behandeling was 1,0 (SD 1,2), een vermindering van 79% ten opzichte van vóór de blaasspoelingen. Acht patiënten (9%) stopten voortijdig met de behandeling vanwege klinisch falen door verschillende oorzaken. Bij twee patiënten die geen verbetering bemerkten door de blaasspoelingen, werd een alternatieve oorzaak voor hun symptomen (anders dan infectie) vastgesteld. Drie patiënten hadden tijdens de behandeling nog steeds last van frequente UWIs, door micro-organismen minder gevoelig of resistent voor gentamicine, zoals *Enterococcus faecalis* en *Candida Albicans*. De drie resterende patiënten hadden terugkerende of aanhoudende symptomen èn positieve urinekweken met dezelfde gentamicine-gevoelige verwekker. Hierbij is een persisterende bron van infectie in de urinewegen aannemelijk: twee mannelijke patiënten met een vermoedelijke chronisch bacteriële prostatitis, en een vrouwelijke patiënt met een vermoedelijke bron in de hogere urinewegen (buiten het bereik van het antibioticum).

Bij twee andere patiënten was geen sprake van klinisch falen, zij hadden namelijk geen klachten tijdens de gentamicine-blaasspoelingen, maar hielden wel continue positieve urinekweken onder de behandeling. Ook bij deze 2 patiënten bleek sprake van een persisterende bron van

infectie in de hogere urinewegen, namelijk geïnfecteerde nierstenen. Na het verwijderen van deze nierstenen werden de urinekweken negatief.

Bij al deze patiënten (met klinisch falen, of met persisterend positieve kweken) was het binnen 6 weken duidelijk dat er minder effect was van de gentamicinebehandeling. Op basis daarvan adviseren wij een proefperiode met profylactisch gentamicine van 6 weken aan te houden. In geval van aanhoudende klachten of persisterend positieve kweken (met een gentamicine-gevoelige verwekker) is aan vullend onderzoek naar een bron in de (hogere) urinewegen gerechtvaardigd (inclusief beeldvorming van de nieren en selectieve urinekweken uit beide urineleiders). Gentamicine-blaasspoelingen kunnen dus ook gebruikt kunnen worden voor diagnostische doeleinden, namelijk om de locatie van een persisterende bacteriële bron vast te stellen.

De behandeling met intravesicaal gentamicine had ook een positief effect op de antimicrobiële resistentie. Het percentage MDR-pathogenen daalde van 79% tot 24% na start van de behandeling, zonder dat een toename van gentamicine-resistentie gezien werd. Deze afname van resistentie van verwekkers van doorbraakinfecties is waarschijnlijk te verklaren door de reductie van het orale antibioticagebruik. De meerderheid van de patiënten (56%) gebruikte geen systemische antibiotica tijdens de gentamicine-blaasspoelingen, en bij de meeste patiënten met een doorbraakinfectie waarbij systemische behandeling wel nodig was, kon vanwege het gunstigere gevoeligheidsprofiel van de verwekkers worden volstaan met orale eerste-lijns-antibiotica.

Uiteraard zou een gerandomiseerd, gecontroleerd onderzoek meer inzicht hebben verstrekt in de werkzaamheid van de preventieve gentamicine-blaasspoelingen. Helaas is onze opzet om een dergelijk gecontroleerde trial uit te voeren niet geslaagd doordat wij te weinig patiënten bereid hebben gevonden deel te nemen aan een gerandomiseerd onderzoek waarbij zij het risico liepen te loten voor de standaardbehandeling (orale antibiotische profylaxe) die eerder bij hen gefaald had. Om deze reden hebben wij de opzet van het onderzoek omgezet naar een prospectieve niet-gecontroleerde trial, waarbij de periode voor de start van de behandeling als interne controle gebruikt werd.

Het is van belang te realiseren dat de deelnemers aan dit onderzoek tot een zeer specifieke patiëntengroep behoorden, met een hoog percentage complexe urologische aandoeningen, zoals patiënten met een niertransplantatie of neoblaas, en dat 59% van de deelnemers reeds intermitterend zelf-katheteriseerde. In de meerderheid van deze patiënten werd de behandeling eerder gecompliceerd door multiresistentie of allergie voor verschillende orale antibiotica. Onze resultaten tonen aan dat gentamicine-blaasspoelingen een effectieve en veilige behandelingsoptie zijn voor deze complexe patiëntencategorie, maar zijn van mening dat eerst de standaardbehandeling zoals geadviseerd door de richtlijnen moet worden geprobeerd.[39]

Recidiverende urineweginfecties door dezelfde bacteriële stam kunnen worden veroorzaakt door re-infectie door bacteriën vanuit de darmflora van de patiënt. In het geval van infecties door multiresistente verwekkers, zou het eradiceren van deze MDR-bacteriën uit de darmflora

van de patiënt een waardevolle aanpak kunnen zijn om terugkerende infecties te voorkomen. In **Hoofdstuk 7** beschrijven wij een casus van een 34-jarige diabetespatiënt met nierfalen waarvoor hij peritoneaal dialyse onderging in afwachting van een nier-alvleeskliertransplantatie, met recidiverende urineweginfecties door een Verona Integron-gecodeerde Metallo-β-lactamase (vim)-positief *Pseudomonas Aeruginosa*.[40] Het enige antibioticum dat bij intraveneus gebruik nog werkzaam was tegen deze verwekker, colistine (MIC 4 mg/ml) is zodanig schadelijk voor de nierfunctie, dat de geplande transplantatie vanwege het hoge infectierisico werd afgelast. Met als doel de patiënt infectievrij te krijgen en de transplantatie alsnog door te kunnen laten gaan, werd hij behandeld met gentamicine blaasspoelingen en transplantatie van fecale donormicrobiota (FMT) in een poging om de MDR *Pseudomonas Aeruginosa* te eradiceren uit het darmkanaal. Deze behandeling voorkwam verdere recidieven van urineweginfecties door MDR *P. Aeruginosa,* zodat de patient terug kon op de transplantatielijst, maar slaagde er niet in om intestinale kolonisatie met een extended-spectrum β-lactamase (ESBL) producerende *E. coli* uit te roeien. Onze bevindingen spreken eerdere positieve publicaties over FMT voor dekolonisatie van MDR Enterobacteriaceae tegen.[41] Analyse van de microbiota van onze patiënt toonde aan dat de diversiteit en samenstelling niet duidelijk verstoord waren door het eerdere gebruik van antibiotica, in tegenstelling tot de verminderde microbiota zoals gezien wordt bij patiënten met terugkerende *Clostridium difficile* infecties (CDI). We veronderstellen dat de verminderde diversiteit geen rol lijkt te spelen bij MDR-kolonisatie, in tegenstelling tot het sterk verstoorde microbioom dat leidt tot terugkerende CDI, waarvoor de behandeling met FMT gebruikelijk gegeven wordt. Daarom zou men kunnen twijfelen aan de werkzaamheid van fecale transplantatie bij patiënten met een normale microbiota diversiteit.

De toename van ESBL-producerende Enterobacteriaceae is een wereldwijde zorgelijke ontwikkeling. Infecties van de urinewegen zijn de meest voorkomende klinische manifestatie van ESBL-producerende *Escherichia coli.* Hoe groot het risico is voor een gezonde niet-symptomatische drager van een ESBL-producerende *E. Coli* op het ontwikkelen van een infectie door deze bacterie is niet bekend. **Hoofdstuk 8** beschrijft ESBL- en AmpC-positieve *E. Coli*-isolaten van patiënten met een fUWI, vleeskuikens, personen die wonen en/of werken op een boerderij met vleeskuikens, en personen uit de algemene bevolking.[42] Multivariate analyse gebaseerd op ESBL/AmpC resistentiegenen, virulentieprofielen en fylogenetische groepen toonde aan dat de meeste fUWI-isolaten tot een duidelijk onderscheidende groep behoren. Uit de resultaten blijkt dat er transmissie plaatsvindt tussen vleeskuikens en personen die wonen/werken op een vleeskuikenbedrijf, maar ook dat de rol van vleeskuikens als een bron van voedselgerelateerde overdracht van multiresistente *E. coli* aan de algemene bevolking en als oorzaak van urineweginfecties waarschijnlijk klein is.

In de afgelopen jaren is een nieuwe vorm van geautomatiseerde, kwantitatieve urinesediment-analyse geïntroduceerd, met een hogere efficiëntie en nauwkeurigheid in vergelijking met de traditionele sediment-analyse. In **Hoofdstuk 9** presenteren wij een retrospectieve case-

record studie, waarin een diagnostische score tool is opgesteld, gebaseerd op de combinatie van de uitslagen van een van deze geautomatiseerde urine analyse methoden (Iris diagnostiek iQ200 Elite) en de klinische symptomen.[43] Deze score tool sloot een urineweginfectie uit in de meerderheid van de patiënten in het derivatiecohort, en kan daarom mogelijk bijdragen aan verbetering van het antibioticumgebruik voor urineweginfecties. Validatie in een nieuw patiëntencohort is vereist voordat deze score in de klinische praktijk kan worden gebruikt.

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CURRICULUM VITAE

Janneke Stalenhoef werd geboren op 4 juli 1977 te Nijmegen als jongste dochter van Marleen en Anton Stalenhoef. Samen met haar broer Martijn groeide zij op in Nijmegen. Na het behalen van haar Gymnasium diploma in 1995 aan het Stedelijk Gymnasium Nijmegen, begon zij met de studie Nederlands Recht aan de Rijksuniversiteit Groningen. Vanaf 1998 combineerde zij deze studie met de studie Geneeskunde. Tijdens haar studietijd was zij werkzaam als onderzoeksassistent bij de vakgroep thoraxchirurgie van het Universitair Medisch Centrum Groningen onder leiding van Prof. Dr. T. Ebels, waar zij werkte aan een onderzoek naar verschillende hartklepprothesen.

In 2002 behaalde zij haar doctoraal Geneeskunde en Nederlands Recht en begon zij aan haar coschappen in het Deventer Ziekenhuis, met een laatste keuzestage op de afdeling interne geneeskunde van het OLVG in Amsterdam.

Na het afronden van het artsexamen bracht zij in 2004 een jaar door in Indonesië, waar zij de interactie onderzocht tussen glucosemetabolisme en immuniteit bij patiënten met tuberculose in een samenwerkingsverband tussen Radboud Universitair Medisch Centrum (Prof. Dr. R. van Crevel) en University of Indonesia in Jakarta (dr. E.J. Nelwan).

In 2005 keerde zij terug in Nederland, om te beginnen met de opleiding tot internist in het OLVG (2005-2009, opleider dr. P.H.J. Frissen) en het AMC (2009, opleider prof. dr. P. Speelman). In 2006 is zij getrouwd met Helmer Schukken, samen kregen zij 2 zoons (Teun 2008 en Ivo 2010). In 2010 vervolgde zij haar opleiding in het LUMC en startte aldaar met het aandachtsgebied infectieziekten (opleiders prof. dr. J.W. de Fijter / prof. dr. J.T. van Dissel). In december 2013 registreerde zij zich als internist-infectioloog, waarna zij als staflid van de afdeling Infectieziekten in het LUMC klinische werkzaamheden combineerde met het onderzoek zoals beschreven in dit proefschrift.

Sinds 1 november 2018 is zij werkzaam als internist-infectioloog in het OLVG in Amsterdam.