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ASYMPTOMATIC BACTERIURIA; PROTECTION AGAINST, AND DIFFERENTIAL DIAGNOSIS TOWARDS SYMPTOMATIC URINARY TRACT INFECTION

Asymptomatic Bacteriuria

Protection against, and differential diagnosis towards symptomatic Urinary Tract Infection

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Abstract
Urinary tract infections (UTIs) are among the most common infectious diseases in humans, with a subset of susceptible individuals who experience recurrent episodes. The increase in antibiotic resistance in gram-negative uropathogens, due to antibiotic overuse, is a strong rationale for developing therapeutic alternatives and to improve diagnostics. In patients with incomplete bladder emptying (e.g. due to spinal lesions and in institutionalized older patients) asymptomatic bacteriuria (ABU) is frequent. In these patient groups diagnosis of UTI is obscured by subjective symptoms being difficult to interpret, and by the already positive urine culture. As ABU is known to be protective against symptomatic episodes, unnecessary treatment should be avoided, and the identification of objective biomarkers to support treatment decision of a suspected UTI episode is thus much needed.

This thesis investigated if the same protective capacity as in spontaneously developed ABU could be induced in patients subjected to deliberate inoculation with the ABU strain *E. coli* 83972, and analyzed the variation of local host responses in patients with *E. coli* 83972 ABU and its possible genetic background, the role of the local inflammatory mediator Interleukin 6 (IL-6) correlation to symptom severity in UTI, and if IL-6 could be used as a diagnostic tool in treatment decision of UTI.

The results demonstrate that E. coli 83972 bacteriuria protects against symptomatic episodes in UTI prone individuals. This was shown in a placebo controlled inoculation study with cross-over design by demonstrating longer time to recurrences and fewer UTI episodes during *E. coli* 83972 bacteriuria as compared with control periods (Paper 1). In patients with long term *E. coli* 83972 ABU the level of mucosal host response to the standardized bacterial challenge demonstrates unique inter-individual specific variation. Genetic analysis suggests this to depend on polymorphisms in specific genes coding for innate immunity (Paper II). Urinary concentrations of IL-6 correlate to symptom severity in UTI, and seem to be the superior biomarker for ABU/UTI differential diagnosis. This was demonstrated by analysis of symptom scoring in patients during long term *E. coli* 83972 ABU and during UTI episodes in the same patients. Furthermore, diagnostic thresholds for IL-6 in differentiating ABU/UTI were analyzed. (Paper III). The use of IL-6 as an added tool in ABU/UTI differential diagnosis was shown to be feasible in a nursing home setting, and reduced together with an educational intervention antibiotic prescription for UTI in a two phase interventional trial (Paper IV).

Key words: Escherichia coli, asymptomatic bacteriuria, urinary tract infection, differential diagnosis, immune response, human genetics, cytokines, interleukin 6

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To Lena, for having an abundance of patience….

Contents

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Sundén, F.**, Håkansson, L., Ljunggren, E. et al.: Escherichia coli 83972 bacteriuria protects against recurrent lower urinary tract infections in patients with incomplete bladder emptying. *J Urol*, 184: 179, 2010

II. Grönberg-Hernandez, J. G., **Sundén, F.**, Connolly, J. et al.: Genetic control of the variable innate immune response to asymptomatic bacteriuria. *PLoS One*, 6: e28289, 2011

III. **Sundén, F.**, Butler, D., Wullt, B.: Triggered urine Interleukin-6 correlates to severity of symptoms in non-febrile lower urinary tract infections. Manuscript, in press, *J Urol*, 2017

IV. **Sundén, F.**, Wullt, B.: Predictive value of urinary interleukin-6 for symptomatic urinary tract infections in a nursing home population. *Int J Urol*, 23: 168, 2016

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Abbreviations

ABU	Asymptomatic bacteriuria
APN	Acute pyelonephritis
CFU	Colony forming unit
CIC	Clean intermittent catheterization
CRP	C-reactive protein
CXCR	Chemokine (C-X-C motif) receptor
E. coli	*Escherichia coli*
ESBL	Extended spectrum beta-lactamase
GSL	Glycosphingolipid
GRO-α	Growth-regulated oncogene alpha
HBP	Heparine-binding protein
IC	Indwelling catheter
IL	Interleukin
IRF3	Interferon regulatory factor 3
LE	Leucocyte esterase
LBP	LPS binding protein
LPS	Lipopolysaccharide
LTCF	Long-term care facility (e.g. nursing home)
MCP	Monocyte chemoattractant protein
mIL-8Rh	Murine interleukin-8 receptor homologue
MSHA	Mannose sensitive hæm-agglutination
NSAID	Non-steroidal antiinflammatory drugs
PAI	Pathogenicity islands
PAMP	Pathogen-associated molecular patterns
pap	Pyelonephritis associated pili
prs	*Pap-related sequence*
SCI	Spinal cord injury
SNP	Single nucleotide polymorphism
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
TNF	Tumor neccrosis factor
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
WBC	White blood cells (estimated by the Leucocyte esterase reaction)
wt	wild type

Abstract

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Introduction

Urinary tract infections

Epidemiology

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans.[1, 2] Women are significantly more likely to experience UTI than men[3] and one-third of all women will have had one UTI treatment by the age of 24.[4] Approximately 50% of all women will experience a UTI at some point in their lifetime and some women suffer multiple recurrences.[4, 5] UTI is rare in the young man but becomes more frequent with higher age, presumably caused by bladder outlet obstruction due to prostatic diseases.[6]

Antibiotics are currently the most effective treatment against acute UTI, but the rapid increase in microbial multiresistance,[7, 8] owing to an overuse of antibiotics,[9–11] is a major concern and an urgent rationale for alternative treatments. The overuse of antibiotics is especially evident in patient groups with a high frequency of ABU, such as hospitalized or institutionalized older patients with multiple diseases, where diagnostic difficulties are prevalent.[9]

The implications of UTIs in the individual patient and the health care are several. In a US multi-state point prevalence study, health care associated urinary tract infections were 12.9%.[12] Data from a Swedish study in women >16 years of age with sporadic or recurrent cystitis in primary care showed a prevalence of 12.5%.[13] The negative effect of UTIs on many aspects of the patient's life quality, such as restriction of daily living and loss of income, interlace with the economic burden of the health care and society. The economic burden on society for UTI, though substantial, can only be estimated. The costs associated with UTI are mainly a result of the high incidence of community acquired UTI.[5] In 1995 the total cost for female community acquired plus nosocomial UTI in the USA was estimated to $1.6 billion.[5] The overall expenditures for the treatment of UTIs in women in the United States, excluding spending on outpatient prescriptions, were approximately $2.5 billion in 2000.[14]

Unlike clinical parameters, there is no normal range or mean value to quality of life (QoL), and it is thus an outcome that can be assessed only by the subjects themselves in relation to their expectations.[15] Studies in different adult populations, including patients with spinal cord injuries, have shown that UTI has a significant effect on various aspects of the patients' QoL in regard to e.g. vitality, social functioning and mental health.[16–19]

Pathogenesis

Uropathogenic *E. coli (Escherichia coli)* (UPEC) is by far the most common (>80%) cause of UTI.[1] In complicated UTI and in patients with compromising disorders or outflow obstruction, gram negative uropathogens like *Klebsiella pneumoniae (K. pneumoniae)* and *Proteus mirabilis (P. mirabilis)* become more frequent.[20] *Enterococcus faecalis (E. faecalis)* is a frequent co-colonizer, or might cause cystitis of its own.[1] In females with uncomplicated UTI *Staphylococcus saprophyticus (S. saprophyticus)* is found in 15% of the cases and typically affects young sexually active women and shows seasonal variation.[21] Group B streptococci are an uncommon pathogen in UTI in young healthy women, but requires treatment in pregnant women.[22]

Diagnosis of UTI

The diagnosis of a UTI can be made by a combination of symptoms and a positive urine analysis or culture. Urine cultures remain the corner stone as the accepted reference standard for the detection and quantification of UTI. A growth of $>10^5$ CFU/mL bacteria detected in two consecutive clean-catched mid-stream specimens is considered significant (Kass' criteria).[23] This bacterial threshold was originally used to define significant bacteriuria in asymptomatic wom-

en and in women with pyelonephritis, but not in women with uncomplicated cystitis. Multiple studies have shown that up to one third of cystitis patients had lower colony counts[24] and lower thresholds, such as 10^4 or 10^3, depending on the type of uropathogen and the type of infection,[25] that are also the recommended guidelines.[26–28] However, in symptomatic women an uropathogen concentration of 10^2 CFU/mL was suggested as sufficient for diagnosis when culture is required.[24, 29, 30] In a study by Hooton in premenopausal women with symptoms of cystitis, bacterial species and colony counts were compared.[31] The presence of *E. coli* in midstream urine was highly predictive of bladder bacteriuria in bacterial counts of as low as 10^2 CFU/mL, showing a positive predictive value of 93%.[31] Interestingly, growth of enterococcus species and Group B streptococci in voided urine was not predictive of their growth in bladder urine and suggest that these organisms are likely to be urethral contaminants instead.[31]

Variety of UTI

The urinary tract is considered to be normally sterile.[32] Bacteria causing UTI are predominantly of fecal origin that after spreading to the perineal and periurethral area, typically ascend the urethra and invade the urinary tract.[33] In most cases the bacteriuria is transient. However, if a strain establishes the result may be a symptom free carrier state, i.e. asymptomatic bacteriuria (ABU). ABU is known to persist for long periods of time (weeks-years) without causing symptoms or renal damage,[34, 35] and may also protect against superinfections with more virulent strains.[36, 37] Conversely, if the infection is symptomatic the symptoms will vary with the location, resulting in acute cystitis in the lower urinary tract and acute pyelonephritis in the upper urinary tract. Acute uncomplicated cystitis is a symptomatic bladder infection characterized by frequency, urgency, dysuria, or suprapubic pain. It is most common in healthy premenopausal and nonpregnant women, and sexual behavior is the primary risk factor both in sporadic and recurrent infections.[5, 38, 39] Acute pyelonephritis (APN) is the least common, but the most severe form of UTI. Bacterial invasion of the renal pelvis occurs when bacteria ascend the urinary tract, resulting in symptoms as fever, renal tenderness, and general malaise. Furthermore, pyelonephritis may affect the kidney function and in patients with concomitant bacteremia it may cause sepsis and be life threatening.[1] Bacteremia occurs in approximately 30% of adults with febrile UTI.[40]

Antimicrobial resistance

Antibiotic resistance has become a worldwide problem. There is a positive association between antibiotic consumption and antibiotic resistance,[41] and the indiscriminate use of antibiotics is considered to be the driving force.[7, 8, 42, 43] In countries with a high antibiotic prescription rate antibiotic resistance has reached a level where many drugs have become ineffective. In a cross-national European database study the total outpatient cephalosporin use varied by a factor of over 250 between the country with the highest (Greece) and the country with the lowest (Denmark) use.[42] The presence of extended-spectrum β-lactamase (ESBL) producing *E. coli* showing resistance to most antibiotics is increasing and the rate of ESBL-producing strains in European countries and in the USA varies between 1 % and 39%, with the prevalence of resistant bacteria increasing over the last decade.[44] In a survey of antibiotic susceptibility in a large community population, ampicillin resistance was observed in 53% of *E. coli* and 28% of *P. mirabilis* isolates. In addition, more than 34% of *E. coli* and *P. mirabilis* isolates were non-susceptible to trimethoprim compared to 20% *Klebsiella* spp., whilst nitrofurantoin resistance was observed in 3% *E. coli* and 15% *Klebsiella* spp.[45]Furthermore, there is an increase in resistance to broad-spectrum antibiotics, particularly to fluoroquinolones and cephalosporins, with the subsequent development of ecological adverse effects (collateral damage), namely the unwanted development

of multidrug-resistant bacteria.[46] Cephalosporins has been linked to subsequent infection with vancomycin-resistant enterococci, ESBL–producing *Klebsiella pneumoniae*, and *Clostridium difficile*.[46] The use of fluoroquinolones has been linked to infection with methicillin-resistant *S. aureus* and with increasing fluoroquinolone resistance in *Pseudomonas aeruginosa*.[46] However, the development of resistance in *E. coli* has not been observed to antibiotics often used in uncomplicated lower UTI such as fosfomycin, nitrofurantoin and pivmecillinam.[8, 47]

Antimicrobial stewardship and long-time care facilities

Infections caused by antimicrobial-resistant organisms are associated with higher rates of treatment failures, prolonged hospitalizations, increased costs, and mortality.[48–50] In addition, the number of approved novel antibiotics have decreased, and it is therefore essential to secure the availability of the currently used antibiotics.[41] In order to preserve antibiotic efficacy, a model called antimicrobial stewardship has been proposed.[43, 51–56] The aim is to reduce the overuse and misuse of antimicrobial agents, enhance patient health outcome, and decrease unnecessary health care costs.[54] Establishing an effective antimicrobial stewardship strategy for the diagnosis and treatment of UTI is important because of its high prevalence.[52, 53, 57] Several strategies have been explored to increase adherence to antibiotic guidelines for the treatment of acute urinary tract infection with variable results.[58, 59] No treatment, use of appropriate antibiotics, reduction of treatment length, and wait-and-see prescriptions are methods that aim to decrease the overall use of antibiotics.

For many years development of antibiotic resistance has been associated with hospital care, but the threat of escalating antibiotic resistance is now also recognized in community settings,[60–62] particularly in long time care facilities (LTCFs; nursing homes) where the antibiotic use for UTI is intense. One of the most important problems in LTCFs is the inappropriate use of antimicrobials to treat UTIs in asymptomatic residents.[63, 64] Almost 50% of asymptomatic nursing home patients are prescribed broad-spectrum antibiotics for suspected UTIs, despite strong evidence demonstrating lack of benefit and potential harm.[9, 65] In addition, over 80 per cent of antibiotic prescriptions written for individuals with an indwelling catheter (IC) were written without evidence of a UTI.[65] The diagnosis of UTIs in elderly LTCF residents is challenging as the high prevalence of ABU and the frequent lack of clear UTI symptomatology makes it difficult to determine whether alterations in clinical status is caused by an UTI.[65–67] Some useful stewardship strategies to improve the use of antimicrobials in LTCFs have been reported[53]. In an interventional study Loeb *et al.* could demonstrate that the rate of antibiotic use was reduced (see below),[68] but this effect seemed to diminish over time.[68] A multifaceted approach was used in the cluster randomized study, including small groups of interactive sessions for nurses, videotapes, written material, outreach visits, and one-on-one interviews with physicians, and to follow strict diagnostic and treatment algorithms. Petterson *et al.*[69] used a similar approach in Swedish LTCFs and found a significant reduction of the fluoroquinolone use (primary outcome) at the end of the study period in the intervention and control groups, but with no difference between the intervention and control homes.[69] However, there were significant differences in some secondary outcomes in favor of the educational intervention, including a reduction of any antibiotic given for all infections, and an increase in a ”wait and see” approach of observation with delayed antibiotic treatment.[69]

Bacterial virulence

Uropathogenic *E. coli*

Escherichia coli (E. coli) is the major pathogen in UTI. Uropathogenic *E. coli* (UPEC) strains are characterized by the expression of several

virulence factors, which are distinctive bacterial properties, products, or structures aiding the organism to overcome host defenses and to invade the urinary tract. Genes coding for virulence factors in *E. coli* are located on mobile genetic elements clustered in particular regions, the Pathogenicity islands (PAI), on the bacterial chromosome.[70] Virulence genes may act in unison and both host and bacterial factors regulate their expression. Traditional classification of *E. coli* strains is based on their expression of cellular markers (serotypes), like lipopolysaccharide (LPS; especially the O-antigen), capsular (K) and flagellar (H) antigens.[71, 72] Early studies have shown that certain (O:K:H) serotypes are associated with pyelonephritis or ABU.[72, 73]

Bacterial adhesion is considered a critical virulence factor in the human urinary tract. The ability of *E. coli* strains to adhere to the uroepithelium varies and is associated with UTI severity, as shown in urine isolates from patients with ABU, cystitis, and pyelonephritis.[74] The adhesive capacity has been attributed to the expression of different adhesive factors, or adhesins, that mediate binding to the uroepithelium, such as Dr, afa and S, P, and Type 1 fimbriae.[75, 76] The two most studied adhesins associated with uropathogenic *E. coli* are Type 1 fimbriae and P fimbriae.

Type 1 fimbriae

Type 1 fimbriae are encoded by the *fim* gene cluster, which is present in most commensal and uropathogenic *E. coli*.[77] The receptor recognizing adhesin FimH, which is located on the tip fibrillum of the fimbriae, mediate binding to cell bound and secreted mannosylated glycoproteins. The signature feature of this binding is its ability to evoke mannose sensitive hemagglutination (MSHA) of guinea pig erythrocytes.[75, 78] In the murine UTI model, Type 1 fimbriae have been shown to enhance bacterial survival and to stimulate mucosal inflammation,[79, 80] yet the evidence of a similar role in the human urinary tract have been contradictory. As there is no significant difference in the *fim* gene frequency between more or less virulent *E. coli* strains in the urinary tract, it has been suggested that the possession of the *fim* gene cluster or expression of type 1 fimbriae does not correlate to disease in humans.[77] Nonetheless, in children with acute pyelonephritis (APN) due to the O1:K1:H7 clone, type 1 fimbrial expression varied with disease severity.[81] On the other hand, type 1 fimbriated *E. coli* 83972 did not trigger an inflammatory response in deliberately colonized UTI prone individuals.[82] Type 1 fimbriae bind to human buccal, ureteral, and vaginal cells, but there is variable evidence of human uroepithelial adherence.[76, 83, 84] Most studies on type 1 fimbriated adhesion have been performed in the murine UTI model, showing that type 1 fimbriae bind to the mouse bladder epithelium.[79] It has been proposed that the attachment of uropathogenic *E. coli* to the uroepithelium is mediated by FimH binding to uroplakins, rigid-appearing protein plaques on the cell surface of the urothelium.[85–87]

P fimbriae

Epidemiological studies have shown a strong correlation between *E. coli* expression of P fimbriae and virulence in terms of inflammatory magnitude and localization.[74, 88–93] The P fimbrial pap gene cluster is found in approximately 80% of pyelonephritis isolates, 30 % of cystitis isolates, 24% of ABU isolates, and 20% of fecal isolates.[75, 90–92, 94] In patients with pyelonephritis with septicemia, the bacterial blood cultures showed a 100% presence of the *pap* gene.[90, 95] Children with recurrent UTI have an increased prevalence of P fimbriated strains in the fecal flora, and the subsequent symptomatic infections are caused by strains expressing the P fimbriae.[91] The P fimbriae mediate attachment to the uroepithelial cells trough binding the Galα1-4Galβ receptor epitope in the globoseries glycolipids.[96, 97] The receptor is found on the uroepithelial cells of the urinary tract,[75] but varies with the individual's P blood group.[98] The receptors are P blood group antigens (P_1, P_2, P^k), which are present on the erythrocytes

depending on the individual's blood group. P fimbriae agglutinate P_1 and P_2 erythrocytes, but fail to agglutinate p erythrocytes.[99] In addition, individuals with the P_1 blood group seem to be at higher risk of developing pyelonephritis with P fimbriated bacteria than individuals of other bloodgroups.[98]

The P fimbriae consist of a rigid shaft and an end fibrillum encoded by the *pap* gene cluster (*pap A–K*). The papA protein is the major structural protein, but the papG adhesin, situated on the tip of the fibrillum, mediates the attachment to the uroepithelial cells.[100, 101] Three classes of P fimbriae with different binding properties have been described.[88, 102] Of clinical relevance in humans are the classes II and III, which predominate in UPEC and differ mainly in type of the G adhesin.[88, 103, 104] The former class carry the $PapG_{IA2}$ adhesin which attach to uroepithelial cells from all P blood group positive individuals,[105] and is strongly associated with APN with bacteremia.[106] Class III carry the $PrsG_{J96}$ adhesin, agglutinates mainly human A_1P_1 erythrocytes, and is associated with cystitis.[105, 107] Class I P fimbriae ($PapG_{J96}$) are uncommon in clinical isolates and without known uro-pathological relevance.[88]

Virulence properties of ABU and cystitis isolates

ABU

During asymptomatic bacteriuria (ABU), bacteria colonize the urinary tract for extended periods of time without causing symptoms of urinary tract infection.[35] ABU thus resembles a state of commensalism, but mostly with a bacterial monoculture rather than a complex flora. The failure of asymptomatic carrier strains to trigger disease associated signaling pathways of the host response has generally been attributed to their lack of virulence. Early studies showed that many ABU strains lacked a defined O antigen, were un-capsulated, and failed to adhere to uroepithelial cells *in vitro.*[72, 74, 108] Later findings showed that the ability to become attached to normal epithelial cells was less in *E. coli* ABU strains in comparison to cystitis or pyelonephritis strains.[74] Molecular studies suggested that this could be explained by ABU strains carrying virulence genes, but failing to express the phenotype as only 20% of ABU strains expressed P fimbriae compared to at least 90% of pyelonephritis strains.[75, 91, 109]

Genome sequencing has revealed that about 50% of ABU strains evolve from virulent uropathogenic *E. coli* (UPEC) clones but fail to express virulence factors, due to reductive evolution by DNA rearrangements, point mutations, and deletions that attenuate virulence. [110, 111] Furthermore, a reduction in total genome size compared to the virulent strains has been observed, [112] suggesting that ABU strains actively undergo reductive evolution in order to adapt to the host environment. Zdziarski *et al.* looked at real-time evolution in the completely genome sequenced non-pathogenic strain *E. coli* 83972 by comparing re-isolates from several patients long-term inoculated with the strain.[112] The prototypic ABU strain *E. coli* 83972 was originally isolated from a girl who had carried the strain during three years without experiencing UTI symptoms,[35] and the strain has been extensively used in host response studies.[93, 110, 111, 113–115] Over time the re-isolates showed multiple mutations in comparison to the original strain, affecting their metabolic and virulence related genes.[112] Further transcriptome and proteome analysis indicated that these genome changes altered bacterial gene expression resulting in unique adaptation patterns in each patient. It was proposed that in prolonged bladder colonization, *E. coli* 83972 adopted its metabolism, iron uptake and stress resistance to the colonized host.[112] In conclusion the authors called for further analyses of the biological relevance of the observed genomic alterations as it could help in identifying potential drug targets to reduce bacterial fitness during symptomatic infections. Furthermore, ABU strains are active modifiers of the host environment by suppressing RNA polymerase II (Pol II)-dependent host gene expression.[116] A recent study in patients inoculated with the ABU strain *E. coli* 83972

revealed a marked suppression of host gene expression in patients who became asymptomatic carriers;[116] the expression of all regulated genes were reduced by >60% within 24h.[116]

Cystitis

Acute uncomplicated cystitis (Cystitis) is the most common form of symptomatic UTI, but a less well-defined disease entity compared to pyelonephritis. Subversion of the fast-acting innate immune response is probably essential for survival during the early stages of infection. Virulence factors increase the fitness of UPEC, but little is known about the possible mechanisms involved. Nonetheless, it has been shown that UTI strains commonly express multiple virulence factors simultaneously, also among isolates from cystitis patients.[76] APN is caused by a restricted subset of UPEC clones, distinguished for example by O:K:H serotypes or *E. coli* reference collection types combined with certain virulence factors with specific functions.[76] Cystitis strains form an intermediary group with respect to these parameters.

Type 1 fimbrial expression has been considered a major virulence factor in acute cystitis as these fimbriae enhance bacterial virulence in the murine urinary tract, [79, 80] but a human inoculation study have so far not verified this role of Type 1 fimbriae in the urinary tract.[82] Interestingly, type 1 fimbriae are also required for aggregation of UPEC into intracellular bacterial communities, which has been suggested to explain the recurrent infections occurring in 20%–30% of the UTIs.[117, 118] Cystitis strains also express P fimbriae, but the frequency of the two uropathogenic classes have varied in different reports.[105, 107, 119]

Toxins such as hemolysin (hly) was identified as an early marker of uropathogenic strains compared to isolates from the fecal flora, as well as aerobactin, one of the first iron-binding proteins to be identified in UPEC.[120] Hemolysin together with cytotoxic necrotizing factor (CNF) enhance uroepithelial damage,[121] including profuse inflammation, extensive shedding of the urothelium, and bladder tissue hemorrhage.

TIR domain containing-protein C (TcpC) is a protein common in most virulent uropathogenic *E. coli* strains and promotes bacterial survival by inhibiting the innate host response downstream of the *TLR4* signaling pathway.[122] As a consequence, secretion of pro-inflammatory cytokines is dampened, and microbial replication is enhanced.

Curli are bacterial surface organelles that bind extracellular matrix and contact proteins of the uroepithelium. These adhesive fibers enhance bacterial biofilm formation on various abiotic surfaces,[123] such as urinary catheters, were the biofilm conveys a survival advantage to the microorganisms.[124]

Host responses in UTI

Innate immunity

The protection against bacterial infection depends on the collaboration of innate and specific immune systems. Innate immunity constitutes the first line of host defense during infection. It is fast acting and respond to bacterial exposure in an immediate and non-specific fashion. In the urinary tract this includes a variety of strategies, such as the mechanical flushing action of the urine flow, the relative impregnability of the epithelial lining, and to activate inflammation. The innate immune response relies on recognition of evolutionary conserved structures, or pathogen associated molecular patterns (PAMPs), on intruding pathogens through pattern recognition receptors (PRRs) on the epithelial cells.[125] Of the PRRs, the Toll-like receptors are the most studied. Among these receptors Toll-like receptor 4 (*TLR4*) plays a key role in initiating innate host response.[126–128] The subsequent epithelial cell activation triggers mucosal inflammation through the secretion of cytokines and inflammatory mediators, such as Interleukin (IL)-1, IL-8 and IL-6, and the recruitment of neutrophils to the urinary tract (pyuria).[129, 130]

Toll-like receptor 4

TLR4 is expressed on the surface of the epithelium throughout the urinary tract.[127] It is a member of the toll receptor family,[131, 132] that plays a major role in the innate immune responses against vast diverse virulence factors. *TLR4* is crucial for the host response to UTI. It recognizes the gram-negative *UPEC* bacterial cell wall component LPS and several non-endogenous and endogenous ligands. In macrophages, optimal LPS activation of *TLR4* requires the co-receptors CD14 and LPS binding protein (LBP). However, in the urinary tract CD14 is lacking and activation of *TLR4* is mediated via binding of Type 1 and P fimbriae to uroepithelial receptors with the subsequent activation of intracellular signaling cascades.[133, 134]

Early discoveries showed that C3H/HeJ mice were highly susceptible to experimental UTI as they failed to clear bacteria from the kidneys.[135, 136] This lack of inflammatory activation was shown to be coupled to a point mutation in the TLR 4-gene (*TLR4*).[137] The subsequent inactivated *TLR4* signaling led to a symptom free carrier state without observed tissue damage, resembling human ABU.[135] Similarly children with ABU have been shown to expresses lower levels of *TLR4* than age-matched controls or children without UTI, suggesting that reduced *TLR4* expression promotes the development of ABU.[138] The results from a study in pediatric patients with ABU and those prone to APN, indicated that the reduced levels of *TLR4* expression in patients with ABU has its origin in *TLR4* promoter polymorphism.[139]

Cytokines

Cytokines are small soluble proteins that function as signal mediators between cells and have a major role in regulating the host defense in response to bacterial infection. In the urinary tract, epithelial cells are responsible for the local production of cytokines.[129, 140–142] In sterile biopsies from the urinary tract preformed IL-8 has been detected, but not IL-6, and in biopsies exposed to virulent *E. coli* both IL-6 and IL-8 were encoutered.[127]

In addition to IL-6 and IL-8, there is an array of other cytokines with detectable urine concentrations during UTI. Acute cystitis has been associated with IL-1α, GRO-α, IL-1β, and TNF-α compared to sterile samples.[66, 143] A battery of urine cytokines were examined in elderly subjects, where a group wise comparison of samples from cystitis and sterile controls detected a significant increase in GRO-α, IL-8, and IL-6 in the cystitis group, but did not find any production of TNF-α, IL-1β, IL-10, IL-12, IL-18, and MCP-1. Although levels of IL-1α was not examined, previous studies have shown elevated titers in acute cystitis.[143, 144] GRO-α is neutrophil chemoattractant[145] and IL-1 is a potent proinflammatory cytokine with pyrogenic property. [146, 147] Uroepithelial cells express IL-1 in two forms: IL-1α, which is mainly membrane-bound and IL-1β, which is a secreted cytokine.[148, 149]

Interleukin 6

Interleukin 6 (IL-6) is a multipotent cytokine that acts in both pro- and anti-inflammatory ways. In systemic release, as seen in febrile UTI and pyelonephritis, IL-6 induces synthesis of acute phase reactants, such as the C-reactive protein (CRP), and acts as an endogenous pyrogen.[150, 151] Locally produced IL-6 in the urinary tract is elevated in symptomatic UTI, and the IL-6 level seems to reflect the severity of the infection.[40, 152–154] In contrast, IL-6 is not triggered in ABU, suggesting its use as a candidate biomarker for differentiating symptomatic and asymptomatic infection.[66, 155]

Interleukin 8

Interleukin 8 (IL-8) was one of the first uroepithelial cytokines to be identified, and it is synthesized by epithelial cells lining all parts of the urinary tract.[156] IL-8 acts as a strong neutrophil chemoattractant.[129, 157] In patients with

E. coli infection urinary IL-8 levels correlate to pyuria.[129, 140] There is a rapid increase in urinary IL-8 concentrations in response to bacterial infection and the levels of urinary IL-8 seem to vary with the type of infection. In a pediatric study, urinary IL-8 levels were higher in febrile UTI than in ABU,[152] and in an elderly population the urinary IL-8 concentrations were shown to be higher in non-febrile UTI as compared to ABU.[66] Urinary IL-8 is elevated in children with renal scarring and vesico-ureteral reflux, even in the absence of infection.[158]

IL-8 mediates its activity by binding the two receptors CXCR1 and CXCR2.[159] Both receptors are expressed on most cells of the innate immune system, including epithelial cells and neutrophils, but their function has been studied primarily in relation to neutrophil activation and neutrophil chemotaxis. CXCR1 has been suggested as the essential receptor involved in neutrophil chemotaxis.[159] In mice, knock out of the IL-8 receptor homologue (mIL-8Rh) resulted in delayed neutrophil chemotaxis and extensive entrapment of neutrophils subepithelially in response to *E. coli* infection.[160, 161] All mice developed signs of pyelonephritis, abscess formation, tissue damage and renal scarring.[160, 161] In the control mice, neutrophils were seen crossing the epithelial barrier into the lumen and infection was cleared without evidence of tissue damage. In APN prone children, the neutrophil surface expression of CXCR1 was low in comparison with age-matched controls without a history of UTI. [162] A study investigating families of APN-prone children and of age-matched control subjects without UTI suggested that susceptibility to APN is inherited and that low CXCR1 expression might predispose to disease.[163]

Pyuria

Neutrophils constitute the majority of the white blood cells in the circulation. Their main role in innate immunity is to migrate to the site of infection and destroy bacteria, mainly through phagocytosis.[159, 160] In response to bacterial infection uroepithelial cells are triggered to secrete the chemokine IL-8 into the subepithelial tissue where it generates a concentration gradient that the neutrophils follow to the site of infection. In UTI the migration is initiated when the CXCR1 receptor on the neutrophil surface binds IL-8.[159] The recruitment of neutrophils into the urine (pyuria) is essential for the clearance of bacteria and the restoration of sterility and mucosal integrity in the urinary tract.[164]

The use of pyuria as a surrogate marker for the detection of UTI has over time replaced bacterial culture in many clinical settings. In clinical practice pyuria is used as a stand-alone test or used to prioritize samples submitted for culture.[165] Pyuria can be evaluated by microscopy,[166] urinary dipstick.[166] or automated methods. The two latter methods are also available for the nitrite test, which is often combined with pyuria to increase the diagnostic accuracy in UTI.[167–169] The use of pyuria as a tool for diagnosing UTI has been questioned[165] as pyuria is present in many other medical conditions[170] and may also be caused by contamination.[1] In addition, pyuria is frequent among persons with asymptomatic bacteriuria, suggesting a possible limited diagnostic value of pyuria in this population.[171]

Initiation of the host response

The pathogenesis in UTI has been described in a two-step model,[172] where the first step is the attachment and activation of the innate host response. The second step is the effector phase, which involves the neutrophil dependent removal of bacteria.

Because the mucosal barrier is inert by nature, the adherence of bacteria to the epithelial cell surface is considered a crucial first step to overcome the inertia.[130] Uropathogenic *E. coli* use specific adherence mechanisms to target the epithelial cells.[75, 142] Epidemiological studies have shown a strong correlation between adherence and disease severity.[74, 173] As ABU strains are non-adhesive, they may avoid breaking the inertia and can thus persist more or less unnoticed in the

urinary tract.[74, 115] During *E. coli* infection, epithelial cell activation is fimbriae mediated.[130, 174]

Among the different virulence associated factors causing pyelonephritis, P-fimbriae mediated adherence has been used as a model system to understand step 1.[172] P-fimbriae show the most direct association with disease severity, being expressed in 70–90% of acute pyelonephritis strains but in less than 20% of ABU strains.[75, 91, 106] P-fimbriae bind to cell surface glycosphingolipid (GSL) receptors and recruit *TLR4* as co-receptors for activation of the epithelial cells.[126]

Specific immunity

Specific immunity is mediated by B- and T-cells and can be activated by innate immunity and exposure to antigens, resulting in the production of specific antibodies and T-cell receptor bearing lymphocytes. It is mainly responsible for elimination of pathogens in the late phase of an infection. Although early studies focused on specific immunity mechanisms, existing data regarding human specific immune responses in the local defense to UPEC are scarce.[175] In healthy adults the urine normally have measurable titers of locally produced secretory (S)–IgA, as well as IgG.[176] In acute pyelonephritis serum antibody titers of mainly IgG and IgM are elevated and urinary antibody titers are predominantly S-IgA,[177] as is the case in cystitis.[178] However, the role of S-IgA in the local specific defense of the human urinary tract is unclear.[179] *In vitro*, urinary S-IgA and IgG from UTI patients were capable of inhibiting UPEC adherence.[180] In UTI, induced antibodies to different properties of *E. coli* virulence have been identified, such as to the O- and K-antigens of LPS, and to the P- and Type 1 fimbriae.[180–183] Specific immunity seems to be of less importance in the early clearance of bacteria in acute UTI.[135] Immunodeficient mice with defective or lack of B- and T-lymphocyte function do not show increased UTI susceptibility.[162] Most individuals with selective IgA deficiency are not prone to urinary tract infection, and the frequency of UTI is not increased in patients with antibody deficiencies like hypogammaglobulinemia.[184, 185]

Host susceptibility

There is a great clinical need to identify UTI susceptible individuals and to distinguish the different mechanisms behind their infection proneness, as this may influence the choice of therapy. Traditionally the urologists focus on ruling out a number of known risk factors for UTI. Vesicoureteral reflux,[186] incomplete bladder emptying due to neurogenic bladder disorder[34] or prostate hypertrophy,[6] and renal stone formation may predispose to increased susceptibility.[187, 188] Concomitantly, the clinicians accept episodes of UTI in a young woman, since being a woman *per se* is considered a risk factor for single and recurrent infections.[3] Other factors which may influence UTI susceptibility are sexual behaviour,[38] recent antibiotic UTI treatment,[38] vaginal microflora,[189] and diet.[190] In addition, susceptibility to UTI is also influenced by the patient´s blood group. Individuals with the P_1 blood group are more prone to develop UTI and carry more P-fimbriated strains in the fecal flora than P_2 individuals.[91, 191] ABO non-secretors with APN are more susceptible to renal scarring.[192]

Furthermore, several studies suggest an association between personal and family history and susceptibility to UTI.[39, 193–195] One study in school girls showed a positive family association in 42% of the patients compared to 11% in controls who did not have a history of UTI.[195] In two population-based case-control studies, Scholes *et al.* found that a history of UTI in female relatives was consistently associated with recurrent urinary tract infection and pyelonephritis in the female study groups.[193, 194] More than 70% of the patients reported at least one female relative with a UTI history compared to 42% in controls.[193] As the risk estimates increased with stronger family history, the authors suggested a genetic component for increased susceptibility to these infections.[193] In addition, behavioral factors such as sexual intercourse history increased

the recurrence risk in the individuals with a UTI history.[193] Studies on possible hereditary factors influencing UTI susceptibility have identified disease-associated genetic variations predisposing for UTI.[139, 163, 196] In one family study, 15% of relatives of pyelonephritis-prone children had a UTI history compared to 3% of relatives of controls.[163] CXCR1 expression was significantly lower in the APN-prone children and in their relatives than in pediatric and adult controls. The reduced CXCR1 expression was associated with polymorphism and mutations in CXCR1.[197]

Predisposition to ABU has been linked to promoter polymorphisms in *TLR4*. In a case-control study Ragnarsdottir *et al.*[139] performed sequencing of *TLR4* in children with ABU, recurrent APN and age-matched controls without UTI. Compared to patients with pyelonephritis and healthy controls, ABU patients had fewer genotype patterns (GPs) and their promoter-sequence variants showed reduced *TLR4* expression.[139] In a second part of the study adults with a first recorded febrile UTI episode some 30 years prior to the study and a subsequent history of recurrent pyelonephritis, were reinvestigated with genetic analysis. Sequencing showed the same *TLR4* single nucleotide polymorphisms (SNPs) as the APN patients in the first part of the study.[139] The authors emphasized the marked difference between children with ABU and APN, and proposed a possible association between *TLR4* promoter sequence variants, promoter GPs and UTI severity.[139]

A study by Fischer *et al.* showed that *interferon regulatory factor 3 (IRF3)* promoter polymorphism might be associated with APN.[196] By genetic screening of innate immune effector genes downstream of TLR4, the authors identified IRF3 as a possible determinant of host susceptibility[196] as *Irf3* knockout mice ($Irf3^{-/-}$) developed a more severe type of infection than wild type (wt) $Irf3^{+/+}$ mice.[196] To further address this question, *IRF3* promoter sequence variation was studied in two UTI-prone patient populations. Group 1 included children with recurrent APN or children with primary ABU. Group 2 comprised adults with a childhood onset of febrile UTI and a following 30-year history of APN. DNA sequencing of *IRF3* promoters from UTI patients revealed variation in the same two positions. However, SNPs for the positions were linked in the study population, but the *IRF3* genotype varied with UTI severity.[196] The homozygous genotype dominated in APN-prone patients (79% in children and 75% in adults) while the heterozygous genotype was more common in primary ABU patients (69%) than those with APN (14%). Transcriptional activity from the APN promoter was about 50% lower compared to the ABU promoter.[196] In all, the authors proposed an association between *IRF3* promoter polymorphism and APN susceptibility, and suggested that reduced *IRF3* promoter activity increases the risk of APN.[196]

Asymptomatic bacteriuria

Epidemiology

ABU is common especially in women, and the overall prevalence is approximately 3.5% among the general population.[5] It occurs in 1% of school girls[198] and is seen in up to 5% of healthy premenopausal women.[5] The prevalence is 2–10% in pregnant women[22, 199] and can reach 16% in patients with Type 1 diabetes.[200] The prevalence increases with age to 3.6–19% in healthy elderly individuals.[201] Among the institutionalized elderly ABU occurs in 15–40 % in men and 25–50% in women,[67, 202] and patients with chronic IC are almost always bacteriuric.[203] Additionally, 23–89% of patients with spinal cord injuries (SCI) have ABU.[34] However, ABU is rare in healthy young men, but increases markedly after the age of 60 years, presumably caused by factors associated with prostate hyperplasia.[6]

Natural course

Historically ABU was considered to be associated with chronic pyelonephritis,[204] and thus to end stage renal disease. Still into the 80's, ABU

was regarded as harmful, but it took a series of large studies to convincingly demonstrate the opposite. In the 60's autopsy studies[1] could decisively show that bacteriuria was not the major cause of chronic pyelonephritis, and screening and epidemiological studies could demonstrate that ABU was harmless in certain populations.[204–206]

Not dangerous

The first obstacle was to define ABU. In the 50´s there was no available method to differ contamination from true, or significant, bacteriuria in urine cultures, and thus impossible to verify the clinical suspicion of a UTI. This dilemma was solved in a study by Kass where he quantitatively determined significant bacteriuria.[23] The threshold of 10^5 CFU/mL in a symptom free female patient or in a female with acute pyelonephritis was considered significant, and lower counts were regarded as contamination (Kass' criteria).[23] In addition, a second urine culture in the symptom free patients showed significant bacteriuria in most of the cases. This result was later verified in repeated consecutive samples, confirming the presence of asymptomatic bacteriuria.[23, 205] ABU is since then defined as asymptomatic carriage of $\geq 10^5$ CFU/mL urine of the same organism detected in two subsequent urine cultures of clean-catched mid-stream samples.[205] Over time the criterion has been modified and is presently mainly applicable to asymptomatic women.[23] For men a single clean-catch voided specimen with $\geq 10^5$ CFU/mL is adequate,[207] and in the case of a single catheterized specimen $\geq 10^2$ CFU/mL is sufficient in both women and men.[34] For patients with a chronic IC $\geq 10^3$ CFU/mL is satisfactory.[208]

Protective effect

The first to show the benefits of non-treatment was Lindberg, who in a series of publications examined schoolgirls with ABU.[72, 198, 209, 210] He investigated the level of CRP and pyuria,[198] the association with residual urine,[210] the differences in *E. coli* strains causing symptomatic and asymptomatic bacteriuria,[72] and their relation to the level of diagnosis.[209] A three year follow-up of the patients, treated and untreated, showed that ABU did not cause pyelonephritis or renal damage in schoolgirls with normal urinary tract imaging.[35] In a later trial Hansson *et al.*[36] demonstrated that non-treatment of asymptomatic children with bacteriuria achieved long-term protection against UTI.[36] Later the concept of non-treatment was shown by Cai *et al.*[211] in a large prospective study in otherwise healthy premenopausal women with ABU and a history of recurrent UTI.[211] Patients were randomized to antimicrobial treatment with a standard antibiotic regimen, or no treatment. At follow-up after 12 months 73.1% of the patients in the treatment group showed recurrence, but only 14.7% of the patients in the non-treatment group. Analyses showed that the use of antibiotic therapy was an independent factor affecting the risk of developing symptomatic UTI, and that the treatment gave a higher probability of developing recurrence in comparison with the non-treatment group. The authors concluded that asymptomatic bacteriuria may be protective and should not be treated in young women.[211]

Non-treatment recommendations

As a consequence of the clinical studies most guidelines recommend non-treatment of ABU.[26, 34] However, risk groups with complicating factors such as abnormalities of the genitourinary tract, or prior to endourological surgery, are excluded from these recommendations.[26, 212, 213] Pregnancy is another exception as untreated ABU is considered a risk factor for subsequent pyelonephritis, preterm delivery, and low birth weight.[22, 26, 34, 199] However, the evidence of an association between ABU and preterm delivery/low birth weight is not clear.[22, 214]

A rare phenomenon: Transition from ABU to UTI

Although ABU has been demonstrated to possess a protective capacity,[35, 36] there is a common clinical observation that patients with verified ABU might have rare episodes of UTI complaints. This could be due to different patients having different protection of ABU (susceptibility variations) or due to the loss of the protective capacity of the bacterial strain. The only known study addressing this problem was by Köves *et al.* [215] In a prospective study the authors investigated whether the non-pathogenic ABU strain *E. coli* 83972, evolved toward virulence during asymptomatic carriage. Isolates from 4 patients who developed UTI symptoms during asymptomatic *E. coli* 83972 carriage were examined. The bacterial genetic profile in the symptomatic re-isolates were unique for each host, but without evidence of common deregulation. Virulence remained unchanged regarding persistence, symptoms, and innate host responses when comparing symptomatic isolates with the wild-type strain in the murine UTI model. It was concluded that the move from asymptomatic carriage to symptomatic UTI was not explained by regained genetic expression of virulence factors.[215]

Differential diagnostics between ABU and UTI

Elderly nursing home residents

UTI is the most common indication for antibiotic treatment in elderly nursing home residents due to a high incidence of infection. The increased number of UTIs and ABUs in this population depends on underlying comorbidities, the use of invasive devices, aging associated changes, and institutional exposure.[216] In addition, the most impaired residents, often with ICs, have the highest frequency of ABU.[217] Neurological diseases occur more frequently with aging and are associated with neurogenic bladder dysfunction. This in turn promotes bacteriuria and increase already present urogenital symptoms.[218] Non-specific symptoms such as changes in mental status and fever are also seen in other conditions.[219, 220] The clinical diagnosis is further impeded by the difficulty to obtain medical history, because many residents are cognitively impaired or have hearing or speech difficulties.[221] The high prevalence of ABU and the frequent lack of clear UTI symptomatology in elderly nursing home residents make it difficult to determine whether alterations in the clinical status are caused by an UTI.[65–67] This diagnostic confusion is an important cause of the antibiotic overuse seen in this patient group.[66, 221, 222] Treatment of ABU does not reduce the incidence of reinfection, genitourinary morbidity or improve survival among the elderly,[217, 223, 224] but may lead to increased antibiotic resistance and increased risk of antimicrobial adverse events.[9]

Spinal lesions

UTI is a prevailing problem in individuals with spinal cord injuries (SCI), who also have a high prevalence of ABU.[34] Patients using clean intermittent catheterization (CIC) and men with sphincterotomy and condom drainage have a 50 % prevalence of bacteriuria, and in subjects with chronic ICs the prevalence is almost 100 %.[34] Both the disease, the interventions to promote bladder emptying, and a low pressure voiding may increase the risk for UTI.[225–227] The type of catheter used in CIC and other measures, such as botulinum toxin injection in the bladder detrusor or urethral sphincter, or antibiotic prophylaxis, do not influence the frequency of symptomatic urinary tract infections.[34, 228] Diagnosing a possible symptomatic UTI in this patient group is difficult due the high frequency of bacteriuria, the lack of clinically typical symptoms and the unique symptoms of SCI, i.e. autonomous dysrefelexia and spasticity, which may also be present without an infection in the urinary tract.[228]

The solution – symptom scoring

What symptoms of UTI should be regarded as indication for treatment? In the nursing home population vague or non-specific symptoms might also be UTI due to clinical practice, as non-specific symptoms are the most common reason for suspecting UTI in nursing home patients. However, in a cross sectional study Sundvall *et al.*[229] investigated the relationship between bacteriuria and newly onset of or increased non-specific symptoms in a nursing home population, and found evidence of vague or non-specific symptoms in UTI unreliable, and not justifying antibiotic treatment.[229] In patients with neurogenic bladder disorder due to SCI the evaluation of symptoms is more difficult as clinical manifestations of UTI are often atypical and nonspecific. In addition, symptoms may involve only aggravation of pre-existing neurological symptoms, whereas some very suggestive symptoms such as fever may have a non-infectious origin.[230] This might also be the case in females with residual urine due to lower motor neuron lesion, e.g. disc prolapse and polyneuropathy, with or without diabetes mellitus. It is not known if the detrusor muscle insufficiency affects symptoms of UTI. Patients on CIC possibly also differ in their symptoms.

In female patients with a normal urinary tract a variety of symptom scoring questionnaires have been developed as clinical tools to assess the diagnosis and severity of acute uncomplicated cystitis (Cystitis).[231–233] In these questionnaires dysuria, urgency, and frequency have been considered the most important symptoms, and are often used in combination with dipstick with the goal to find appropriate treatment algorithms.[169, 234–236] Recently Alidjanov *et al.*[233] validated a self-reporting questionnaire for clinical diagnosis of cystitis in female patients, including QoL and additional health conditions, symptomatic changes and outcome assessment. However, validation was only due to clinical evaluation without taking biomarkers into account. Another study[18] investigated the social and economic burden of recurrent UTI on health-related QoL using an anonymous, self-administered web-based survey across five European countries. In the questionnaire dysuria, urgency, and frequency were included together with an additional 6 predefined symptoms relating to UTI. At least 2 symptoms were required for the diagnosis of a UTI. Interestingly, there was a significant difference in the reported symptoms from each country.

Signs and symptoms of UTI in individuals with SCI on CIC are generally considered to have poor sensitivity and specificity.[237] This assumption was addressed in a study by Massa *et al.*,[237] where they determined the validity, accuracy, and predictive value of some signs and symptoms of UTI in individuals with SCI using CIC, and the same individuals accuracy at predicting their own UTI. The main outcome was the incidence of UTI defined as bacteriuria ($>10^5$ CFU/mL) plus the presence of at least one sign or symptom in accordance with the National Institute on Disability and Rehabilitation (NIDRR) consensus statement.[238] "Autonomic dysreflexia" had high specificity (99%) but low sensitivity.[237] "Increased spasticity" had a specificity of 77.2% and a sensitivity of 17.2%.[237] The specificity and sensitivity of "pyuria" were high with a moderate predictive value. Taking into account all the 12 signs and symptoms, the participants overall accuracy of predicting their own UTI was 66.2% with a positive predictive value of 32.6%.[237] The authors concluded that the participants were much better at predicting when they did not have a UTI than when they did have a UTI.

The solution–biomarkers

Heparin-binding protein (HPB) is an inflammatory mediator released from secretory vesicles and azurophilic granules of activated neutrophils. It activates macrophages, monocytes, and has antimicrobial activity, mainly against Gram-negative bacteria.[239] In clinical studies, elevated plasma levels of HBP have been associated to circular failure and sepsis.[240] Its potential as a urinary biomarker for UTI was investigated in a series of studies by

Table 1. *Clinical trials using urine concentrations of Interleukin(IL-) 6 to discriminate between different forms of UTI; sensitivity and specificity of urine IL-6 at different cut off values.*

First author, year	Population, number of cases/controls	Conditions studied	Cut off level[a]
Sheu JN, 2006[245]	Children 1–10 years old, 78/12	Pyelonephritis vs Lower febrile UTI	>22 pg/mL
Rodrigues LM, 2008[247]	Children, 0–14 years old, 18/17	Pyelonephritis vs Lower UTI	>15 pg/mL >1.8 pg/mL
Azab S, 2016[248]	Children 0–14 years old, 155/30	Pyelonephritis vs Lower UTI	>20 pg/mL >2 pg/mL
Hedges S, 1992[244]	Adults, 29/10	Pyelonephritis vs Controls	>20 units/mL
	Adults, 29/ 22	Pyelonephitis vs ABU	>20 units/mL
Kjölvmark C, 2012[241]	Children, 0–18 years old, 15/33	UTI vs no UTI	10 pg/mL
Kjölvmark C, 2014[243]	Adults, 105/47	Cystitis vs no UTI	30 pg/mL
Rodhe N, 2009[66]	Elderly nurisng home residents, 16/24[c]	ABU vs Cystitis	>30 pg/mg
Kjölvmark C, 2016[242]	Elderly nursing home residents, 24/38[d]	ABU vs Cystitis	≥30 pg/mL ≥10 pg/mL

a) Concentrations of IL-6 in pg/mL (ng/L) or as units/mL. In Rodhe et al, 2009, IL -6 was adjusted for the Creatinin concentration in urine (pg/mg Creatinine). b) Not stated if the cystitis was non-febrile or not.

Kjölvmark *et al.*[241–243] Analysis from one of these studies in children with pyelonephritis revealed increased concentrations of HBP. Analysis further indicated a HBP level of 32 ng/mL as a viable cut-off level for detection of UTI.[241]

Few studies have investigated the role of IL-6 in differentiating between non-febrile symptomatic UTI and ABU.[66, 242] (Table 1)Rodhe *et al.*[66] investigated urinary levels of different cytokines in elderly community-dwelling patients with acute cystitis as compared to individuals with ABU or individuals without bacteriuria. The aim was to find a possible diagnostic tool to discriminate between ABU and acute non-febrile UTI. Distinctly higher levels of IL-6 were found in patients with non-febrile UTI than in those with ABU, and a cut-off level of 30 pg/mg was proposed.[66] Similarly Kjölvmark *et al.*[243] found IL-6 to significantly discriminate between non-febrile UTI and ABU, and suggested a cut-off level of 30 pg/mL.[242] Figure 1 summarizes the role of IL-6, IL8 and neutrophils as local and systemic biomarkers in UTI, and suggests the possible role of IL-6 as a urine biomarker differentiating between UTI and ABU.

Sensitivity (%)	Specificity (%)	Main results of the study
86	81	Patients with pyelonephritis had significantly higher levels of IL-6 compared to patients with lower febrile UTI.
38.9 77.8	94.1 58.8	Children with pyelonephritis had significantly higher IL-6 levels than children with lower UTI[b].
39.9 79.9	95.1 57.2	Urinary IL-6 levels were significantly higher in patients with pyelonephritis than in patients with lower UTI[b].
86	100	The levels of IL-6 were significantly elevated in patients wtih pyelonephritis compared to controls with sterile urine.
nd	nd	IL-6 could not discriminate patients with pyelonephritis from individuals with ABU.
73.3	84.1	Patients with UTI (pyelonephritis and cystitis) had significantly higher levels of IL-6 compared to patients without UTI.
52	92.9	IL-6 levels were significantly elevated in patients with cystitis compared to patients with no UTI.
81	96	Patients with cystitisb had significantly higher levels of IL-6 compared to patients with ABU.
38 46	82 68	Residents with cystitis had significantly higher levels of IL-6 compared to residents with ABU.

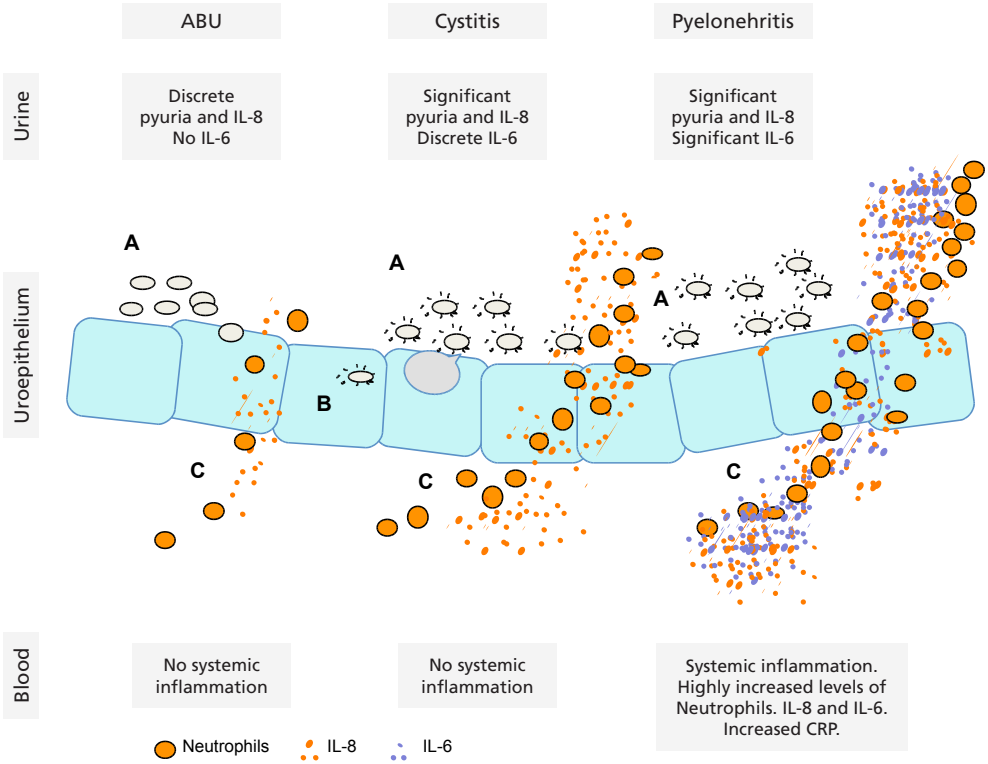
c) Aged matched control population with community aquired cystitis diagnosed at a Primary Health Care Centre. d) Aged matched control population with community aquired cystitis diagnosed at two Primary Health Care Centre offices and at two hospital emergency departments.

Correction for Creatinine

The use of urinary concentrations of IL-6, adjusted or unadjusted for creatinine concentration, varies in studies.[40, 66, 152, 244–248] However, in a cross-sectional study by Sundvall *et al.*[168] a correlation between unadjusted urine IL-6 concentrations and creatinine adjusted IL-6 concentrations was found in 421 nursing home residents. Pearson's correlation coefficient was 0.86 ($p<10^{-6}$), indicating a high correlation between creatinine adjusted and unadjusted urinary IL-6 concentrations in this study population.[168] The grade of correlation for urinary HBP is not known.

The solution – algorithms

Long term care settings, especially nursing homes, are increasingly recognized as reservoirs of antibiotic-resistant bacteria.[53] The empirical use of antibiotics is frequent in the absence of microbiology results or even in the absence of a definitive diagnosis of infection. Experts believe that many of the antibiotic prescriptions written in nursing homes may be inappropriate. In



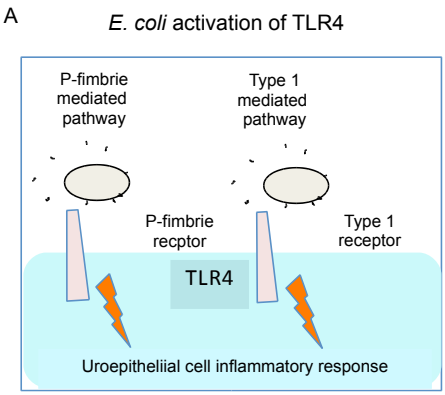


Figure 1. Schematic figure outlining bacterial challenge and activation of the host response in urinary tract infections. (A) In asymptomatic bacteriuria, there is no or only inefficient activation of the uroepithelium. In symptomatic urinary tract infection, bacterial contact with the uroepithelium is mediated by P or type 1 fimbriae and its receptors on the uroepithelium. Toll-like receptor 4 recognizes the gram-negative uropathogens, the uroepithelial cell is activated, and inflammatory mediators (interleukin [IL] 6 and IL-8) are produced.[154] (B) In recurrent cystitis, intracellular bacterial fabrics have been suggested.[117] (C) Neutrophils from the circulation transmigrate, guided by expressed IL-8 receptors (CXCR), by following the concentration gradient of IL-8 to the place of infection to combat the bacteria by phagocytosis.[159]
ABU = asymptomatic bacteriuria; CRP = C-reactive protein; IL = interleukin; TLR4 = toll-like receptor 4; UTI = urinary tract infection. Published with permission from European Urology Supplements, Elsevier B.V.

a study by Phillips *et al.* over 80% of antibiotic prescriptions for catheterized individuals were written for individuals with ABU.[65] Increased antimicrobial stewardship efforts are indicated to reduce unnecessary urinary catheterization, unnecessary diagnostic testing and inappropriate prescribing of antimicrobials for ABU in LTCFs and other institutional settings.[53, 249, 250](Table 2) Minimum criteria for initiating antibiotics in nursing home residents have been proposed.[216] The Loeb minimum criteria (LMC),[251] developed by a consensus conference and published in 2001, are proposed standards for the initiation of antibiotics in long term care settings. For residents without an IC a minimum of acute dysuria alone or fever is required, together with at least one of the following criteria: New or worsening urgency, frequency, suprapubic pain, gross hematuria, costovertebral angle tenderness, or urinary incontinence. In a patient with IC the presence of at least fever or new costovertebral tenderness, shaking chills with or without identified cause, or new onset of delirium is required for treatment initiation.[251]

In a multifaceted interventional study the effect of the LMC on antibiotic prescription rate for UTI in a nursing home population was investigated.[68] In the study the LMC was used both as a diagnostic algorithm for ordering urine cultures and as a treatment algorithm for prescribing antibiotics. The number of antibiotic prescriptions was significantly lower in the intervention arm than in the control arm, but this difference seemed to wane during the 12-month follow-up.[68] The authors suggested that the reduced difference in prescription rate during the final months of the study could be due to weariness of the health care provider with the intervention.[68] The LMC appears not to be widely in use. The adherence to LMC was investigated in a cross-sectional analysis by Olsho *et al.*[252] The prescription rate in nursing homes was followed during a 3-month period, representing > 100,000 resident-days. The authors found little evidence of adherence to the LMC by the prescribers as only 10% of the prescriptions for UTI were in accordance with the criteria.[252]

Another interventional study in a nursing home population used only a multifaceted educational strategy to evaluate its impact on the rate of prescriptions for UTI.[69] The educational intervention significantly reduced the overall proportion in antibiotic prescriptions and also influenced the proportion of infections handled by physicians as ”wait and see”. However, there was no evidence of a significant change in the primary outcome variable (the prescribing of fluoroquinolones).[69] To conclude the moderate effect of the intervention, the authors raised a question to consider in future studies in nursing home settings; is it the nurse or the physician who has the most influence on antibiotic prescribing?

Specificity and sensitivity of urinary biomarkers in studies done

In spite of the obvious need to have an objective biomarker to distinguish between ABU and UTI not many studies have been performed, much due to the complexity of the problem. We know that ABU causes a low but significant host response and that the responses are triggered in UTI.[66, 155] However, the variation of the magnitude differs in both UTI and ABU with individual differences, creating difficult differential diagnosis. IL-6 has been suggested as a biomarker to be used for differentiating between colonization and symptomatic infection.[253] Nanda *et al.*[253] described in 2009 the flaws in present methods to differentiate between types of UTI, e.g. between lower UTI and ABU, and pointed to the need for novel biomarkers that can assist in the diagnosis and determination of disease severity for UTI.[253] IL-6 was suggested as a good marker candidate.[253] This was also supported by other investigations repeatedly demonstrating low or a lack of urinary IL-6 in ABU, but a frequent triggering in non-febrile lower UTI.[66, 155]

Rodhe *et al.*[66] studied a cohort of elderly community dwelling persons with ABU and one

Table 2. *Randomized controlled trials (RCTs) to reduce potentially inappropriate antibiotic prescribing in Long-term care facilities (LTCFs).*

References, country	Study design, condition studied	Target group	Interventions
Loeb *et al.*, 2005[68] USA	Cluster randomized: 24 nursing homes, UTI	Physicians, nurses	1. Diagnostic & treatment algorithm for urinary infection. 2. Small group interactive sessions for nurses: videos, outreach visits. 3. One to one interviews with physicians. 4. Pocket cards and posters with algorithms.
Monette *et al.*, 2007[249] Canada	Cluster randomized controlled trial; 8 LTCFs. Multiple infectious conditions including UTI.	Physicians	Interventional group: 1. Mailing antibiotic guide and individual prescribing profile past 3 months to 36 physicians. Antibiotic courses given by physicians characterized as adherent or nonadherent. 2. Repeat mailing 4 months later.
Pettersson *et al.*, 2011[69] Sweden	Cluster randomized controlled trial; 58 nursing homes, UTI	Physicians, nurses	1. Developed guidelines in focus groups with local physician, nurse. Evaluation of guidelines in pilot study with revision. 2. Small educational sessions – physicians, nurses.
Naughton *et al.*, 2001[250] USA	Randomized controlled; 10 LTCFs, Pneumonia	Physician only or multi-disciplinary group (pysician, nurses and nurse practitioners)	1. Small group consensus process for guideline development with physician/nurse practitioners. 2. Nurses/nurse practitioners: 1 hour training session on guidelines. 3. Laminated pocket cards summarizing guidelines. 4. Laminated posters with guidelines by telephone.

open care cohort with cystitis, the majority being women in both. He investigated an array of urine cytokines and found significant differences between IL-6, IL-8 and GRO-α when comparing UTI and ABU, but IL-1 β did not differ.[66] IL-6 was the preferred marker in terms of sensitivity and specificity (Table 3). In addition, urine Leukocyte esterase (LE) was investigated using a cut off value of >2, which increased the sensitivity and specificity of LE as a biomarker.[66] IL-6 (cut-off >30 pg/mg) together with LE (cut-off >2) resulted in a combined sensitivity of 69% and specificity of 100% (95% confidence interval 42–88). The author concluded that the combination of IL-6 and LE is a possible approach to increase accuracy in differentiating ABU from cystitis.

Kjölvmark *et al.*[241] studied urinary Heparin-Binding Protein (HBP), an inflammatory mediator which in previous trials had shown association with bacterial infections. In a pediatric population, HBP and IL-6 were found to sig-

Main outcome measure	Main result of the study
Number of antimicrobials prescribed for suspected urinary tract infections.	The rate of antimicrobial use for suspected urinary tract infections was significantly lower in the intervention arm than in the usual care arm (1.17 vs. 1.59 courses per 1000 patient days). However, the effect decreased over time.
Primary outcome: adherence to the prescribing guide according to five criteria; choice of antibiotic according to diagnosis, dosage, duration, frequency and dose adjustment according to creatinine clearance.	1. Nonadherent prescription reduction interventional vs. control group: 20.5% vs. 5.1%. 2. Likelihood of prescription of nonadherent antibiotics: physicians in the interventional group were 64% less likely to prescribe nonadherent antibiotics than those in the control group (odds ratio 0.36, 95% confidence interval 0.18–0.73).
Primary outcome: Number of fluoroquinolone prescriptions for UTI. Secondary outcome: Number of antibiotic prescriptions, proportion of a wait-and-see approach.	The number of quinolone prescription decreased in the intervention homes, but not significantly. The proportions of infections treated decreased significantly, and the proportion of wait-and-see approach increased significantly in the intervention homes compared to the control homes.
Antibiotic use for nursing home acquired pneumonia and antibiotic use in adherence with the guidelines. Outcomes were measured for 5 months post intervention.	1. No differences in antimicrobial use consistent with guidelines between two randomized groups. 2. In a pre/post analysis: a) Antibiotics meeting guidelines 50% vs. 81.8% (p-0.06) for multi-disciplinary group and 65% vs 69% (p=0.73) for physician only. b) No change in 30 day mortality or hospitalization.

nificantly differ between UTI and non-UTI.[241] However, HBP was the preferred marker compared to IL-6 with better sensitivity and specificity indicating its usefulness in diagnosing UTI in children. A second study in an adult population showed similar results.[243] In a follow-up study,[242] a large cohort of nursing home residents with ABU were compared to patients in open care and in a hospital emergency department with symptoms of acute cystitis. IL-6, but not HBP, significantly discriminated between cystitis and ABU[242] (Table 3). The authors concluded that urinary HBP is associated with UTI and may be a useful diagnostic marker for UTI, but is inferior to IL-6 in differentiating between symptomatic and asymptomatic infection.

Specificity and sensitivity of biomarkers in ABU and UTI

The specificity and sensitivity of urinary IL-6 has demonstrated rather high specificity when

Table 3. *Sensitivity and specificity with 95% confidence intervals for some urinary biomarkers and their cut off values when discriminating between acute cystitis and ABU in elderly individuals.*

			Cut off	Sensitivity (%)	Specificity (%)
Rodhe, 2009[66]	IL-8	(pg/mg)[a]	>285	63	96
	IL-6	(pg/mg)[a]	>30	81	96
	WBC	(>2)[b]	>2	88	79
Kjölvmark, 2016[242]	HBP	(ng/mL)	≥30	92	33
	IL-6	(pg/mL)	≥30	38	82
	IL-6	(pg/mL)	≥10	46	68
	WBC	(WBC/µL)	>2	71	42

a) IL -8 and IL-6 adjusted for the Creatinin concentration in urine (pg/mg Creatinine).
b) The Leukocyte-esterase reaction on a semiquantitative scale for the assesment of White Blood Cells (WBC) counts in urine : 1 = 10–25 leukocytes/µL urine; 2 = approximately 75 leukocytes/µL urine; 3 = approximately 500 leukocytes/µL urine; 4 ≥ 500 leukocytes/ µL urine.

populations are clear-cut, but low sensitivity. In other words, ABU patients might have high IL-6 levels if something unknown is wrong in the urinary tract, e.g. stones or interstitial cystitis, and symptomatic patients might, or might not, have increased IL-6. However, the cause of triggered or not triggered IL-6 in a UTI episode has never been examined.

Non-antibiotic treatment alternatives for recurrent UTI

Clean Intermittent Catheterization

UTI remains the most common infection occurring in patients with spinal cord injury (SCI).[254] Renal failure and sepsis secondary to recurrent urinary infection were previously the most common causes of death for these patients, but with advances in voiding management, urinary infection is now an infrequent cause of mortality. The use of Clean intermittent catheterization (CIC) is by many considered the voiding management of choice as it will secure a volume-adapted, low-pressure emptying of the bladder with a low risk of UTI.[238] Studies have confirmed that bladder management with an IC *vs.* no IC for patients with SCI results in more urological complications, such as bladder and renal stones, urinary tract infection, strictures and erosions, and bladder cancer.[255] Adherence to the use of CIC requires different efforts adapted to the individual's particular needs and preconditions, but adequate information about its advantage over other voiding managements cannot be underrated. This includes the reduced risk of UTI and septicemia, reduced upper urinary tract complications and the safety of CIC.[256] Complimentary therapies or interventions may also be required for good compliance, such as professional councelling,[257] anticholinergic medication in cases of incontinence despite reduced fluid intake, reconstructive hand surgery to improve hand function,[258] or botulinum injections to decrease urethral sphincter tone.[259] Because most SCI patients make their choice of a definitive voiding meth-

od within 2 years post injury,[256] this time frame seems to be an opportunity to actively alleviate the patient's functional or psychological barriers towards CIC as a bladder drainage method.

Hormonal replacement therapy

The postmenopausal reduction in ovarian estrogen secretion is often associated with vaginal atrophy, which is considered to be associated with diffuse genitourinary symptoms and increased susceptibility to vaginal colonization with uropathogens.[4, 260] The potential role of estrogen in postmenopausal women with recurrent UTI was investigated by Raz and Stamm in a double-blind, placebo controlled trail of topically applied intravaginal estrogen cream.[261] Several observations were made after 8 months of treatment; in the group given estrogen the incidence of UTI was significantly reduced compared to the placebo group and lactobacilli absent in vaginal cultures prior to treatment reappeared after one month in 60% of the cases.[261] Likewise, the vaginal pH decrease significantly as well as the colonization of *Enterobacteriaceae* spp, which was more or less unchanged in the placebo group.[261] Similar effect was shown in another trail using an estrogen-releasing vaginal ring, which reduced the proportion of women with a UTI by about one third.[262] However, as treatment with antibiotic prophylaxis over long periods of time may increase the risk of developing antibiotic resistance, trials with non-antibiotic measures such as topical estrogens prior to initiation of antimicrobial prophylaxis are recommended in guidelines.[26]

Bacterial interference

Bacterial interference refers to the antagonism between bacterial species during the process of surface colonization and acquisition of nutrients.[263] The initial event in establishing colonization or infection is bacterial adherence to the various epithelial surfaces of the genitourinary tract, which has led to studies aimed at blocking adhesion events as a manner to prevent UTI. Competitive exclusion of uropathogens with receptor analouges,[264] vaccines against fimbriae,[183] non-pathogenic microbes,[37, 265–270] or commensal organisms (probiotics) like lactobacilli[271] has been suggested as possible approaches to reduce the risk of UTI.[264]

Lactobacilli

Lactobacillus spp. are the dominating microorganism of the urogenital flora of healthy premenopausal women.[272] Lactobacilli may protect the vagina and periurethral area from colonization by uropathogens through a variety of mechanisms, perhaps reflecting their role in the microbial community. These include the ability to resist vaginal microbicides, including spermicides, maintain an acidic pH, direct killing of pathogens by producing hydrogen peroxide, and bacteriocins antagonistic to pathogen growth, as well as acting as competitive inhibition of bacterial adherence.[273] Several trials have been conducted using various lactobacillus species to recolonize the vagina in women with recurrent UTI, utilizing vaginal suppositories or oral administration, with mixed results. In one study 48 women were randomized to vaginal suppositories containing *L. casei v rhamnosus*, twice weekly or given placebo, the lactobacilli prophylaxis did not show an advantage in terms of UTI prevention compared to placebo.[274] In a randomized trial where women received either trimethoprim-sulfamethoxazole 480 mg once daily or oral capsules containing *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 twice daily, the rate of UTI recurrences did not differ between the two groups.[275] In a recent phase 2 double-blind placebo-controlled trial, 100 premenopausal women with at least one prior UTI in the last year were randomized to receive *Lactobacillus crispatus* containing intravaginal suppositories (Lactin-V; Osel) or placebo following antimicrobial treatment for an acute UTI.[276] 15% of the women receiving Lactin-V had at least 1 UTI compared with 27% of the

women receiving placebo.[276] Moreover, high-level *L. crispatus* vaginal colonization in women who received Lactin-V was associated with a significant reduction in recurrent UTI compared to high-level colonization pattern in women who received placebo.[276] The authors concluded that *L. crispatus* showed a promising prophylactic effect against recurrent UTI, and suggested that rapid restitution of the vaginal microbiota with Lactin-V after antibiotic treatment could be an advantage over repopulation of the vaginal flora with endogenous *L. crispatus.* The *Lactobacillus* spp. with the best prophylactic potential is still unclear. Present European guidelines recommend prophylactic use of only Lactobacillus spp. previously tested in studies and only within investigational trials.[26]

Vaccines

Various vaccines proposed to provide protection against UTI caused by *E. coli* or other uropathogens are being explored. Although it appears that a prior UTI fails to elicit a protective host immune response in man, data from animal models have indicated that immunization with uropathogenic *E. coli* (UPEC) is feasible.[277, 278] Ideally the target of a UPEC vaccine most likely needs to be highly immunogenic, expressed by the bacterium during infection and be surface-exposed in order to be recognized by the host immune system.[279] Furthermore, the candidate vaccine should be pathogen-specific in order to avoid targeting commensal *E. coli* in the gastrointestinal tract. Vaccinating with whole or lysed fractions of inactivated pathogens has been successful against other human pathogens, such as *Bordetella pertussis* (whooping cough) and *Vibrio cholerae* (cholera).[280, 281] The vaginal vaccine Urovac® contains 10 heat-killed uropathogenic bacterial species, including six different serotypes of uropathogenic *E. coli, Proteus vulgaris, Klebsiella pneumoniae, Morganella morganii,* and *Enterococcus faecalis*. The vaccine induces primarily immunoglobulin G and immunoglobulin A in the urogenital tract, thereby reducing potential colonization of the vagina and bladder with uropathogens.[282] Urovac was tested in three trials in 220 women compared primary immunization with placebo, or primary immunization with booster immunozation.[282–284] Primary immunization consisted of 3 vaginal suppositories at weekly intervals followed by 3 additional placebo suppositories at monthly intervals. In booster immunization the placebo suppositories were replaced with vaccine suppositories. Primary immunization alone did not reduce UTI recurrence. However, following the booster immunizations there was a prolonged time to the first recurrence of UTI, compared to primary immunization only or placebo.[282–284]

OM-89 is an oral immunostimulant containing extract of 18 different serotypes of heat-killed uropathogenic *E. coli* which stimulates innate immunity by increasing lymphocytes proliferation, macrophage phagocytosis, and the upregulation of dendritic cells.[285] Four placebo-controlled clinical studies have evaluated the vaccine's safety and efficacy.[286–289] The risk ratio for developing at least one UTI was significantly lower in the active treatment group and the mean number of UTIs was reduced by half compared to placebo. A meta-analysis of five clinical trials showed that OM-89 is significantly more effective than placebo in preventing recurrent UTI.[290] The most common adverse effects reported are headache and gastrointestinal symptoms.[288, 290]

NSAID and symptomatic treatment

Uncomplicated lower UTI in women are common in general practice.[291] Empirical treatment is often promptly initiated in those with typical symptoms, but evidence indicates that many women with UTI symptoms will recover spontaneously without treatment.[292] An alternative treatment strategy was applied in a double-blind randomized pilot study comparing antibiotic treatment to non-steroidal anti-inflammatory drug (NSAID) treatment in women with

uncomplicated UTI.[293] The women were randomized in either ciprofloxacin or ibuprofen groups for 3 days. If the symptoms were unresolved or worse during the trial period treatment was stopped and (another) antibiotic prescribed. There was no significant difference in total symptom score or proportion symptom free at day 4.[293] In a large multicenter study in a primary care setting women with symptoms suggesting UTI were randomized to treatment with either ibuprofen or fosfomycin for three days to investigate if NSAID treatment could reduce antibiotic prescribing.[294] Almost two thirds of the patients treated with ibuprofen did not receive antibiotic treatment during the four-week follow-up.[294] However, they had a significantly higher symptom burden than the women in the antibiotic group. Five women developed pyelonephritis after ibuprofen treatment compared to one treated with fosfomycin.[292] The difference was not significant as the study was underpowered for this specific complication. One ibuprofen related severe adverse event was reported in one woman due to gastrointestinal bleeding. The authors concluded that treatment with ibuprofen could not be recommended as a first line approach.[294] Despite this study result, the authors suggested that ibuprofen might be a treatment option that could be discussed with women with mild to moderate symptoms within a delayed prescription strategy and as a shared decision.[294] A Scandinavian randomized study evaluating ibuprofen vs. mecillinam treatment in uncomplicated female UTI has been completed, but these results has not yet been published.[295]

Present Investigations

Aims of this thesis

- To investigate whether a model ABU strain can establish in the lower urinary tract and if it carries a protective effect against superinfection of more virulent strains causing episodes of symptomatic UTI.
- To characterize the human innate mucosal host response to the prototypic ABU strain *E. coli* 83972. In addition, we investigated the association between the prototypic ABU strain with genetic polymorphisms affecting the host immune response.
- To investigate the correlation between urinary IL-6, IL-8 levels and neutrophil numbers and the severity of UTI symptoms. and the severity of UTI symptoms. Diagnostic thresholds for IL-6 were identified that differentiates between symptomatic and asymptomatic infection.
- To measure urinary IL-6, IL-8 and pyuria in a UTI-prone ABU population to determine diagnostic thresholds. The clinical utility of urine IL-6 as an added diagnostic tool for ABU and UTI differential diagnostics in an elderly institutionalized nursing home patient cohort was investigated.

Paper I. Escherichia coli *83972 bacteriuria protects against recurrent lower urinary tract infections in patients with incomplete bladder emptying.*

Background

Incomplete bladder emptying increases the susceptibility for bacterial infections in the urinary tract, such as asymptomatic bacteriuria (ABU) and symptomatic urinary tract infections (UTI).[296, 297] Individuals prone to ABU are at increased risk of repeated and unnecessary antibiotic treatments, due to positive urine cultures and an already present low local host inflammation caused by ABU.[298] It has been proposed that the protective effect of ABU may be due to bacterial interference, where bacteria of low virulence inhibit symptomatic UTI, possibly by competing for nutrition's or production of toxic molecules.[264] Based on these findings a protocol to deliberately establish bacteriuria in the lower urinary tract was created as an alternative to

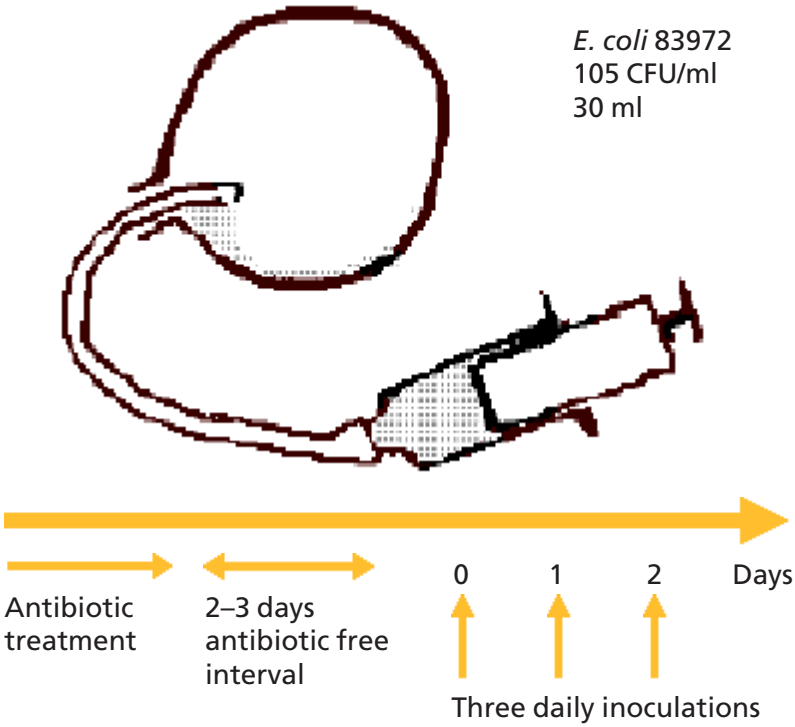


Figure 2. The human inoculation protocol. If the patients urine is not sterile, antibiotic treatment is administrated. After an antibiotic free interval the patient is catherized, the bladder is emptied, an inoculation with 30 ml E. coli 83972 (10^5 CFU/ml) is deposited in the bladder and the catheter is removed. If bacteriuria is not established, or depending on the study protocol, the procedure may be repeated daily for two days. Published with permission from Cellular Biology, Wiley Online Library.[93]

conventional therapy in patients with recurrent complicated UTIs (Figure 2).[299,300] The inoculation protocol has also successfully been used as a research model for *E. coli* host interaction, and on the influence of virulence factors for bacterial establishment and for induction of the host response.[301] In this 2-phase placebo controlled study, with a cross over design, patients with incomplete bladder emptying and recurrent urinary tract infection received inoculations with the non-pathogenic ABU strain *E. coli* 83972 to evaluate its therapeutic effect. The study received ethical vetting board approval (LU 742-01, 19/12/2001).

Results

A total of 20 patients fulfilled the criteria for evaluation in both phases of the study. There were no significant side effects of *E. coli* 83972 establishment or any febrile UTI episodes in either study arm. In phase 1 of the study patients were randomized using a blinded, placebo-controlled protocol with crossover to *E. coli* 83972 or saline inoculation. The time to the first UTI was compared in the same patients after *E. coli* 83972 establishment and after saline or failed *E. coli* 83972 establishment. There were 23 UTI reports from the 20 patients, with 8 patients re-

Table 4. *Clinical trials utilizing* E. Coli *83972 inoculation for bacterial interference.*

Reference	Study design	Population	Condition	Drainage method[a]	Outcome	Main results of the study
Hull R, 2000[268]	Pilot study; prospective, nonrandomized	21 (3 females, 19 males)	Spinal cord injury, ≥ 1UTI preceding year	13 CIC 6 SPC 2 EC	Safety and feasibility of bacterial interference using *E. coli* 83972 inoculation to prevent urinary tract infection.	• Successful colonization: 13/21 • Mean duration of colonization: 12.3 months (range 2-40) • No UTIs reported during colonization. • Mean number of infections pre/post colonization: 3.1 per year/0.
Darouiche, 2001[266]	Pilot study; prospective, nonrandomized	44 (4 females, 40 males)	Spinal cord injury, recurrrent UTIs	19 CIC 11 SPC 8 EC 6 IC	Safety and efficacy of *E.coli* 83972 inoculation in preventing symptomatic urinary tract infection.	• 30/44 (68%) successfully colonized ≥ 1month (mean 8.7 months). • No UTIs due to *E.coli* 83972. • Mean UTI rate pre/post successful colonization: 3.77 vs 0.06 symptomatic UTIs/patient-year ($p < 0.001$). • Rate of UTI colonized vs non-colonized: 0.06 vs 1.80 episodes/patient-year (mean, $p < 0.001$). • Mean time to first UTI after successful colonization vs failed colonization : 7.4 months vs 3.4 months.
Darouiche, 2005[267]	Pilot trail; prospective, randomized, placebo-controlled, double-blind	27 male patients; 21 cases/ 6 placebo	Spinal cord injury, ≥ 2 UTI episodes preceding year	8 CIC 3 SPC 16 IC	The efficacy and safety of bacterial interference during a 12 month post *E. coli* 83972 inoculation period vs placebo/unsuccessful colonization.	• 13/21 (62%) successfully colonized ≥ 1 month. • Significantly reduced number of UTIs during follow up in experimental group vs. placebo group (mean 1.6 vs 3.5, p=0.036). • No evidence of UTI/septicaemia due to E.coli 83972. • Likelihood of 1 UTI episode during 1 year follow-up: 46% in experimental group vs 96% in non-colonized group (p=0.01).

a) CIC= Clean intermittent catheterization, SPC= Suprapubic catheter, EC= External condom, IC= Indwelling catheter

porting UTI from both study arms; only one after established *E. coli* 83972 bacteriuria and six after saline or failed *E. coli* 83972 inoculation. Median time to the first UTI was significantly longer after *E. coli* 83972 inoculation compared to saline or failed *E. coli* 83972 inoculation (11.3 vs. 5.7 months, sign test p= 0.0129). All patients proceeded to phase 2 of the study, where we recorded the number of UTI episodes during 12 months with *E. coli* 83972 and 12 months without *E. coli* 83972 using a blinded, observational protocol. Additional inoculations with *E. coli* 83972 or saline were given to patients with less than 12 months in either study arm during phase 1. Evaluation was performed when the patients had spent 202 (mean 10.1 per patient) months with *E. coli* 83972 bacteriuria and 168 months (mean 8.4 per patient) without *E. coli* 83972. The number of reported UTIs was significantly lower in the same patients with *vs.* without *E. coli* 83972 bacteriuria (13 and 35 UTI reports, respectively, paired t test p= 0.009, CI 0.31–1.89).

Conclusions

This paper demonstrates that deliberately colonization with *E. coli* 83972 protect patients with incomplete bladder emptying from recurrent urinary tract infection. The results indicated that this strategy could be a feasible treatment alternative to antibiotics in carefully selected individuals with incomplete bladder emptying, who are prone to urinary tract infection.

Paper II. Genetic control of the variable innate immune response to asymptomatic bacteriuria.

Background

Although ABU is known to possess protective capacity,[35, 36] there is a common clinical observation that patients with verified ABU do have rare episodes of UTI complaints. This may lead to uncertainty about the extent of innate immune reactivity (e.g. pyuria and cytokine content in the urine) during ABU and use of local host response parameters as a basis for diagnostic and therapeutic decisions. Interestingly, variations in the local host response noted in individuals with ABU, may also be influenced by genetic factors such as polymorphisms in the *TLR4* and *IRF3* promoters that were previously associated to ABU.[196]

In this study, we analyzed the urinary host response parameters from 23 patients with incomplete bladder emptying that participated in a placebo-controlled study of therapeutic *E. coli* 83972-inoculation (see *Paper I)*. The analysis was performed prior, during and after repeated *E. coli* 83972 inoculations. In addition, patients were genotyped for specific promoter polymorphisms in *IRF3* and *TLR4* that accumulate in ABU-prone individuals. The study received ethical vetting board approval (LU 742-01, 19/12/2001).

Results

233 bacteriuric urine samples were analysed. Neutrophil numbers and IL-8 concentrations were significantly increased ($p<0.0001$) compared to sterile samples, but IL-6 was not affected. In spite of a common bacterial challenge (*E. coli* 83972) the levels of IL-8 and neutrophil counts varied significantly between individuals. In addition, the inter-individual differences in host response magnitudes were identical upon re-infection suggesting an individual unique genetic predisposition to the degree of innate immune reactivity.

In the extended cytokine/chemokine screening, the neutrophil chemoattractant GRO-α was detected at increased levels, in addition to IL-8. Furthermore, chemoattractants involved in mast cell or eosinophil (RANTES, Eotaxin-1), monocyte (MCP-1), and T cell (RANTES, IP-10) chemotaxis were detected at increased levels. The inflammatory regulators IL-1-α and soluble IL-1 receptor analogue, and the T lymphocyte/dendritic cell product sIL-2Rα were detected

with variable increase compared to sterile control samples. 19 proteins, a majority of them involved in specific immunity, were not detected. Five of the 11 polymorph-associated *TLR4* genotypic patients with ABU[139] had lower neutrophil numbers (p=0.0014), IL-6 (p<0.0001), IP-10 (p<0.0001), MCP-1 (p=0.008), and sIL-2Ra (p<0.0001) concentrations compared to the remaining group. The heterozygous *IRF3* promoter genotype associated with ABU was carried by four of the eleven patients, showing lower neutrophil counts (p=0.01) and lower concentrations of IL-6 (P=0.0007) and MCP-1 (P=0.0001) than the remaining six patients.

Conclusions

This paper demonstrated that genetic polymorphisms in genes regulating innate immunity have a significant impact on the magnitude of the host response during bacterial colonization of the urinary tract. The results also indicate that innate immune mediators are involved in the host-specific, low immune response in ABU.

Paper III. Triggered urine Interleukin-6 correlates to severity of symptoms in non-febrile lower urinary tract infections.

Background

During ABU, urine levels of IL-8 are increased, but IL-6 is mostly absent or at least, very low (see *Paper II*), suggesting that IL-6 could act as a candidate in differential diagnostics between symptomatic UTI and ABU. Recent studies have addressed the diagnostic difficulties of UTI in ABU prone populations.[302, 303] Several biomarkers were previously investigated, including IL-8 and IL-6, as well as neutrophil counts, estimated by the leukocyte-esterase reaction (LE).[66, 241,] In these studies urine levels of IL-6 have been identified as the most promising marker for UTI diagnosis,[66, 242] but when specificity and sensitivity has been evaluated, the result are moderate.[242,] In this longitudinal cohort study, we included 23 patients that were long-term inoculated with *E. coli* 83972, 20 of the included patients had previously participated in a randomized controlled trial (see *Paper I)* and three patients inoculated in open protocols were added to the data. The patients had incomplete bladder emptying due to lower motor neuron (n=12), or spinal lesions (n=11), and were followed with regular monthly symptom-scoring and urine sampling, analyzed for urine culture, IL-6, IL-8 and neutrophil counts. The patients were monitored for 260 months with, and 198 months without *E. coli* 83972. At the time of the study laboratory reference values for IL-6 and IL-8 were <15 ng/L and <200 ng/L, respectively. The study received ethical vetting board approval (LU 742-01, 19/12/2001).

Results

23 patients, alternatively with and without *E. coli* 83972 long-term bacteriuria reported 17 and 36 episodes of UTI, requiring 20 and 50 antibiotic treatments, respectively ($p < 0.01$). 37 of the non-febrile UTI episodes had complete data on host response parameters and symptom scoring prior to, and after antibiotic treatment, demonstrating prompt relief of symptoms with scoring returning to ABU levels ($p < 0.0001$). In addition, 62 episodes were reported with minor urinary tract symptoms without the need for treatment, of which 35 (56%) were positive for urine culture and with complete data on host response parameters.

The 37 reported UTI episodes were accompanied by an increased host response with elevated mean IL-6, IL-8 and neutrophil counts. In a pooled comparison to the mean ABU values, IL-6 was the only parameter with a significant increase in UTI ($p = 0.0371$).

To detect immune response differences in the same patient to UTI versus ABU, we paired the patient's own ABU mean host response with

their own UTI response. IL-6 levels were higher in both patient groups during symptomatic infections (p=0.0021 and 0.0215 respectively) while IL-8 was only significantly higher in patients with residual urine (p=0.0290). There was no difference in urine neutrophil counts in either of the patient groups.

The subjective symptoms assessed by the patients were stratified according to severity as measured by symptom scoring, and correlated to the host response. To compensate the subjective base-line variation of scoring between individuals, we calculated fold-changes (FC) of each UTI score to the mean ABU score in the same patient. UTI episodes were divided into "low-scoring UTI" (FC < 1.5) and "high-scoring UTI" (FC ≥1.5). There was no difference between the ABU host responses to low-scoring UTI responses. However, when the same comparison was made between ABU and high-scoring UTI, the concentrations of IL-6, IL-8 and neutrophil counts were dramatically increased (p=0.0015, p=0.0196 and p=0.002 respectively).

A threshold level of 25 ng/L urine IL-6 was identified as a marker for UTI in the need for antibiotic treatment (see below). To investigate whether this threshold level could differentiate between low-scoring and high-scoring UTI, the 37 UTI reports were stratified according to the IL-6 threshold. The common scoring points between the patient groups were analyzed and we observed a clear-cut correlation between symptom severity and elevated urine IL-6. There was no change in scoring between periods of sterile urine and ABU except for "urine properties". Patients with spinal lesions also reported spasticity and autonomic dysreflexia, which did not differ significantly in any comparison between patients with sterile urine, ABU or UTI.

Sensitivity and specificity for IL-6, IL-8, neutrophil counts and semi-quantitative data on White Blood Cells (WBC) as estimated by the Leucocyte esterase (LE) reaction, were analyzed for differential diagnostic capacity of ABU versus all UTI episodes, or in only high-scoring UTI episodes. ROC curve analysis was performed to identify optimal diagnostic thresholds. As expected, the sensitivity and specificity pattern of all biomarkers were poor, or at most moderate when tested in all UTI episodes. However, in high-scoring UTI, IL-6 (cut-off 25 ng/L) and urine neutrophils (cut-off $100x10^4$/ml) demonstrated good to excellent sensitivity (77% and 92% respectively) and specificity (93% and 79%, respectively). To investigate the usefulness of combined markers, easy to use in clinical practice, the combination of IL-6 and WBC (cut-off ≥ 2) was tested, demonstrating excellent sensitivity and specificity (83% and 93%, respectively).

Conclusions

Triggered IL-6 correlated to UTI symptom severity. In addition, the results indicate that IL-6 could be used to differentiate between UTI and ABU.

Paper IV. Predictive capacity of urinary interleukin-6 for symptomatic urinary tract infections in a nursing home population.

Background

UTI is the most common indication for antibiotic treatment among institutionalized older adults.[65] The high prevalence of ABU and the frequent lack of clear UTI symptomatology in elderly nursing-home residents result in diagnostic difficulties.[65] Antibiotic treatment is often given to determine if unspecific symptoms relate to bacteriuria.[20, 229, 304] Trials of antibiotic stewardship programs have been tried on nursing home patients, where optimized diagnostic routine methods and improved decision-making algorithms have reduced antibiotic treatments,[68, 69] but with the interventional effect decreasing over time.[68] Thus, there is an obvious need for improved clinical standards and introduction of

new diagnostic biomarkers to distinguish ABU from symptomatic UTI.[243, 253] Urinary concentrations of IL-6 have been suggested as a biomarker differentiating between ABU and UTI.

In the first part of this study an ABU nursing home cohort was monitored for 12 months to identify urinary inflammatory threshold values defining the transition from ABU to UTI. In a second part of the study residents from 10 nursing homes with a total of 368 beds were invited to participate in a two-phase interventional trial, to investigate IL-6 as an added diagnostic tool for treatment indication in suspected UTI. During the first phase (*Year 1)* all antibiotic prescriptions were registered. After *intervention* with introduction of IL-6 as a biomarker for UTI, the monitoring was repeated during the second phase (*Year 2*). The sample size was calculated to detect a 30% reduction of antibiotic prescriptions with a significance of $p<0.05$. The primary endpoints were identification of diagnostic thresholds for UTI, and if IL-6 as a biomarker for UTI could reduce antibiotic treatments. At the time of the study laboratory reference values for IL-6 and IL-8 were <15 ng/L and <200 ng/L, respectively. The study received ethical vetting board approval (Dnr 341/2005, 12/9/2005 and Dnr 439/2007, 24/9/2007).

Results

ABU cohort

A total of 22 UTI episodes in 13 patients (five women and eight men of which four had IC resulted in antibiotic treatment. Urine cultures showed uropathogenic growth in 18 out of 22 (82%) of the UTI episodes. After antibiotic treatment, significant subjective improvement was recorded by symptom scoring ($p < 0.0001$). 13 of the 18 UTI episodes with a positive urine culture, from eight patients, had complete data on urinary IL-6, IL-8 and WBC before, during and after the symptomatic episode. When the host response during UTI was compared with the ABU responses from all patients, only IL-6 showed a significant increase ($p =0.0008$). A clear difference was seen in the magnitude of ABU host responses when ABU patients with and without UTI episodes were compared; the UTI-prone patients had higher ABU host responses. When ABU and UTI host responses were compared only in the UTI-prone patients, once again IL-6 was the only marker with a significant increase ($p =0.042$). In contrast, when ABU host responses from patients without any reported UTI episode were compared with ABU responses from the UTI prone patients, highly significant increases were detected in all three inflammatory parameters. To investigate the triggering of host response during transition from ABU to UTI in individual patients, we calculated the mean ABU host response in each of the UTI-prone patients, and compared it with the response at the symptomatic episode in the same patient. In this paired analysis, only IL-6 showed a significant increase ($p = 0.017$). In conclusion, IL-6 was the only marker distinguishing between ABU and UTI in the ABU patients with symptomatic episodes.

Nursing home cohort

During the first phase of the study (*Year 1)* 137 antibiotic treatments for suspected UTI was prescribed to 84 patients (mean 1.04 treatments/1000 patient days). Uropathogenic growth was revealed in 105 of 113 (93%) obtained cultures. After *intervention* with introduction of IL-6 as a biomarker for UTI the monitoring was repeated. During the second phase of the study (*Year 2)* a total of 110 antibiotic treatments for suspected UTI was given to 72 patients with positive cultures, and in six patients with negative urine cultures (mean treatments 0.81/1000 patient days). The reduction of antibiotic treatments, as compared with year 1, was 20% (ns). The rate of repeated prescriptions and the time interval between recurrences did not differ between the 2 years of the study.

In a subsequent analysis the IL-6 (cut-off 25 ng/L) predictive capacity for UTI episodes requiring antibiotic treatment versus ABU was calculated. Sensitivity and specificity was 57% and

80%, respectively. The positive predictive value was 52% (95% CI 42–60) and the negative predictive value was 83% (95% CI 78–87).

Conclusions

Urinary interleukin 6 could be a promising biomarker to detect the transition from asymptomatic bacteriuria to symptomatic urinary tract infection in older adults. The educational intervention, including the introduction of IL-6 as an additional diagnostic tool, reduced the number of antibiotic prescriptions by 20%. The reduction was not significant possibly due to the study being statistically underpowered. Further larger studies with robust methodology are warranted to determine whether development for near to patient testing would be worthwhile.

Discussion

Historically, the symptom-free bacterial colonization (ABU) of the urinary tract was considered to be the cause of recurrent cystitis and chronic kidney infections.[1] Later, epidemiological studies proved the irrelevance of these fears, and ABU not only to be harmless,[1, 35] but also to possess a protective effect against ascending symptomatic super-infections.[36, 211] In spite of this well-established knowledge ABU is in clinical practice unnecessarily eradicated due to difficulties in deciding whether to treat or not to treat bacteriuria in patients with diffuse symptoms.[222] Moreover, the increased use of antibiotics in the society drives the selection of antimicrobial resistance, creating an urgent rationale for the development of alternative treatments.[42, 305] We have in this thesis investigated the capacity of deliberately established ABU with an apathogenic isolate, *E. coli* 83972, to act as an alternative treatment of recurrent UTI, the individual variation of innate immune response to ABU, and the potential use of the acute-phase reactant IL-6 as a biomarker for UTI in patients with ABU. The same study population (UTI prone patients with incomplete bladder emptying and/or neurogenic bladders) was used for paper I–III. Paper IV analyzed an elderly population in nursing homes facilities.

In the first study (*Paper I*) we demonstrated that deliberate bacteriuria with a model ABU strain can be established, that it carries a protective effect similar to that of naturally selected strains, and that individuals prone to UTI benefit from this approach by a significant reduction of symptomatic UTIs, a finding also demonstrated in previous observational studies, and in a later RTC (Table 4). Furthermore, the safety of the ABU strain as a treatment option was verified. In our study colonization failure occurred in a limited number of patients, something also seen in other similar studies.[266–268] These patients might eliminate the inoculated bacteria due to a more active antimicrobial host defense in the urinary tract than those in whom *E. coli* 83972 bacteriuria developed and who might have an attenuated antibacterial response. We chose to use a self-reporting definition of UTI in our study as we believe self-reporting was the most adequate method to use, i.e. well-adjusted to the everyday clinical practice and providing an excellent nonbiased marker of UTI episodes. A previous study by Massa *et al.* showed that the positive predictive value of self-diagnosing in patients with SCI and neurogenic bladder was low.[237] However, the reliability of self-diagnosing recurrent UTI was verified in this study by the high rate of uropathogenic growth in urine cultures from UTI reports in patients without *E. coli* 83972 bacteriuria, and by the prompt decrease in symptom-scoring post treatment.[306] In the second phase of the study patients were given additional inoculations with the ABU strain or saline in order to fulfill the study protocol, and consequently this study phase was observational. However, the study blinding was not compromised by this approach, as the patients were prepared at study inclusion for random re-inoculations in both study arms. Furthermore, as the re-inoculations were given after failed colonization or spontaneous clearance of *E. coli* 83972, it was impossible for the patients or the study group to know

whether *E. coli* 83972 was present in the urine. The ABU treatment was accompanied by a low innate immune response in the bladder[66, 155] but minor irritative symptoms were reported by 50% of the study population equally distributed in both study arms. The host response in patients with ABU obscures the clinical significance of positive reagent strip results, particularly when the patient's UTI symptomatology is unclear or diffuse. Thus, there is a need for alternative diagnostic biomarkers, distinguishing ABU that does not require treatment from symptomatic UTI.[243, 253] Neutrophil numbers in urine vary greatly among patients with ABU and the diagnostic value of pyuria has been debated in this patient group.[1] In order to use host response parameters as a basis for diagnostic and treatment decisions, more information about the variability of the host response in ABU is needed. In *Paper II* we characterized the immune response repertoire in patients who developed bacteriuria with the ABU strain *E.coli* 83972. By selecting this patient group, the study could in a unique way standardize the bacterial challenge to one single strain (*E. coli* 83972), and thus isolate the host-driven differences in the innate immune response in each individual. We found neutrophils and IL-8 to be significantly increased in *E. coli* 83972 bacteriuric samples compared to sterile samples, but IL-6 levels were not affected. The levels of IL-8 and neutrophil counts were low, but varied significantly between individuals, and the inter-individual differences in host response magnitudes were identical upon reinfection. In the extensive cytokine/chemokine screening for 31 immune-related proteins, a total of 13 proteins were detected in varying concentrations during *E. coli* 83972 ABU, with the majority being involved in chemotaxis. Of the remaining 18 proteins, none were detectable in sterile or bacteriuric samples. The majority of these proteins were involved in specific immunity, indicating that the low host response during ABU is mainly the result of innate immune activation. Our results suggest that ABU elicits a variable and low innate host response, the level of which is host specific, and that immune response measurements could add both qualitative and quantitative information when differentiating between ABU and symptomatic UTI. Furthermore, genetic variations seem to influence the innate host response in ABU. Previous studies have reported ABU to be linked to promoter polymorphisms in *TLR4* and *IRF3*, as a difference was observed between patients with symptomatic infections and ABU.[139, 196] We defined *TLR4* and *IRF3* promoter polymorphisms in eleven of the subjects in our study population and found 9 of these patients to carry genotypes associated with primary ABU, as revealed by lower levels of urinary IL-6 and neutrophil infiltration.

To better understand the well-known difficulties in differentiating between ABU and UTI by the use of biomarkers in urine, we investigated the correlation of the host response and symptom scoring in *Paper III*. In this study we analyzed 37 UTI reports from patients monitored during long-term *E. coli 83972* bacteriuria and control periods (for study population, see paper I). The 37 UTI episodes were selected due to having complete data on all host response parameters (IL-6, IL-8, WBC, neutrophils). In a first analysis we demonstrated IL-6 to be the only parameter that increased significantly during UTI, as compared to ABU host responses in both pooled and paired comparisons. In previous studies specificity and sensitivity of IL-6 have been evaluated and the results were moderate at best (see *Paper IV*).[242,] In the present study the sensitivity of IL-6 was clearly improved when we stratified the severity of UTI by comparing symptom-scoring during ABU and UTI. The IL-6 threshold of 25 ng/L differentiated between UTI with low-scoring and with high-scoring symptoms, resulting in high specificity and sensitivity, and with sensitivity further improved by the combination of WBC. Interestingly, patients with more severe symptoms of lower UTI reported subjective sensations of fever, although in our study no febrile UTI episode was reported or objectively registered. This is in line with the pyrogenic effect of

IL-6[151] suggesting that severe symptoms associate with "deeper tissue engagement",[156] however still being clinically defined as lower, non-febrile UTI. Our finding suggest the low sensitivity of IL-6 threshold values commonly found in non-febrile lower UTI, depended on the mixed spectra of the disease, ranging from low-scoring (IL-6 negative) to high-scoring (IL-6 positive) symptomatic UTIs.

In our last study, *Paper IV*, we investigated if IL-6 could be used in clinical practice as a tool for deciding to treat or not to treat suspected UTI in a nursing home population. The diagnosis of UTIs in elderly nursing home residents is challenging due to the high prevalence of ABU and the frequent lack of clear UTI symptomatology. Studies available have identified IL-6 to be the most interesting marker candidate differentiating between ABU and UTI.[66, 243] In the first part, or the *ABU cohort* part of the study, the role of urinary IL-6, IL-8 and pyuria in the same elderly population during ABU and during symptomatic UTI episodes were investigated. The results showed that urine concentrations of IL-6, but not IL-8 or neutrophil numbers, differentiated between asymptomatic and symptomatic periods of bacteriuria in pooled and intra-individual comparisons. This led us to suggest an IL-6 threshold of 25 ng/L for initiating antibiotic UTI treatment in the second part of the study, the *Nursing home cohort* study, where it was introduced to the clinicians as an added decision tool in an interventional UTI treatment protocol in our complete nursing home population. Implementation of the protocol led to a marked reduction (20%) of antibiotic prescriptions. However, the reduction was not significant, probably due to the study being undersized in order to statistically confirm the achieved reduction of antibiotic prescription. Other limiting factors in our study were the restricted number of UTI episodes analyzed with complete data on mucosal host responses before, during and after symptomatic episodes, and the open exploratory character of the interventional part of the nursing home cohort study, in which we could not identify bias from the increased use of urine culture or the increased awareness of the ABU protective effect. Despite these limitations the present results show urine IL-6 to be the a promising marker of an objective UTI diagnosis in patients with an ongoing inflammatory response caused by ABU.

When accuracy of the inflammatory markers to predict UTI in our ABU prone study population was analyzed, the sensitivity and specificity of IL-6 (cut-off 25 ng/L) were found to be poor and moderate, respectively. One evident cause of the moderate specificity was the intra-individual several-fold fluctuation of the ABU baseline responses in the UTI-prone patients, producing false positive values of all markers, including IL-6. The poor sensitivity was possibly caused by the low IL-6 concentrations in many patients treated for UTI. Hypothetically, this could be the patients that were unnecessarily treated, without significant "deep" tissue infection,[156] and which instead could have benefited from a wait-and-see approach. In our separate *ABU cohort* study, with the UTI diagnosis controlled by symptom scoring, only 23% of the UTI episodes were below the proposed cut-off of 25 ng/L, suggesting an improved sensitivity of this threshold when stricter criteria for UTI diagnosis was applied. Although the effect of the diagnostic use of IL-6 could not be isolated in the present pilot study, it does confirm feasibility and potential clinical usefulness of the approach. In addition, all responsible doctors interviewed after the study regarded urinary IL-6 as a useful diagnostic tool that supported a wait-and-see approach.

Future perspectives

We showed that bacterial interference, utilizing *E.coli* 83972 inoculation, could be an attractive treatment alternative to antibiotics in patients with lower urinary tract dysfunction and incomplete emptying. Future studies are needed to further define individual variation and

host response genetics in inoculated patients, and phenotypic and genotypic adaptation of *E. coli* 83972 in the same hosts. Hypothetically individually tailored *E. coli* 83972 could then be inoculated in patients with fitting immune properties to achieve an optimally "silent" co-habitation in the host, protecting against symptomatic superinfections. Other study goals would be to enhance, by discrete transformation of factors responsible for bacterial colonization, *E. coli* 83972 persistence in the human lower urinary tract. Indirectly, our study also strongly supports the non-treatment approach of spontaneously developed ABU in patients prone to UTI.

The pursuit of novel biomarkers to differentiate ABU from UTI needs to be prioritized in order to protect ABU prone populations from unnecessary antibiotic exposure. Our results suggest urinary IL-6 (cut-off 25 ng/L) to detect the transition from ABU to symptomatic UTI. Further studies should explore if urine IL-6 can stratify UTI into groups with different needs for treatment, and if "IL-6 negative" episodes have a more benign outcome and could benefit from alternative non-antibiotic treatments.

Nursing home populations are especially exposed to antibiotic overuse due to the diagnostic difficulties in distinguishing ABU from bacteriuria in need of antibiotic treatment. Our results indicate urine IL-6 as being the most promising marker of an objective UTI diagnosis in patients with an ongoing inflammatory response caused by ABU. Furthermore, we showed the feasibility of using an educational intervention program to reduce antibiotic prescribing in older adults. Further studies should test, by close safety monitoring, the concept of IL-6 as a marker for treatment indication or a wait-and-see approach in suspected UTI in cohorts large enough to detect the reduction of the antibiotic prescriptions.

Populärvetenskaplig sammanfattning

Urinvägsinfektion (UVI) är en av de vanligaste bakteriella infektionerna hos människan.

Antibiotika är för närvarande den mest effektiva behandlingen mot akut UVI, men effektiviteten hotas av den snabbt ökande antibiotikaresistensen i samhället. En viktig orsak till ökningen av resistensen är överanvändning av antibiotika. Således finns ett stort behov att utveckla alternativa behandlingsmetoder, istället för antibiotika. Urinvägarna är normalt sterila, d.v.s. utan bakterieväxt. Bakteriell kolonisering kan leda till symtomatisk infektion (cystit med enbart lokala symtom och utan feber, eller pyelonefrit med hög feber och allmänpåverkan) eller en helt symtomfri kolonisering, s.k. asymtomatisk bakteriuri (ABU). Överanvändning av antibiotika är mest påtaglig i grupper med hög förekomst av just ABU, t.ex. sjukhusvårdade och institutionaliserade multisjuka äldre eller individer med nedsatt blåstömningsförmåga, som ryggmärgsskadade. ABU är ovanligt hos barn och friska medelålders vuxna, men är vanligt hos äldre och förekommer hos omkring hälften av personer vårdade i äldreboende, samt hos individer med ryggmärgsskada och blåsfunktionsrubbning. Forskning har visat att ABU är ofarligt, att ABU bakterier är ”snälla” och att de dessutom kan skydda mot UVI genom att helt enkelt blockera utrymmet för att mer ”elaka” bakterier ska kunna nå urinvägarna. Därför skall ABU inte behandlas, förutom hos gravida kvinnor och personer som skall genomgå kirurgiska ingrepp i urinvägarna.

Diagnos av nedre UVI grundas främst på de symtom som patienten anger, i kombination med snabbtester av urinen. Snabbtesterna visar om vita blodkroppar finns i urinen, och ger en ungefärlig indikation på bakterieväxt. Vid tveksamhet skickas också en urinodling, som dock kräver 2–3 dagar innan resultatet är klart. För ett snabbt behandlingsbeslut är således läkaren beroende av främst de symtom patienten anger samt de ganska osäkra snabbtesterna av urinen. Vita blodkroppar kan exv. föreligga i urinen även hos patienter utan cystit. Det finns stora patientgrupper som har svårt att säkert ange symtom vid misstänkt UVI, exv. äldre som p.g.a. demens eller stroke kan ha svårt att kommunicera. Ryggmärgsskadade har också svårtolkade symtom, då skadan bl.a. orsakar känselnedsättning i olika omfattning. Dessa patientgrupper har som vi redovisat dessutom ofta ABU. Hos dessa patientgrupper finns således två svårigheter vid diagnos av UVI; dels är det svårt för läkaren att värdera patientens symtom och dels är snabbtesterna av urinen närmast oanvändbart eftersom bakterieväxt föreligger antingen patienten har en behandlingskrävande UVI eller inte. Således föreligger ett starkt behov av att utveckla objektiva tester, s.k. biomarkörer, för att säkert kunna avgöra om antibiotikabehandling krävs. Dessutom föreligger naturligtvis ett stort behov av att utveckla alternativ till antibiotikabehandling för att ytterligare undvika resistensutveckling hos bakterierna.

När bakterier når urinvägarna aktiveras ett lokalt immunförsvar i urinblåsans slemhinneceller. En mängd olika substanser bildas i slemhinnan för att reglera och initiera inflammationen. Cytokiner, som är små proteiner, fungerar som signalmediatorer mellan celler och har en viktig reglerande funktion av inflammationen. De två viktigaste cytokinerna i detta sammanhang är interleukin 6 (IL-6) och 8 (IL-8). Urinkoncentrationerna av IL-6 i urinen är förhöjda vid UVI och nivåerna återspeglar svårighetsgraden av infektionen. Vid ABU är IL-6 nivåerna däremot oftast omätbara eller mycket låga. IL-8 är en s.k. kemokin som utövar en stark dragningskraft på de vita blodkropparna. De vita blodkropparna är avgörande för bekämpningen av bakterierna vid en UVI. När IL-8 frisätts från slemhinnecellerna byggs en koncentrationsgradient upp i underliggande vävnad, längs vilken de vita blodkropparna vandrar till infektionsområdet. Väl där dödar de bakterierna genom fagocytos (”cellätande”). De vita blodkropparna försvinner sedan ut i urinen (pyuri), något vi

märker genom att urinen blir grumlig och illaluktande vid UVI. Eftersom IL-8 behövs för att ”locka” de vita blodkropparna till platsen för infektionen, finns det en korrelation mellan IL-8 koncentrationer och antalet vita blodkroppar i urinen. Även vid ABU föreligger ett svagt värdsvar i form av mätbara nivåer av IL-8 och vita blodkroppar i urinen, vilket förklarar varför mätning av vita blodkroppar eller IL-8 i urin inte är ett säkert bevis på en symtomatisk UVI. Däremot har IL-6 låga nivåer vid ABU, men stiger vid symptomatisk UVI. Urinkoncentrationen av IL-6 ter sig därför som en lämplig markör för att påvisa UVI hos personer med ABU.

I delarbete 1 undersökte vi om en avsiktlig inokulering av urinblåsan med en ”snäll” ABU bakterie ger samma skydd mot symtomgivande UVI som naturligt utvecklad ABU har. Vi rekryterade patienter med blåstömningsrubbningar av olika slag (p.g.a. svag blåsa eller ryggmärgsskada) och som hade upprepade besvärliga UVI. Vi använde vi oss av ett tidigare utvecklat protokoll för bakterieinokulering av urinblåsan. Efter antibiotikabehandling för att göra urinen steril sprutas, via tillfällig kateterisering, en bakterielösning (ca 30 ml) in i urinblåsan. Bakteriestammen som används, *Escherichia coli* (*E. coli*) 83972, är en mycket välstuderad ofarlig tarmbakterie med ABU-egenskaper, och som har påträffats hos en ung kvinna med ABU under flera år utan att ha orsakat några som helst biverkningar. I tidigare studier har visats att *E. coli* 83972 inokulering ger ABU under långa perioder (veckor–år) i urinblåsan, förutsatt att ofullständig blåstömning föreligger. *E. coli* 83972 är känslig för alla typer av antibiotika och innehåller en plasmid (ringformad DNA-molekyl) som möjliggör identifikation.

För att undersöka skyddseffekten av *E. coli* 83972 ABU använde vi oss av en studie med randomiserad dubbel-blind cross-over design. Patienterna lottades slumpmässigt till att få antingen koksalt eller bli inokulerade med *E. coli* 83972. Efter halva studieperioden bytte patienterna grupp och kunde därmed fungera som sina egna kontroller. Varken patienterna eller de som utförde inokulationerna hade vetskap om det var koksalt eller *E. coli* 83972 som gavs. Vi följde patienterna under sammanlagt två år med målet att de skulle vara inokulerade med *E. coli* 83972 eller ha fått koksalt under perioder på ett år vardera. Resultatet visade att vårt antagande stämde; tid till första UVI var signifikant längre och antalet UVI under hela studieperioden var signifikant färre med *E. coli* 83972 ABU inokulering, jämfört med då försökspersonerna fick koksalt.

Det är i klinisk praktik känt att patienter med ABU har olika grader av lokalt immunsvar. Denna variation försvårar för läkaren att avgöra om en ABU patient med ABU har en behandlingskrävande UVI eller bara sin ”vanliga” ABU. **I delarbete 2** karaktäriserade vi därför värdsvaret noggrant hos individer som inokulerats med *E. coli* 83972 ABU samt fastställde huruvida de olika individuella nivåerna av värdsvaret vid ABU påverkades av bakomliggande variation (polymorfism) i de gener som avgör immunsvaret. Under ABU med *E. coli* 83972 var nivåerna av IL-8 och neutrofiler signifikant förhöjda jämfört med sterila urinprover. Urin IL-6 nivåerna var däremot inte förhöjda under ABU. Dessutom varierade koncentrationerna av IL-8 och neutrofiler mellan försökspersonerna, tydande en genetisk orsak till variationen av immunsvaret. Detta antagande styrktes när vi såg samma individuella mönster av immunsvar vid upprepad inokulation. Man skulle kunna likna immunsvaret vid ett ”fingeravtryck” – alla individer har ett likartat svar men med en individuell variation (och känslighet), unikt för just den individen. Vid undersökning av bakomliggande genetiska faktorer fann vi förekomst av genetisk variation (polymorfism) kopplad till tendens att utveckla ABU hos en majoritet av patienterna.

I delarbete 3 studerade vi sambandet mellan symtom och svårighetsgrad av UVI och identifierade diagnostiska gränsvärden för IL-6, IL-8 och vita blodkroppar vid ABU och UVI. Patienterna fick fylla i symtomformulär (symtom-score) och lämna urinprov för bestämning av bakterieväxt, IL-6, IL-8, och vita blodkroppar varje månad samt vid symtom på UVI. Resultaten visade att

endast IL-6 nivåerna var genomgående signifikant förhöjda vid UVI vid analys av hela grupper, samt inom individer med både ABU och UVI. Vi kunde också påvisa att förhöjt IL-6 mycket tydligt kunde identifiera UVI episoder med svårare symtom. Nästan alla patienter med UVI episoder med höga symtompoäng hade också ett IL-6 gränsvärde överstigande 25 ng/L. Detta betyder möjligtvis att IL-6 i framtiden skulle kunna användas för att skilja ut patienter med UVI som absolut ska ha antibiotika. Patienter med UVI med låga IL-6 nivåer skulle då istället kunna erbjudas enbart avvaktan eller kunna behandlas med eventuella alternativ till antibiotika.

Slutsatser av delarbete 1–3

- Medveten kolonisering med *E. coli* 83972 skyddar personer med ofullständig blåstömning mot återkommande urinvägsinfektioner. Resultaten tyder på att denna metod skulle kunna fungera som ett behandlingsalternativ till antibiotika hos väl utvalda individer med inkomplett blåstömning och UVI-benägenhet.
- Genetisk polymorfism (variation) i gener som reglerar det naturliga immunförsvaret har en signifikant inverkan på värdsvaret vid bakteriell kolonisering av urinvägarna. Resultaten tyder också på att naturliga immunmediatorer är inblandade i det värdspecifika låga immunsvaret vid ABU.
- Aktiverat IL-6 korrelerar till svårighetsgraden av symtom vid UVI. Dessutom pekar resultaten på att IL-6 kan användas för att skilja ABU från UVI.

I delarbete 4 undersökte vi om urin IL-6 kunde användas som ett extra diagnostiskt verktyg för att skilja ABU från UVI i en sjukhemspopulation. I en första del av studien identifierade vi 36 sjukhemsvårdade personer med ABU och studerade deras värdsvar i urinen (IL-6, IL-8 och vita blodkroppar) vid ABU respektive UVI under ett års tid. Resultaten visade att urinkoncentrationer av IL-6 var bättre på att särskilja ABU och UVI än IL-8 och vita blodkroppar. Utifrån resultaten identifierade vi en IL-6 koncentration på 25 ng/L som gräns för behandlingskrävande UVI.

I den andra delen av studien ingick 368 personer boende på 10 sjukhem i Lunds kommun. Första året registrerades all antibiotikaförskrivningen p.g.a. UVI (år 1). Därefter utfördes intervention med introduktion av IL-6 som ett extra diagnostiskt verktyg, samt en pedagogisk del i form av undervisning av de ansvariga doktorerna om ABU och dess skyddande effekt, UVI och dess uppkomst, samt om sambandet mellan IL-6 i urinen och svårighetsgraden av urinvägsinfektion.

Efter interventionen registrerades antalet antibiotikabehandlingar under ytterligare ett år (år 2). Vi fann en reduktion av antalet behandlingar med 20 % jämfört med år 1. Minskningen var inte signifikant (säker) – för att detta skulle fastställas krävdes nämligen en reduktion av behandlingarna med 30 %. Vi kunde dock med vår studie visa att IL-6, tillsammans med den utbildningen som gavs i samband med interventionen, är möjligt att använda som diagnostiskt verktyg och att det tycks minska antalet onödiga UVI behandlingar. Samtliga läkare som deltog i studien fann IL-6 användbart eller mycket användbart vid diagnostik av UVI i denna patientgrupp.

Slutsatser av delarbete 4

- Interleukin 6 i urin är en lovande biomarkör för att påvisa övergången från asymtomatisk bakteriuri till urinvägsinfektion hos äldre personer.
- Den pedagogiska interventionen, innefattande introduktionen av IL-6 som ett extra diagnostiskt verktyg, reducerade antalet antibiotikakurer med 20 %. Reduktionen var inte signifikant, troligtvis beroende på att studiepopulationen var för liten.
- Ytterligare större studier med robust metodologi behövs för att säkert fastställa nyttan av IL-6 som diagnostiskt verktyg för behandlingskrävande UVI hos patienter som är benägna att utveckla ABU.

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