Treatment duration and prognostics in febrile urinary tract infection

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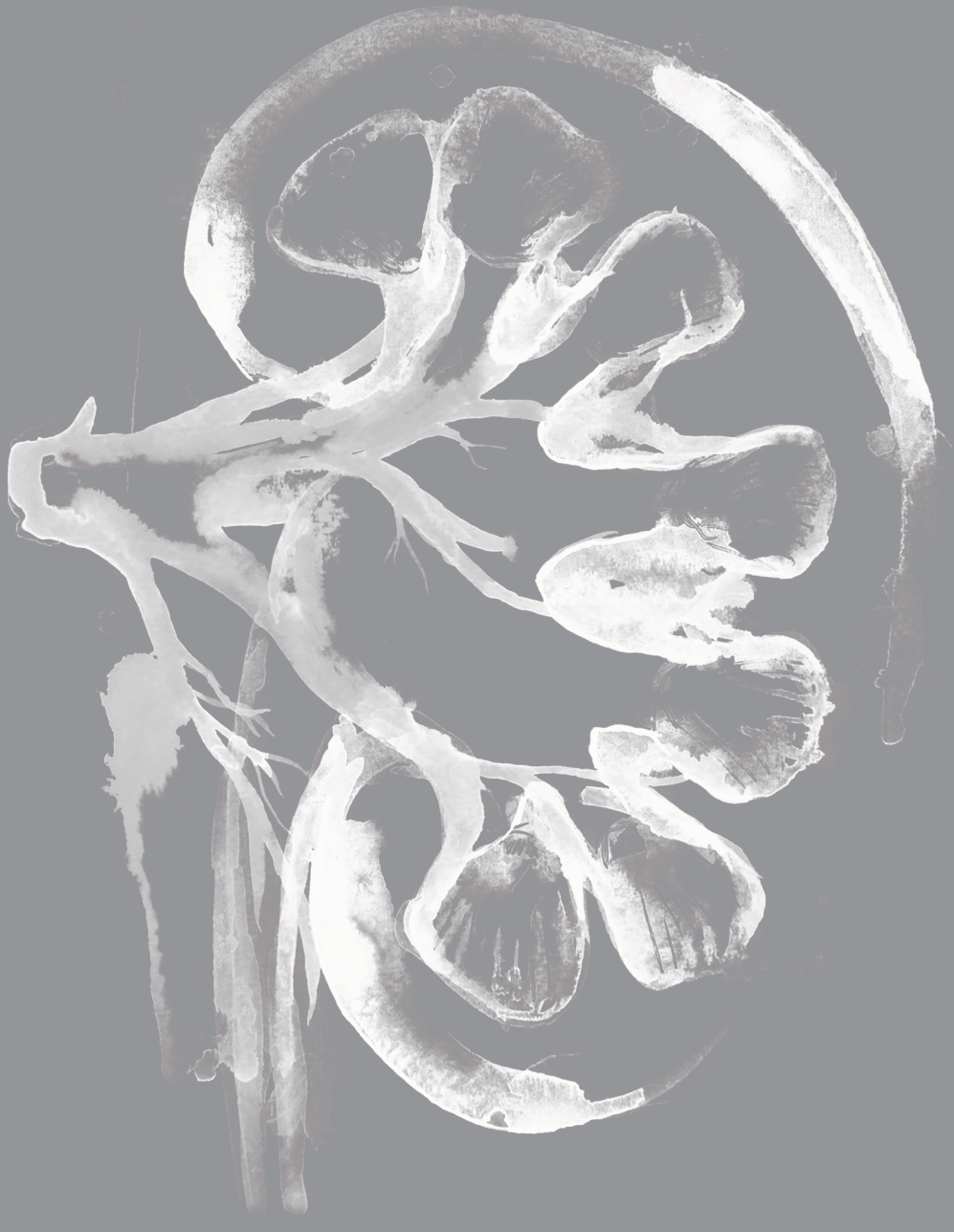

Summary and general discussion

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

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

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

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

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

Risk factors for antimicrobial resistance

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

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

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

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

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

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

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

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

Optimal duration of antimicrobial therapy

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

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

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

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

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

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

Prediction of a complicated course

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

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

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

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

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

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

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

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

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

The role of genetics in UTI

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

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

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

Future directions of research

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

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

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

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

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