Urinary tract infection in small children: aspects of bacteriology, vesicoureteral reflux and renal damage

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La science n’a pas de patrie,

parce que le savoir est la patrimoine de l’humanité,

le flambeau qui éclaire le monde.

Louis Pasteur

ABSTRACT

Background: Urinary tract infection (UTI) is a prevalent bacterial infection in children. The diagnosis is based on growth of bacteria in urine specimen and treatment is chosen out of knowledge of the present antimicrobial resistance situation. Vesicoureteral reflux (VUR) is a well-known risk factor for UTI in children. Besides acute discomfort of UTI, long-term consequences associated with renal damage may occur.

Research questions: What is the relation between UTI, VUR and renal damage? How has bacterial resistance to oral antimicrobials changed over time? What is the significance of a low bacterial count? How does renal damage develop over time?

Methods: The study was retrospective, population-based and included children below 2 years of age with first time symptomatic UTI. The data files were analyzed. Recorded were clinical and laboratory parameters at index UTI including symptoms, duration of fever, highest measured temperature, highest C-reactive protein, sampling method, bacterial count, bacterial findings, antibacterial resistance, treatment and occurrence of recurrent UTI. All radiological and scintigraphic investigations were reexamined. The grade of VUR and renal damage was classified.

Results: A significant relationship between renal damage and severity of VUR was found. During a 10-year period the *E.coli* resistance to trimethoprim increased from 5 to 17%, while it remained unchanged low to nitrofurantoin and cefadroxil. Bacterial count below the significant level of 100,000 CFU/mL was found in 19% of the children and these children had similar rate of high grade VUR and renal damage as those with higher bacterial number. In children with renal damage 19% had regressed and 19% progressed at a median follow-up time of 8 years. Those who progressed had more severe renal damage at the index DMSA scan, a higher rate of VUR grade III-V and more often recurrent UTI.

Conclusions: Children with high grade VUR are risk subjects for permanent renal damage. The *E.coli* resistance to trimethoprim has increased significantly and trimethoprim is no longer appropriate as a first-line drug for empirical treatment. The possibility of UTI should be considered also with low bacterial count. This information should also be considered in the development of new guidelines. Children with severe renal damage, high grade VUR and recurrent UTI are at risk for progression of renal damage.

Keywords: Children, Urinary tract infection, Vesicoureteral reflux, Renal damage, Bacterial count, Recurrence, Antibiotic resistance, Urine sampling

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Urinvägsinfektion (UVI) är en av de vanligaste bakteriella infektionerna under de första levnadsåren. UVI med njurengagemang kan leda till permanent njurskada med risk för framtida men i form av nedsatt njurfunktion och högt blodtryck. Diagnosen UVI fastställs genom fynd av bakterier i urinprov. Njurskada diagnostiseras med gammakamera-undersökning av njurar, DMSA scintigrafi. En känd riskfaktor för njurinfektion är förekomst av backflöde av urin från urinblåsa till njurar, vesikoureteral reflux (VUR).

Vetenskapliga frågeställningar: Vilka samband föreligger mellan UVI, VUR och njurskada? Hur har bakteriell resistens mot de vanligaste per orala antibiotika utvecklats över tid? Vilken betydelse har låga bakterietal? Hur utvecklas njurskada över tid?

Metod: Studien inkluderade alla barn under 2 år med förstagångs-UVI som sökt på akutmottagningen på Drottning Silvias barn- och ungdomssjukhus 1994-2003. Vid journalgenomgång registrerades symtom, feberduration, högsta temperatur, högsta CRP, metod för urinprovtagning, bakterietal, bakterieart, resistensmönster, behandling och förekomst av recidivinfektion. Alla röntgen och gammakameraundersökningar eftergranskades. Graden av VUR och njurskada klassificerades.

Resultat: Man fann signifikant samband mellan graden av VUR och förekomst av njurskada. *E.coli´*s resistens mot trimetoprim ökade mellan 1994 och 2003 från 5% till 17%, medan resistensen mot nitrofurantoin och cefadroxil kvarstod oförändrat låg, under 1%. Bakterietal under signifikansgränsen 100.000 bakterier/ml förkom hos 19% av barnen. Dessa barn hade likartad förekomst av höggradig VUR och njurskada som de med högre bakterietal. Hos barn med njurskadan, med en medianuppföljningstid av 8 år, förbättrades njurskadan hos 19% medan man hos 19% såg en försämring. Hos de som försämrades var det vanligare med höggradig VUR, recidiverande UVI och mer uttalad njurskada vid den initiala undersökningen.

Konklusion: Barn med höggradig VUR är riskpatienter avseende permanent njurskada. *E.coli*-resistensen mot trimetoprim har ökat kraftigt varför trimetoprim inte längre kan betraktas som förstahandsval vid UVI hos barn. UVI med låga bakterietal är relativt vanligt och dessa patienter missas om man tillämpar höga signifikansgränser. Detta bör beaktas vid utformning av framtida riktlinjer för UVI hos barn. Barn med höggradig VUR, recidiverande UVI och uttalad njurskada har ökad risk för försämring av njurskadan över tid.

LIST OF PAPERS

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

I. Swerkersson S, Jodal U, Sixt R, Stokland E, Hansson S. Relationship among vesicoureteral reflux, urinary tract infection and renal damage in children. *J Urol. 2007; 178: 647-51.*

II. Swerkersson S, Jodal U, Åhrén C, Hansson S. Urinary tract infection in small outpatient children: the influence of age and gender on resistance to oral antimicrobials. *Eur J Pediatr. 2014; 173: 1075-81.*

III. Swerkersson S, Jodal U, Åhrén C, Stokland E, Hansson S. Urinary tract infection in infants: the significance of low bacterial count. *Pediatr Nephrol. 2016; 31: 239-45.*

IV. Swerkersson S, Jodal U, Sixt R, Stokland E, Hansson S. Urinary tract infection in small children: the development of renal scarring over time. *Submitted*

CONTENT

ABBREVIATIONS

ABU	Asymptomatic bacteriuria
CFU	Colony-forming units
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
DMSA	^{99m}Tc-dimercaptosuccinic acid
DRF	Differential renal function
GFR	Glomerular filtration rate
E.coli	*Escherichia coli*
ESBL	Extended spectrum β-lactamase
OR	Odds ratio
ROC	Receiver operating characteristic
SD	Standard deviation
Sp	Species
SPA	Suprapubic aspiration
UTI	Urinary tract infection
VCUG	Voiding cystourethrography
VUR	Vesicoureteral reflux

1 INTRODUCTION

Urinary tract infection (UTI) is a common disease affecting especially infants and young children. The clinical presentations are diverse, from an unaffected infant with asymptomatic bacteriuria to a severely septic child. Historically the disease was associated with significant mortality and long-term morbidity.

This thesis is dealing with aspects of diagnosis, treatment, investigation and prognosis of urinary tract infection in small children.

1.1 Historical perspective

Of the Hippocratic aphorisms, circa 400 BC, several relates to nephrology. *"When in fevers the urine is turbid like that of a beast of burden, in such a case there either is or will be headache"*. This could be a description of pyelonephritis as could the following aphorism. *"When there is a farinaceous sediment in the urine during fever, it indicates a protracted illness"*. Calculus in the urinary tract are quite clearly described and for children with bladder stones states *"Children get stone from milk if it is not healthy, but is too hot and bilious . . . and I say that it is better to give children wine, much diluted, for it has a less heating and drying effect. . ."*. [1]

For a long time the tools for diagnosing kidney disorders were mainly clinical symptoms and visual analysis of urine (uroscopy). Treatment consisted of different diets, laxatives and venesectio, but performed were also surgical procedures like drainage of pus, catheterization and removing obstructive stones.[2] In the 16th century several texts on pediatric diseases were published but most of them only briefly were dealing with kidney disorders. This was also true for the following centuries, e.g. in the text of von Rosenstein (1764), considered to be the first modern text on pediatrics, urinary tract diseases are not mentioned at all.[3] No major progression concerning the causes and treatments of kidney diseases was made until the middle of 19th century. Even if bacteria were described already in the 17th century by Leeuwenhoeks it was not until 1860's and Pasteur's discovery of microorganisms as the origin of fermentation and Koch's development of technics to isolate bacteria in pure culture, that the science of bacteriology

really started.[2] Several bacteria were discovered and were connected to specific infections. In 1886 Theodor Escherich published his work about bacteria in the gastrointestinal tract of children, "Die Darmbakterien des Säuglings und ihre Beziehungen zur Physiologie der Verdauung", where he also described Bacterium coli commune, later named Escherichia coli.[4]

With the introduction of the "germ theory of disease" the interest of antiseptic technics increased. In 1867 Joseph Lister published his works on aseptic use of Phenol, carbolic acid, in surgery. During the coming years several aseptic substances for internal use were tested. Paul Ehrlich, honoured by the nobel price, was one of the pioneers of chemotherapy. In 1909 he prepared Salvarsan, the first effective cure against syphilis. With the introduction of Sulfanilamid in 1937 the first effective treatment of urinary tract infections was established. This was followed by nitrofurantoin in 1953 and ampicillin in 1962, both then effective antibiotics against the most common uropathogens.[5]

In descriptions of small children with febrile urinary tract infection from the pre-antibiotic era mortality rates around 20% were reported. An equal portion had incomplete recovery and 60% healed spontaneously, often after weeks of severe illness.[6] This scenario was dramatically changed with the access of efficient antimicrobials.

1.2 Epidemiology

The first Swedish study on the epidemiology of UTI in children was intiated by Jan Winberg.[7] The aggregated morbidity risk of UTI was calculated from all children up to 11 years admitted to the Children's Hospital in Göteborg with the diagnose of UTI between 1960 and 1966. This study showed a minimal incidence of 1.1% for boys and 3% for girls. A study of children 7 years of age found a cumulative UTI incidence of 1.7% in boys and 7.8% in girls.[8] In a Swedish quality assurance project, covering 65% of the Swedish population under 2 year of age, the minimal incidence of symptomatic UTI was 2.2% for boys and 2.1% for girls.[9]

In both boys and girls UTI is most prevalent during the first years of life, after that it is still common in girls but rare in boys (figure 1).[7,10]

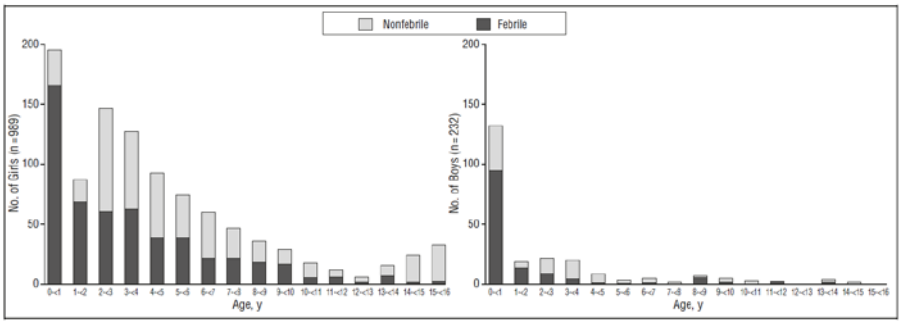

Figure 1. Age distribution at first symptomatic urinary tract infection in girls (left) and boys (right). (Wennerström. Arch Pediatr Adolesc Med. 1998; 152:879-883). With permission.

1.3 Diagnostic

The ideal means for diagnosing UTI should be easy to handle, inexpensive, comfortable for the children, have high sensitivity and specificity, and well discriminate between kidney involvement and low UTI.

1.3.1 Clinical symptoms

The symptoms of UTI are often unspecific. Most infants with UTI presents with fever and irritability, but also vomiting, feeding problems and lethargy are frequent symptoms.[11] Furthermore, bacteremia is not uncommon; 4-9% of children with UTI are reported to have a positive blood culture.[12,13]

1.3.2 Urine tests

The nitrite test detects nitrite producing bacteria. Most Gram-negative bacteria produce nitrite reductase which reduces nitrate to nitrite, while Gram-positive bacteria such as enterococci do not produce this enzyme. Accordingly, the nitrite test identifies mainly Gram-negative bacteria. Furthermore, to detect nitrite there must be sufficient amount of bacteria in the specimen. Short bladder incubation time with low bacterial number reduces the rate of positive tests. In summary the nitrite test has a high specificity for UTI, but poor sensitivity. Meta-analyses of conducted studies have shown specificity from 76% to 100% and sensitivity from 16% to 88%.[14,15]

Leukocyte esterase is an enzyme present in granulocytes. The detection of esterase indicates pyuria, but the leukocyte esterase test is non-specific for UTI as leukocytes in urine may be present as a result of other infections. Conversely, with a short bladder incubation, true bacteriuria may give a negative test result. In meta-analysis specificity ranged from 69% to 98% and sensitivity from 38% to 100%.[14,15]

1.3.3 Inflammatory markers

Besides the presence of fever as a clinical marker of inflammation, laboratory biomarkers have been used for discriminating UTI localized to the lower urinary tract from UTI with kidney involvement. Most used serum biomarkers have been C-reactive protein (CRP) and procalcitonin. They have shown significant association with both acute kidney involvement and late renal scarring.[16-18]

1.3.4 Urine sampling

A urine culture with growth of microbes is a prequisite of UTI diagnosis in children. Therefor it is crucial to obtain representative urine specimen without contamination of microbes from localizations outside the urinary tract. Suprapubic aspiration (SPA) was introduced 1959 as a safe and reliable method for urine collection and this method has become the “gold standard” for urine sampling.[19] In studies comparing midstream sampling with SPA the correspondence between the methods was good with specificity from 75% to 100% and sensitivity 75% to 100%.[20-24] Only a few studies have compared bag samples with catheter specimen and the results were diverse with contamination rates from 7.5% to 63%.[25,26] Urine collection by catheter is regarded as a reliable sampling method with low risk of contamination, but there are no studies comparing this method with SPA.

1.3.5 Definition of bacteriuria

In the 1950s Kass studied bacteriuria in women.[27] He found 2 population groups, those with bacterial number below 10,000 colony-forming units (CFU)/mL, regarded as contamination, and those with bacterial counts more than 100,000 CFU/mL representing true UTI. The 2 groups overlapped at about 10,000 CFU/mL. From these results he recommended 100,000 CFU/mL as a dividing line which since then has been the commonly used

cut-off level. However, the accuracy of this cut-off level has been questioned as more recent studies have found lower bacterial counts in patients with symptomatic UTI.[28,29] In a study of children less than 2 years of age with urine specimen obtained by bladder catheterization a cut-off level of 50,000 CFU/mL was found to well discriminate among true UTI and contamination.[30]

In recently published national guidelines on the management of UTI in children the recommended sampling methods and definition of bacteriuria are varying (table 1).[31-37] However, there are some studies indicating that with the proposed definitions of bacteriuria a substantial number of children with true UTI will be missed.[38-40]

Table 1. Recommended cut-off levels for bacterial count related to sampling method in published urinary tract infection guidelines.

Guideline	**SPA** **CFU/mL**	**Catheter** **CFU/mL**	**Clean catch** **CFU/mL**
ESPU 2015[35]	any growth	$\geq 10^3$- 5 x 10^4	$\geq 10^4$-10^5
Canada 2014[37]	any growth	$\geq$5 x 10^4	$\geq 10^5$
AAP 2011[33]	$\geq$5 x 10^4	$\geq$5 x 10^4	not defined
Italy 2011[34]	not defined	$>10^4$	$\geq 10^5$
NICE 2007[32]	not defined	not defined	not defined
France 2007[31]	$\geq 10^3$	$\geq 10^3$	$\geq 10^5$
Germany 2007[36]	any growth	10^3->10^4	10^4->10^5

1.4 Bacteriology

The organisms causing UTI in children are almost exclusively inhabitants of the large intestine. Organisms are ingested during delivery and some of these will be established in the intestinal microbiota of the neonate. Other organisms colonizing the neonate are derived from the environment such as from family members and hospital staff. The colonization pattern is influenced by delivery mode, feeding modes, family structure and other environmental factors. As the gut environment of the neonate includes

oxygen, facultative bacteria dominate the first flora, while anaerobes are prevented from growing. Consequently, common in the neonates flora are *E.coli*, enterococci, *Klebsiella* and *Enterobacter*. With the expansion of facultative bacteria the oxygen in the gut will be consumed and the environment gets more suitable for anaerobic organisms. As a result the anaerobs will finally outnumber the facultative bacteria. As a consequence the gut microbiota gets more stabilized which makes it harder for new bacteria to establish and proliferate. This phenomenon is termed colonization resistance. Consequently, this colonization resistance is weak in neonates and young children but develops during childhood.[41,42]

1.4.1 Enterobacteriaceae

This family includes the most prevalent uropathogens such as *Escherichia coli (E.coli), Klebsiella, Enterobacter and Proteus.* All are Gram-negative rods and facultative anaerobes. Furthermore, all ferment glucose and can also generate energy by reducing nitrates to nitrites. Enterobacteriaceae are part of the normal gut flora. They possess strong virulence factors such as an outer cell membrane, adhesion molecules, pili or fimbriae, biofilm production, immune evasion mechanisms and production of different toxins. Uropathogenic *E. coli* also express pyelonephritis-associated pili, which are required for colonization of the kidney.[43]

1.4.2 Enterococci

Enterococci are Gram-positive cocci and facultative anaerobes belonging to the normal intestinal flora. They produce adhesion factors and biofilm, which make them easily attach to urinary catheters. Enterococci are resistant to cefalosporins as they produce a penicillin-binding protein with low affinity to these agents.[44]

1.4.3 Other microbes

Other infrequent pathogens such as fungi, *Pseudomonas, Staphylococci, Hemophilus Influenzae* and *Stenotrophomonas maltophilia* mostly indicate a compromised urinary tract such as posterior urethral valves, other obstruction or high-grade vesicoureteral reflux (VUR).[43,45]

1.5 Oral antibiotics

Antimicrobial drugs may have different mechanism of action. Most oral drugs used in the treatment of UTI in children belong to 3 categories of action: interference with cell wall synthesis, protein synthesis inhibition and inhibition of metabolic pathway.[46]

1.5.1 β-lactam agents

This group includes penicillins, cephalosporins and carbapenems. The drugs have different ability to penetrate the various layer of the cell wall, which is one explanation for intrinsic resistance of Gram-negative bacteria to some of the β-lactam agents. Drugs penetrating into the membrane become strongly bound by penicillin-binding proteins, enzymes necessary for cell wall synthesis, and thus disrupting integrity, shape and cell division.[47]

1.5.2 Trimethoprim

Trimethoprim inhibits dihydrofolate reductase that converts dihydrofolic acid to tetrahydrofolic acid, an essential stage in bacterial purine, and ultimately, DNA synthesis. Sulfonamid may have a synergistic effect by inhibiting another enzyme, dihydropteroate synthetase, which is involved in the same pathway.[48,49]

1.5.3 Nitrofurantoin

The mechanisms of antibacterial activity of nitrofurantoin are not well-understood, but it appears to have multiple ways of action. It inhibits bacterial enzymes involved in energy production and cell wall synthesis and it binds to ribosomal proteins which causes inhibition of bacterial protein synthesis.[50-52]

1.6 Antibiotic resistance

Microorganisms produce antibiotic substances and many antibiotics in clinical use are of environmental origin. In the microbial communities these natural antibiotics may have different functions; inhibit growth of other microorganisms, modulate interaction within microbial communities and delivering signals for intermicrobial communication. Bacteria may also

possess resistance determinants. Thus, also resistance to antibiotics has the origin in the natural environment.[53]

1.6.1 Mechanisms of antibiotic resistance

The microbes may use different mechanisms to avoid the effects of antibiotics: inactivation of the active molecule, modification of the target of action and reduction of the concentration of the drug.[54]

In resistance to β-lactam antibiotics the resistant bacteria produce β-lactamase that inactivates β-lactam. The separate drugs in the group of β-lactam agents differ in sensitivity to β-lactamase. With the combination of clavulanic acid, a β-lactamase inhibitor, most types of β-lactamase can be inactivated. However, multidrug-resistant microbes producing extended-spectrum β-lactamase (ESBL) are resistant to most β-lactam agents and some ESBLs are also resistant to clavulanic acid. ESBLs are plasmid-encoded and the plasmids are transferable between microbes. The plasmids may also carry other resistant genes with activity against other groups of antibiotic such as aminoglycosides and sulphonamides.[43,55] Enterococci have natural resistance to β-lactam antibiotics as they express penicillin-binding proteins that bind weakly to β-lactam drugs.[44]

Acquired resistance to trimethoprim is mainly mediated by plasmid transferred genes, encoding dihydrofolate reductase resistant to trimethoprim. Other possible mechanisms are through efflux pumps reducing cellular drug concentration and porins regulating the influx of drugs.[56-58]

Even though nitrofurantoin has been on the market since more than 50 years there is no significant development of resistance to *E.coli*. However, *Klebsiella* and *Enterobacter* are often and *Proteus* always resistant to nitrofurantoin. The mechanisms behind this resistance are unclear. Nitrofurantoin is often effective against ESBL-producing *E.coli*.[50]

1.6.2 *E.coli* resistance to antibiotics

The bacterial resistance pattern in Sweden has since 1996 been reported by the Swedish Annual Resistance Surveillance and Quality Control. Between 1996 and 2014 urine samples, from mainly adult patients, showed an increasing resistance for *E.coli* isolates to trimethoprim from around 10% to

20% and to ampicillin from around 20% to 35%, while to nitrofurantoin the resistance has remained at an unchanged low level. At the same time *E.coli* resistance to cefadroxil has increased from almost no resistance to around 5%, which reflects the spread of ESBL-producing Enterobacteriaceae (figure 2). Since the first reported ESBL-positive *E.coli* in Sweden 2007 the number has increased each year by 9 to 33%.[59,60]

Internationally the ratio of trimethoprim resistant *E.coli* varies with figures around 20-40% in Europe and United states and over 50% resistance in Turkey.[61-64] Reports on the global carriage rates of ESBL-producing bacteria show a steady increase in all regions with carriage rates of over 50% in Southeast Asia.[65]

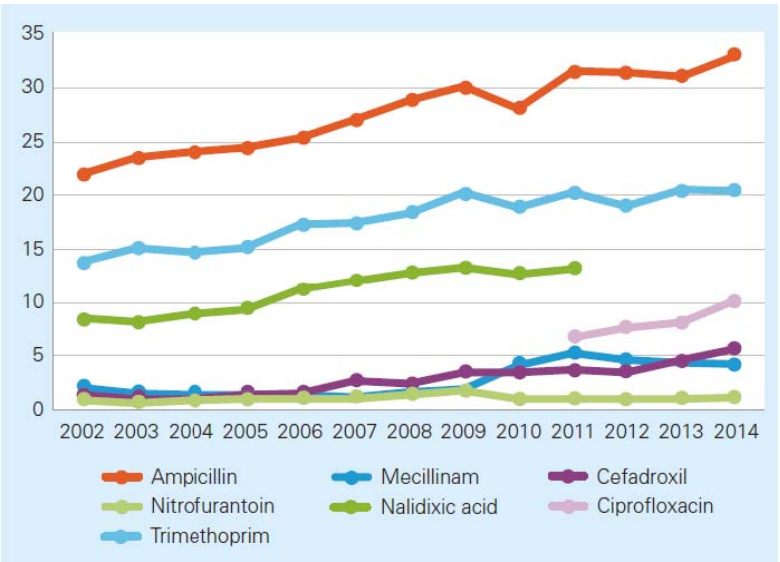

Figure 2. Proportion ,%, of resistant E.coli isolates from urine in adults and children in Sweden, 2002-2014. (Swedres-Svarm 2014. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden 2014)

The evolving antimicrobial resistance are driven by both appropriate and inappropriate use of ant-infective medicines for human and animal health and food production. In an international perspective the situation regarding antimicrobial resistance is favourable in Sweden. The main reason to this is a comparable modest prescription of antibiotics. Since 1992 the total sales of

antibiotics on prescription has decreased by 40%, with the most pronounced decrease in the age group of children 0 to 4 years (figure 3).

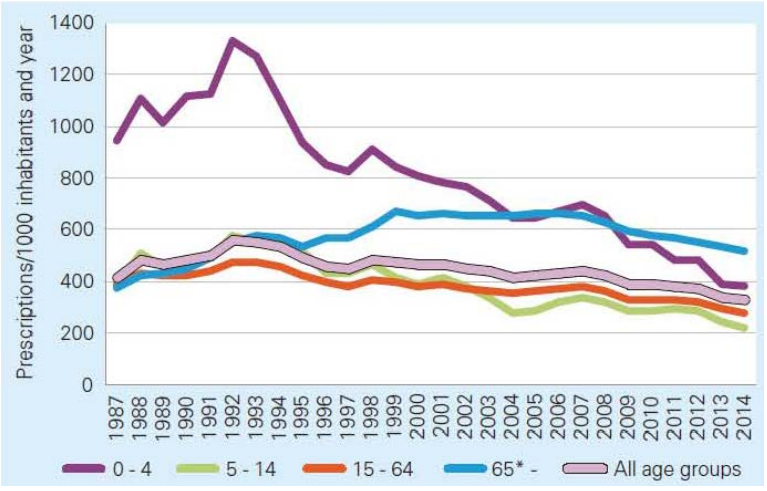

Figure 3. The sales of antibiotics for systemic use in out-patient care 1987-2014, prescriptions/1000 inhabitants and year in different age groups.(Swedres-Svarm 2014. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden)

1.7 Vesicoureteral reflux

VUR is the pathological retrograde flow of urine from the bladder into one or both ureters and the renal pelvis. There are different techniques for diagnosing VUR of which voiding cystourethrography (VCUG) is the most established. The VUR is graded according to the extent of retrograde flow and dilatation of ureter, renal pelvis and calyces. The most used classification was introduced by the International Reflux Study in Children and defines VUR as grade I if reflux of urine to ureters only; grade II if reflux to ureter, pelvis and calyces without dilatation; grade III if mild or moderate dilatation of ureter and renal pelvis; grade IV if mild or moderate dilatation of ureter and renal pelvis with obliteration of the sharp angel of the fornices but maintained papillary impressions; grade V gross dilatation with papillary impressions not visible in the majority of the calyces (figure 4).[66]

The terms low-grade or non-dilated VUR is used for VUR grade I-II and high-grade or dilated-VUR for grade III-IV.

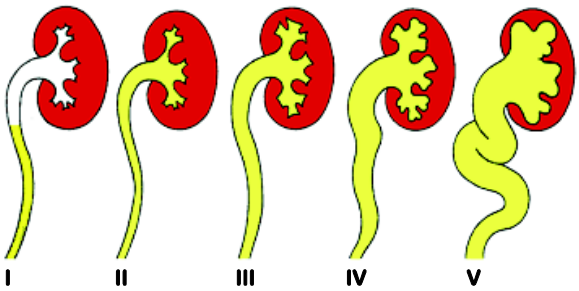

Figure 4. Classification of vesicoureteral reflux according to the International Reflux Study in Children.[66]

Studies from the 1950s and 1960s of normal children indicated incidence of VUR of less than 1% and around 30% in children with UTI.[67,68] However, more recent studies have shown that VUR is common also in children with several different disorders, such as anorectal malformation and hypospadias.[67] Furthermore, studies comparing children with verified and improbable UTI showed similar frequency of VUR in both groups.[69,70] Therefor the low incidence of VUR in normal children has been question and also the association between VUR and UTI.[67,71]

VUR has a high tendency to spontaneous resolution as shown in several studies, with resolution rate around 50% after 6 months in VUR grade III and up to 40% in grade IV-V at 1 year.[10,72] However, some studies have shown considerable lower resolution rate in high grade VUR especially in girls.[73,74]

1.8 Renal damage

Microbes invading the urinary tract normally attach to the mucosa and initiate a host response with release of chemotactic substances leading to neutrophil influx and eventually elimination of the bacteria. In contrast, depending on bacterial virulence and host factors, the response may be exaggerated with severe infection with pronounced inflammation. As a consequence focal ischemia with release of cytokines and toxic metabolites may lead to

irreversible renal damage. Involved in this process may be both anatomical and genetically determined inflammatory factors.[75-77]

Traditionally renal scarring was diagnosed through urography identifying kidney parenchyma thinning and contour defects. Today the most widespread method of revealing renal damage is by isotope technic, especially ^{99m}Tc-dimercatosuccinic acid (DMSA) scintigraphy. The isotope is accumulated in the tubular cells and the renal parenchyma is visualized by using a gamma camera (collimator). Renal damage is visualized as one or more up-take defects. The method estimates also the percentage differential renal function (DRF) of the separate kidney. The lowest normal value for DRF is 45%, thus a normal DRF range is 45% to 55%.[78] Up-take defects may be seen at the acute-phase of a UTI as a consequence of local inflammation. These defects may be reversible and regress during a time period of up to 6 months.[79-81] Permanent renal damage is defined as an up-take defect remaining after the acute-phase.

Renal damage may be acquired as a sequel after acute UTI or of congenital origin established already in utero. Previous studies found the acquired form more prevalent among girls, while the congenital damage seems to be associated with high-grade VUR and more often found in boys.[72,76,82]
DMSA scan cannot distinctly differentiate between acquired and congenital renal damage. Acquired damage can only be diagnosed if a DMSA scan has been performed both before and after a UTI, which rarely is the case.[83] Therefor there are controversies regarding the impact of permanent renal damage and its association to VUR and UTI.[76,84] However, a review of published articles on the risk of renal scarring in children with a first UTI showed that 57% (95% CI: 50-64) had changes on acute-phase DMSA scan and 15% (95% CI: 11-18) had evidence of renal scarring on follow-up DMSA scan. Children with VUR were significantly more likely to develop pyelonephritis and renal scarring compared with children without VUR.[85]

1.8.1 Evolution of renal damage

In the North American Pediatric Renal Trials and Collaboration Studies (NAPRTCS) 2008, renal scarring associated with VUR, reflux nephropathy, was seen in 5% of kidney transplanted and 3.5% of dialysis treated children.[86] In contrast, a national study of Swedish children with glomerular filtration

rate (GFR) below 30 mL/min/1.73 m^2 did not reveal any children with renal damage associated with VUR or UTI.[87]

In a recent review of long-term consequences of UTI the prevalence of impaired renal function at long-term follow-up varied between 0 and 56%.[88] Of 1029 children included in prospective studies chronic kidney disease (CKD) was present in 55 and in 43 of these impaired GFR was already found at the beginning of follow-up. One study found no deterioration of GFR after 16-26 years if unilateral scarring, but a mean decrease of GFR from 94 to 84 mL/min/1.73 m^2 in 7 patients with bilateral scarring.[89] In a recent study of 86 women with 35 years follow-up after UTI in childhood a significant decrease in GFR was found only in presence of bilateral damage, while in patients with unilateral damage renal function remained unchanged.[90]

Only a few studies have analyzed the development of renal damage over time by serial DMSA scans. In the International reflux study 287 children with VUR grade III and IV were followed during five years by repeated DMSA scans.[91] In 31 (11%) children a decrease in differential renal function (DRF) of >3% occurred, while in 8 (3%) renal lesions improved. Deterioration was more prevalent if bilateral VUR grade IV, occurrence of recurrent UTI and age < 2 years.

In a recent study of 108 children with VUR grade III-V followed for 5 years serial DMSA scans revealed a decrease in DFR of more than 6% in 18% of the children and recovery of focal lesions in 5%.[92] Risk factors for deterioration were prenatal diagnosis, reduced GFR at start, recurrent UTI and VUR grade IV-V. Finally, in the Swedish reflux trial of 203 children with VUR grade III-IV followed for 2 years new scars or >3% decrease of DRF was seen 24 (12%) of whom 15 had recurrent UTI.[93]

Contrasting to these studies, all including children with high grade reflux, Parvex et al followed 50 children with an abnormal DMSA scan 6 months after acute pyelonephritis.[94] At follow-up DMSA scan after 3 years 8 (9%) kidneys of initially 88 scarred renal units showed complete and 56 (63%) partial resolution.

2 AIM

The aim of this study was to analyze:

— What is the relation between urinary tract infection, vesicoureteral reflux and renal damage?

— How has bacterial resistance changed over time?

— What is the significance of a low bacterial count?

— How does renal damage develop over time?

3 PATIENTS

The Queen Silvia Children´s Hospital has a long tradition of special interest in children with UTI and a UTI clinic was created already in the 1960s. The children´s hospital is the only hospital for children in the Göteborg region. During the period of the study the total population of the region was around 800,000, including 20,000 children below 2 year of age. The majority of small children with UTI in the city is handled at the emergency room of the children´s hospital and then followed at the UTI clinic according to a standardized protocol.[7,95]

Paper I

Eligible were children below 2 years of age diagnosed with a first time symptomatic, culture-verified UTI at the Queen Silvia Children´s Hospital from January 1989 through December 1993. By search of files of the UTI clinic children were selected who within 3 months from the UTI were investigated by ultrasound, VCUG and DMSA, and with a second DMSA scan at 1 to 2 years. Excluded were children with urogenital or anorectal malformations, neurological disease and if obstruction was suspected on ultrasonography.

Paper II-IV

Included were children below 2 years of age, consecutively diagnosed at the emergency room of the Queen Silvia Children´s Hospital from January 1994 through December 2003 with a first-time symptomatic, culture-verified UTI. The selected children were identified through search of the data files of the Clinical Bacteriological laboratory at Sahlgrenska University Hospital of all urine specimens with positive urine cultures taken at the emergency department of the children´s hospital during the study period. For children with significant growth of bacteria the clinical data from the documents at the UTI clinic were analyzed. Only children with symptomatic UTI were included. Excluded were children with asymptomatic UTI, urinary tract obstruction, other urogenital malformation, neurogenic bladder or severe neurological or systemic disease.

In all 2287 children with a positive urine culture were identified. Of those 1037 were considered as contamination because of growth of mixed organisms, a second negative specimen before antibiotics were given or

insignificant bacterial count. Asymptomatic bacteriuria was found in 99 children and 148 children fulfilled the exclusion criteria. Thus, 1003 children were included in the study (figure 5). All boys were uncircumcised.

Paper II

All 1003 patients were included. In addition 2 children with Wilms tumor and one with neurogenic bladder were incorrectly entered in this paper.

Paper III

Included were children below 1 year of age with UTI diagnosed by urine collection through suprapubic aspiration. In the original population 449 children were diagnosed by SPA. Of those 3 children were above 1 year of age and information about bacterial count was missing in 16 (figure 6).

Of the included 430 children 385 were investigated by DMSA scan. Of those 43 children were excluded in the analysis of permanent renal damage; 8 children had an early abnormal scintigraphy but no follow-up DMSA scan, 35 had recurrent UTI before follow-up investigation.

Paper IV

This paper comprises children with an abnormal DMSA scan performed at least 90 days after the index UTI and with a follow-up DMSA scan after more than 2 years. Of the background material of 1003 children, 869 had a DMSA scan performed, while in 134 children DMSA scan was not done; 20 children had moved to other residence, 32 declined examination, in the remaining 82 children median CRP was 20 mg/L and median of highest measured temperature was 38.5°C. In 92 children with abnormal index DMSA scan a follow-up DMSA scan after 2 years was missing; in 74 (68 minor, 6 moderate damage) the doctor or parent decided not to perform another DMSA scan, 16 are followed by other care-giver and in 2 children information about drop out is missing. Because of difficulties in evaluating changes of renal status 2 children with horseshoe kidney, 6 heminephrectomized and 2 nephectomized children were excluded (figure 7).

Table 2. Summary of included patients in paper I-IV.

	Paper I	Paper II	Paper III	Paper IV
Number of patients	303	1006	430	103
Boys, n (%)	163 (54)	494 (49)	275 (64)	46 (45)
Sampling method, n				
Suprapubic aspiration		449	430	52
Catheter		9		
Midstream		247		28
Bag		197		10
Unspecified		104		13
VCUG , n	303	908	407	100
DMSA scan, n	303	n.s.	342	103
Duplex		42	22	10
VUR surgery				
Deflux injection		4	1	3
Neoimplantation		6	4	5
Other surgery				
Nephrectomy		2	0	
Heminefrectomy		6	3	

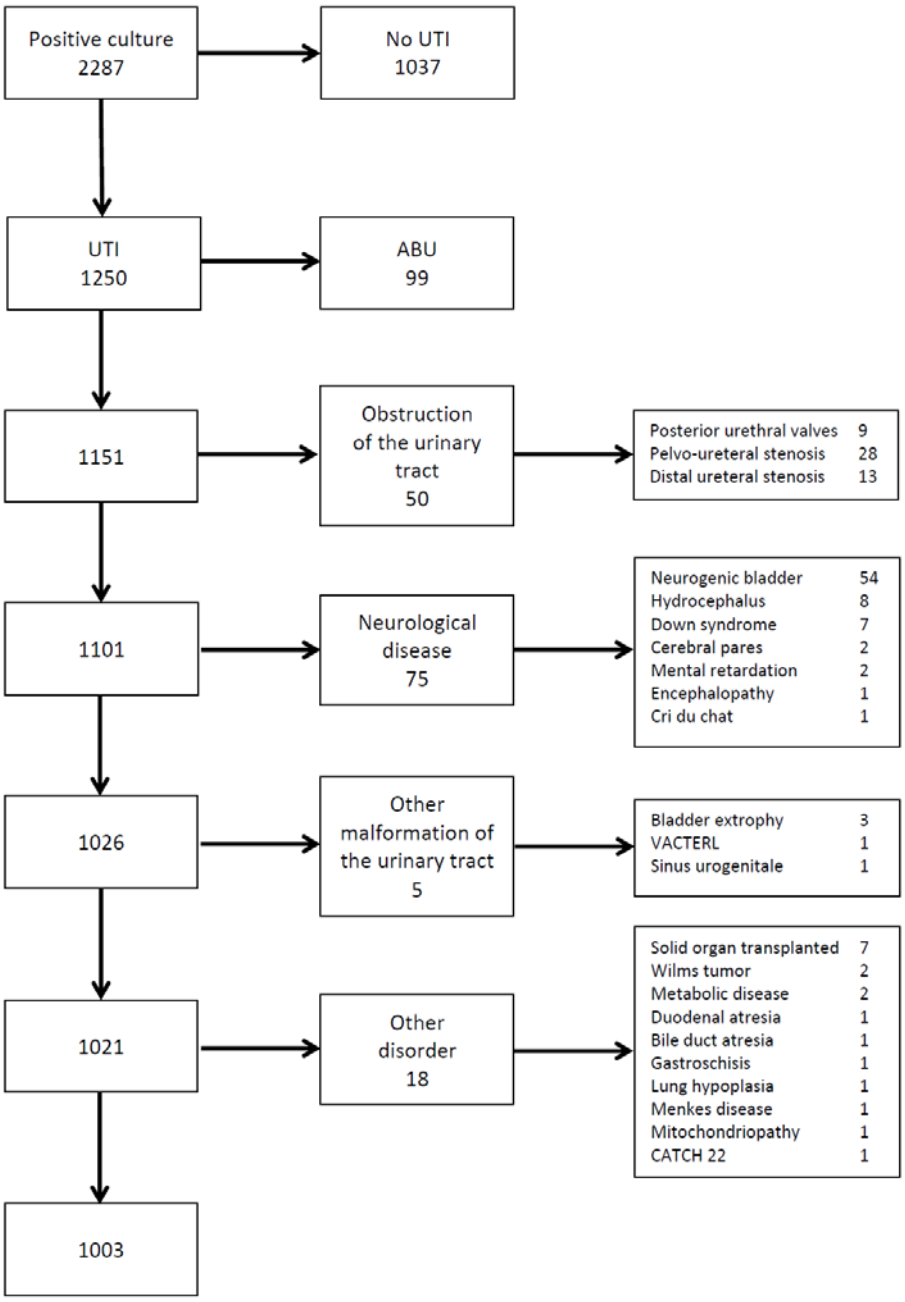

Figure 5. Flow-chart over included children in paper II-IV.

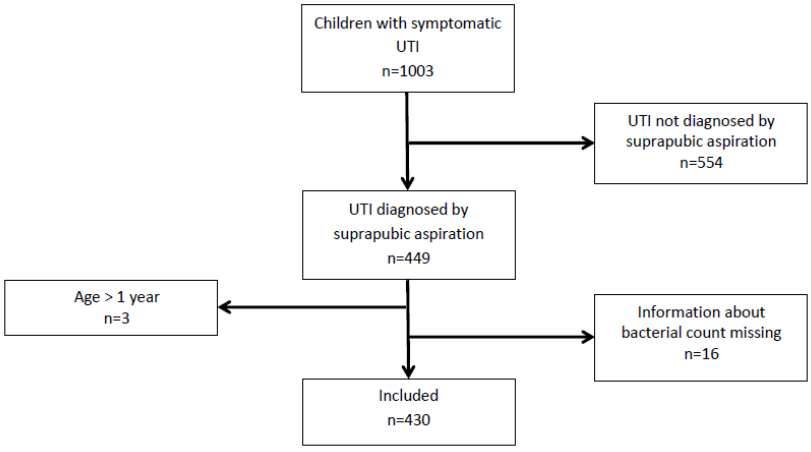

Figure 6. Flow chart od included infants in paper III

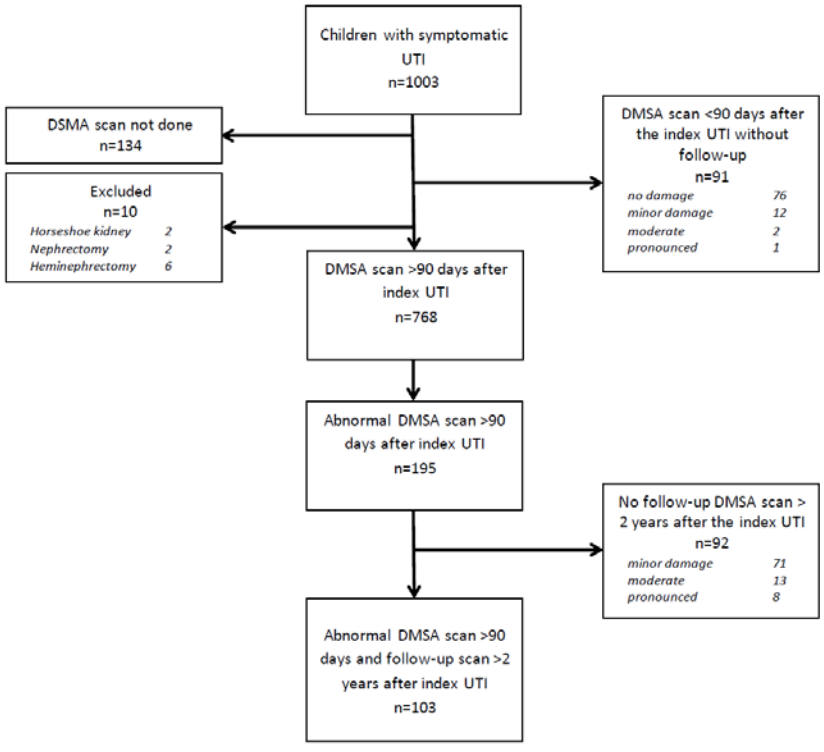

Figure 7. Flow chart of included children in paper IV.

4 METHODS

4.1 Data collection

For the eligible children the data files of the UTI clinic were analyzed and for those included in the study clinical and laboratory parameters at index UTI were recorded including symptoms, duration of fever, highest measured temperature, highest measured CRP, serum creatinine, method of urine collection, bacterial count, bacterial findings, antibacterial resistance, results of urinalysis, antibiotic treatment and occurrence of recurrent febrile UTI. Febrile UTI was defined as temperature ≥38.5 C°.

4.2 Urine culture and susceptibility testing

All urine cultures were analyzed at the Clinical Bacteriological Laboratory at Sahlgrenska University Hospital. Bacterial typing was performed according to standard bacteriological methods. Susceptibility testing using disc diffusion was done in accordance with the recommendations of the Swedish Reference Group of Antibiotics.[96]

Significant bacteriuria was defined as growth of a single species of at least 100,000 CFU/mL in two midstream or bag samples, 10,000 CFU /mL in one catheter sample, or any bacterial growth in urine from suprapubic aspiration sample.

For trimethoprim and nitrofurantoin, isolates reported as intermediate sensitive were grouped as resistant, whereas for cefadroxil intermediate sensitive isolates were grouped as sensitive.

4.3 Imaging

The objective of performing voiding cystourethrography (VCUG) is to diagnose VUR. The VCUG was done in accordance with the standard procedures at the pediatric radiology department. All investigations were reevaluated by the same radiologist and VUR grade was classified according to the definitions proposed by the International Reflux Study in Children.[66]

The purpose of DMSA scintigraphy is to detect renal damage. DMSA scan was performed in agreement with the guidelines of the Pediatric Committee of the European Association of Nuclear Medicine.[96] Static renal scintigraphy was performed 3 to 4 hours after injection of DMSA in a dose of 1 MBq/kg body weight (minimum 15 MBq). Three images were obtained: posterior and oblique left and right posterior. All examinations were reevaluated by the same nuclear medicine specialist. A kidney without up-take defects and with a differential renal function (DRF) of 45% or more was classified as normal. Renal damage was classified as minor if one or more up-take defects and DRF of 45% or more, moderate if DRF 40-44% and as pronounced if DRF less than 40% (figure 8). In cases with bilateral defects or renal duplication, an arbitrary classification was done to the same categories. Furthermore, renal abnormalities were also classified as focal or generalized.

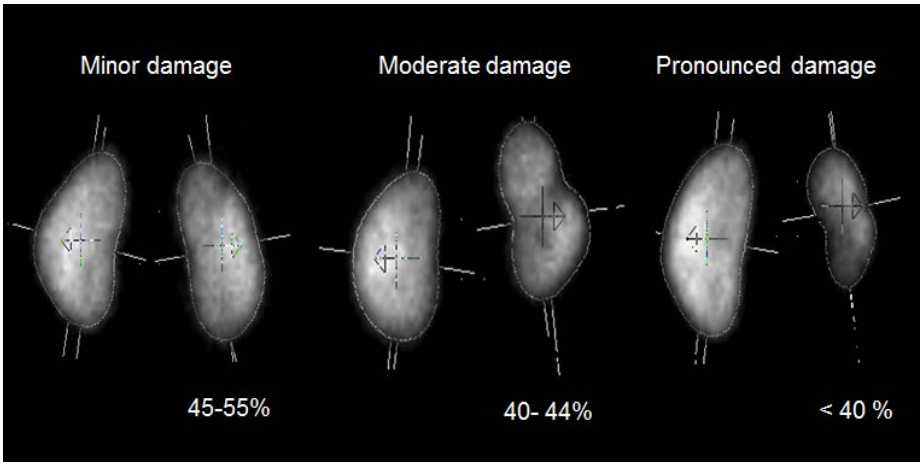

Figure 8. Classification of renal damage used in the study.

In separating permanent renal damage from transient up-take defects the time interval between acute UTI and DMSA scan is of importance. Conducted studies have shown variable time for acute defects to resolve and the recommended minimal time after an acute UTI to perform a DMSA scan varies from 3 to 6 months.[97] In paper III a minimal time interval of 6 months was chosen as it was of importance to include only those lesions that could be considered as permanent. In paper IV, where change in renal status between

the first DMSA scan after the acute phase of UTI and a follow-up DMSA scan was the objective, a shorter minimal interval of 3 months was chosen. In this paper there was no difference in renal outcome in the group with time interval of 3 to 6 months compared those with an interval of more than 6 months.

Children with normal early DMSA scan, within 3 months of UTI, without recurrent febrile UTI were considered to have normal kidneys as endpoint. This was based on a previous study showing that a normal DMSA scan in the acute phase of UTI did not deteriorate if there was no recurrence.[12]

The evolution of renal damage in paper IV was assessed by evaluating the changes of up-take defects and DRF. A decrease in DRF of more than 3% was regarded as progression of renal damage. The motive for choosing this cut-off level is the study by Piepsz et al. showing that 2-3% difference in DRF corresponds to one standard deviation.[78] The evolution of renal damage was classified into three groups: regression if up-take defects on index DMSA scan had partially or completely resolved at follow-up, progression if more than 3% decline of DFR between index and follow-up DMSA scan and unchanged in the remaining cases.

4.4 Statistical methods

The distribution of continuous variables is given as median, minimum and maximum and categorical variables as number and percentage. All significance tests were two-sided and conducted at the 5% significance level except for interaction analysis in the logistic regression model in paper II where $p<0.1$ was used for statistical significance. The statistical analyses were performed using SAS® software version 9.3.

Paper I For comparisons between groups Wilcoxon's two-sample test was used. The Mantel-Haenszel chi-square test was used to analyse the trend in a contingency table. Spearman's rank correlation coefficient was used for correlational analyses. Relative risks with 95% confidence intervals were calculated in order to detect differences between VUR grades. Stepwise logistic regression was used for multivariable purposes.

Paper II The Fisher's exact test was used for comparisons between 2 groups with dichotomous values. The Mantel-Haenszel chi-square test was used for ordered categorical variables. Group comparisons were performed using the Wilcoxon sign rank test. To select independent predictors for change of trimethoprim resistance over calendar years, variables were entered into a stepwise logistic regression model.

Paper III For comparison between two groups Mann-Whitney U-test was used for continuous variables, Fisher's exact test for dichotomous values and Mantel-Haenszel chi-square test for ordered categorical variables. In order to select independent associated factors to low bacterial count all significant univariable variables were entered into a multivariable stepwise logistic analysis.

Paper IV For comparison between the three groups of evolution of kidney damage the Mantel-Haenszel chi-square test was used for dichotomous variables and the Spearman correlation test for ordered categorical and for continuous variables. In the assessment of factors associated to progression of kidney damage univariable analysis was done by logistic regression and all significant univariable variables were entered into a multivariable stepwise logistic analysis.

5 RESULTS

5.1 The relation between VUR, UTI and renal damage - paper I

Included in this paper were 303 children. The characteristics at the index UTI are shown in table 3.

Table 3. Characteristics of 303 children with first-time symptomatic UTI.

	Boys	**Girls**
Number	163	140
Age at index UTI, months, median (range)	3.1 (0.1-19.9)	8.5 (0.1-22.6)
Temperature ≥38.5°C, n (%)	118 (73)	128 (91)
CRP, mg/L, median (range)	49 (5-290)	65 (5-290)
VUR, n (%)		
No VUR	127 (78)	96 (68)
VUR I-II	14 (9)	30 (21)
VUR III-V	22 (13)	14 (10)

5.1.1 Vesicoureteral reflux

VUR was found in 22% of the boys and 31% of the girls. Boys had a higher proportion of dilated VUR, 22 of 36 (61%), while in girls non-dilated VUR was more prevalent, found in 30 of 44 (68%). This gender difference was significant ($p<0.01$).

There was a significant relation between VUR grade and recurrent febrile UTIs. Febrile recurrence occurred in 21 of 223 (9%) children without VUR, in 7 of 44 (16%) with VUR grade I-II and in 8 of 36 (22%) with VUR grade III-V ($p=0.02$).

There was also a significant relation between VUR grade and the level of maximum CRP at index UTI ($p<0.05$ in boys, $p<0.01$ in girls) (figure 9).

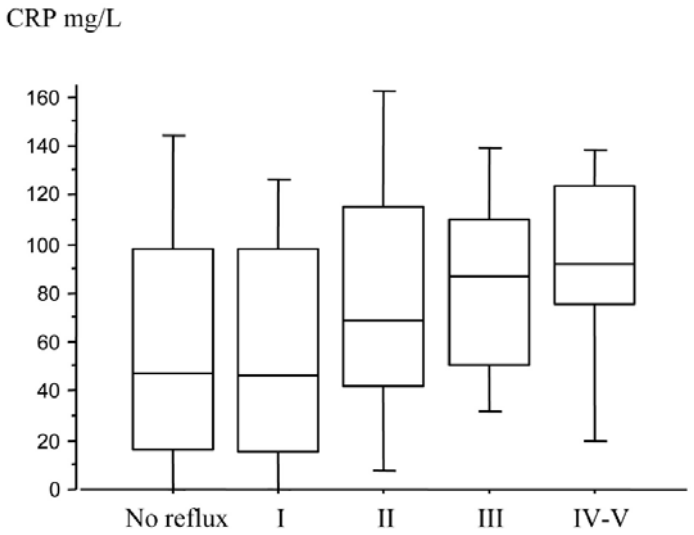

Figure 9. Level of C-reactive protein in relation to VUR grade. Results presented as box plots indicating medians with lower and upper quartiles. Whiskers show 10th and 90th and percentiles. With permission.

5.1.2 Renal damage

At follow-up 1 to 2 years after index UTI 80 of 303 children (26%) had abnormal DMSA scan. The proportion of renal damage was similar in boys and girls, 38 of 163 (23%) and 42 of 140 (30%), respectively (p=0.2).

There was a significant association between DMSA defects and grade of VUR; abnormal DMSA scan was fund in 43 of 223 (19%) children without VUR, in 3 of 13 (23%) in VUR grade I, in 13 of 31 (42%) in VUR grade II, in 13 of 27 (48%) in VUR grade III and in 8 of 9 (89%) in VUR grade IV-V ($p<0.001$). The relative risk of abnormal DMSA scan in relation to VUR grade is shown in figure 10. However, 15 of 26 children with dilating VUR, 14 with grade III and 1 with bilateral grade IV, had normal DSMA scan at follow-up.

Children with renal damage had increased frequency of recurrent febrile UTI, found in 16 of 80 children (20%) with renal damage compared to 20 of 223 (9%) with normal kidneys ($p<0.01$).

There was a correlation with both maximum temperature and maximum CRP at the index UTI and the occurrence of renal damage at follow-up ($p<0.05$ and $p<0.001$, respectively).

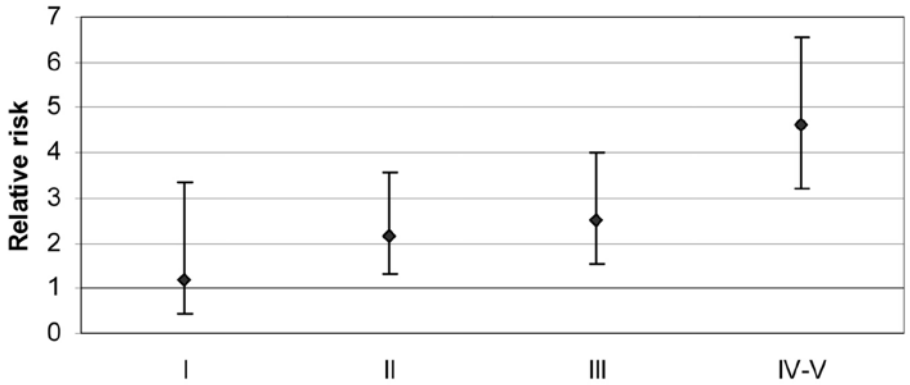

Figure 10. Relative risk and 95% CI of abnormality on follow-up DMSA scintigraphy in infants with different grades of VUR (I to V) compared to infants without demonstrable VUR. With permission.

5.1.3 The relation between VUR, UTI and renal damage

VUR grade, temperature and CRP level at index UTI, and recurrent febrile UTI were all significantly associated to renal damage at DMSA scan performed after 1 to 2 years. When analyzing these factors in a stepwise logistic regression model, VUR was the only independent factor for renal damage in boys ($p<0.0001$) and both VUR and CRP in girls ($p<0.05$ and $p<0.001$, respectively).

5.2 Bacterial resistance - paper II

The material in this paper included 1006 children. The characteristics are shown in figure. Temperature below 38.5°C together with CRP <20 mg/L was found in 148 children, 95 boys and 53 girls with median age of 2.7 and 10.4 months, respectively.

Table 4. Characteristics of 1006 children at first-time symptomatic UTI.

	Boys	**Girls**
Number	494	512
Age at UTI, months, median (range)	3.4 (0.2-23.6)	9.3 (0.2-23.9)
Temperature ≥38.5°C, n (%)	346 (70)	424 (83)
CRP, mg/L, median (range)	50 (5-430)	73 (5-380)
CRP ≥20 mg/L, n (%)	349 (71)	413 (81)
Urine sampling method		
Suprapubic aspiration	282	167
Catheter	4	5
Midstream	119	128
Bag	53	144
Not documented	36	68
Vesicoureteral reflux		
No VUR	387	345
Grades I-II	27	64
Grades III-V	46	39

5.2.1 Bacteriology

Bacterial findings are shown in table 5. *E.coli* was more prevalent in girls ($p<0.0001$). Non-*E.coli* infection was associated with severity of VUR; in children without VUR non-*E.coli* was found in 38 of 732 (5%), in VUR

grades I to II in 2 of 91 (2%) and in VUR grades III-V in 25 of 85 (29%) ($p<0.0001$).

Table 5. Bacterial species at first-time UTI according to gender.

	Boys n (%)	**Girls n (%)**
Gram-negative bacteria		
Escherichia coli	439 (89)	489 (96)
Klebsiella species	26 (5)	11 (2)
Proteus species	9 (2)	6 (1)
Enterobacter species	7 (1)	1
Pseudomonas species	0	1
Hemophilus influenzae	0	1
Gram-positive bacteria		
Enterococci	8 (2)	3
Staphylococcus aureus	3	0
Coagulase negative staphylococci	2	0

5.2.2 Bacterial resistance

The resistance to most used antibacterial oral drugs in children is shown in table 6. The resistance to trimethoprim was 14% for *E.coli* but low for other common uropathogens. In contrast the *E.coli* had a high sensitivity to nitrofurantoin and cefadroxil, but there was total resistance to cefadroxil for enterococci and to nitrofurantoin for *Klebsiella*, *Proteus* and *Enterobacter*.

5.2.3 *E.coli* resistance to antibiotics

Between 1994 and 1996 the *E.coli* resistance to trimethoprim increased from 5 to 17% ($p<0.05$). From 1996 to 2003 the resistance to trimethoprim stabilized around 15%, whereas the resistance to cefadroxil and nitrofurantoin has remained at a low level under 1% (figure 12).

When analyzing the relation between age and resistance it was found that *E.coli* resistance to trimethoprim increased around 9 months of age, from a

resistance rate of 11 % below 9 months to 19% over 9 months ($p<0.01$) (Figure 11).

The influence of age and calendar year on trimethoprim resistance was analyzed in a logistic regression model. Both variables were found to be significantly related to trimethoprim resistance ($p<0.05$). However, when analyzing boys separately no relation was found, while in girls both calendar year and age was significantly associated with trimethoprim resistance ($p<0.01$ and $p<0.05$, respectively) (Figure 13).

Table 6. Number of isolates resistant to oral antibacterial agents at first-time UTI.

	Trimethoprim	**Cefadroxil**	**Nitrofurantoin**
Escherichia coli, n=928	131	2	3
Klebsiella species, n=37	2	1	37
Proteus species, n=15	1	3	14[a]
Enterobacter species, n=8	0	5	8
Enterococci, n=11	0	11	0

[a] One missing

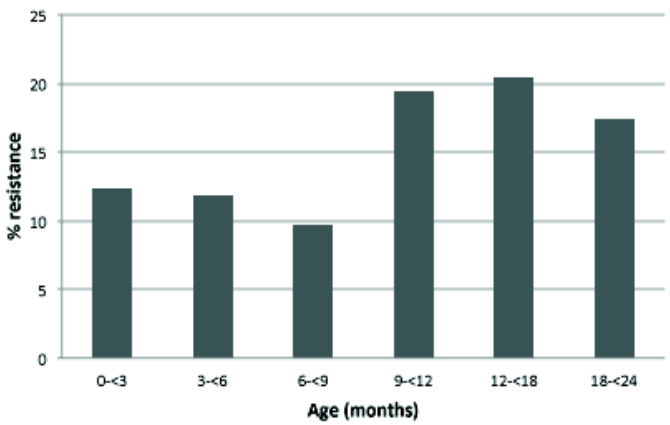

Figure 11. Trimethoprim resistance in E.coli related to age at first-time UTI. With permission.

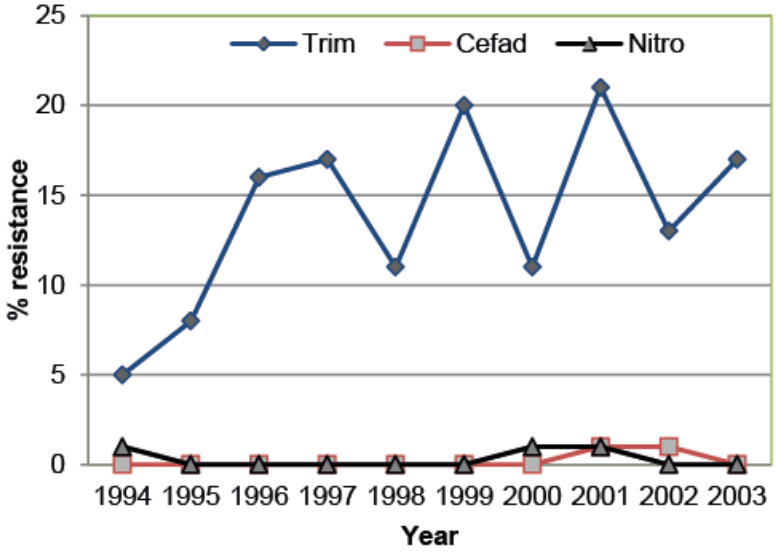

Figure 12. E.coli resistance to trimethoprim, cefadroxil and nitrofurantoin related to calendar year of isolation. With permission.

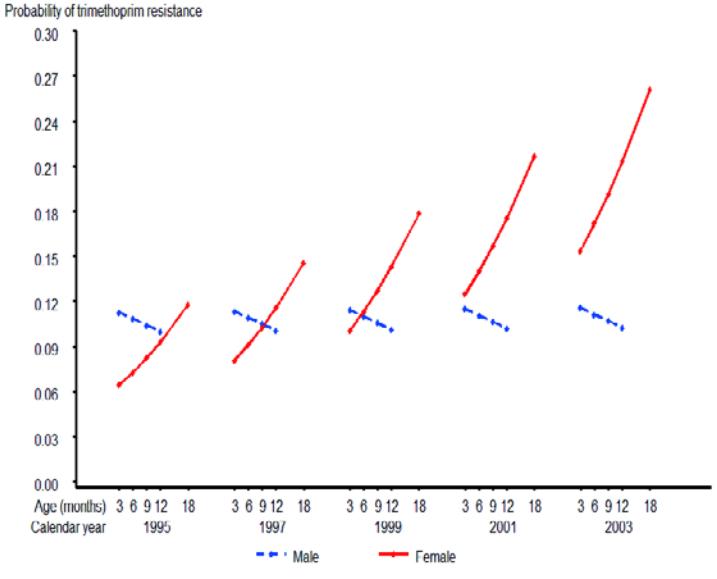

Figure 13. Probability of trimethoprim resistance for E.coli in relation to gender, age and calendar year at diagnosis. With permission.

5.3 The significance of low bacterial count – paper III

Included were 430 infants under 1 year of age, all diagnosed by urine samples obtained through suprapubic aspiration. The bacterial count is shown in table 7. The children were divided in two groups, a low bacterial group with bacterial count <100,000 CFU/mL and a high bacterial group with bacterial count ≥100,000 CFU/mL.
Low bacterial count was found in 83 (19%) of the infants.

Table 7. Number of colony-forming units per milliliter in urine obtained by suprapubic aspiration. 1n 430 infants with symptomatic urinary tract infection.

Bacterial count	**Boys n=275**	**Girls n=155**	**Total n=430**
<1000 CFU/mL	4	2	6
1000 - <10,000 CFU/mL	11	5	16
10,000 - <50,000 CFU/mL	34	18	52
50,000 – <100,000 CFU/mL	5	4	9
≥100,000 CFU/mL	221	126	347

Characteristics of the two groups are shown in table 8. There was no difference between the groups concerning gender, age or duration of fever. Infants in the low bacterial group had significantly lower temperature and CRP than in the high bacterial group. Similarly, CRP <20 mg/ml was found in 42 (52%) of 83 infants in the low bacterial group. Totally 22 infants, 19 boys and 3 girls, had both temperature <37.5°C and CRP <10 mg/mL. Of those, 10 infants had urine cultured because of poor weight gain.

Urinalysis revealed lower proportion of pyuria and positive nitrite test in the low bacteria group. The nitrite test showed a strong relation to bacterial count. The test was negative in all samples with bacterial count < 10,000 CFU/mL, positive in 10% with 10,000-<100,000 CFU/mL and in 45% with ≥ 100,000 CFU/mL.

UTI caused by non-*E.coli* species were more prevalent in the low bacteria group ($p<0.001$).

The frequency and severity of VUR were similar in the groups, as was the frequency of renal damage. The prevalence of recurrent febrile UTI within 24 months of the index UTI was higher in the high bacterial count group, but not at a significant level ($p=0.17$). However, the samples were small.

Probable factors associated with low bacterial count were analyzed in a stepwise logistic regression model. The significant factors related to a low bacterial count were CRP <20 mg/L, odds ratio 3.06 (95% CI 1.78-5.35; $p<0.0001$), absence of pyuria, odds ratio 2.49 (95% CI 1.22-5.08; $p=0.008$) and non-*E.coli* infection, odds ratio 2.50 (95% CI 1.13-5.52; $p=0.021$).

Table 8. Clinical data according to bacterial count.

	< 100,000 CFU/mL n=83	**≥100,000 CFU/mL n=347**	**p-value**
Gender, boys, n (%)	54 (65)	221 (64)	0.9
Age, months, median (range)	4.1 (0.3-12)	3.8 (0.2-12)	0.83
Duration of fever, days, mean (range)	2.7 (0-14)	2.5 (0-14)	0.76
Highest temperature , °C, median (range)	38.7 (37.0-40.7)	39.2 (36.6-41.3)	0.0025
CRP, mg/L, median (range)	17 (5-260)	65 (5-320)	<0.0001
CRP <20 mg/L, n (%)	42 (52)	76 (22)	
Pyuria, n (%)	62 (78)	314 (92)	0.0005
Positive Nitrite test, n (%)	6 (8)	154 (45)	<0.0001
E.coli, n (%)	67 (81)	326 (94)	0.0006
Vesicoureteral reflux, n (%)			
No VUR	60 (81)	269 (81)	1.00
Grades I-II	7 (10)	27 (8)	
Grades III-V	7 (10)	37 (11)	
DSMA scan abnormality, n (%)	12 (17)	62 (23)	0.33
Minor	*5 (7)*	*39 (14)*	
Moderate	*3 (4)*	*11 (4)*	
Pronounced	*4 (6)*	*12 (4)*	
Recurrent febrile UTI, n (%)	5 (6)	41 (12)	0.17

5.4 Evolution of renal damage – paper IV

The material comprises 103 children, 46 boys and 57 girls, with abnormal DMSA scan performed at least 90 days after the index UTI and who had a follow-up DMSA scan after more than 2 years.

The children were divided in 3 groups according to evolution of renal damage: regression, unchanged or progression. The characteristics at the index UTI is shown in table. There were no significant differences between the groups regarding gender, age or highest CRP. Non-*E.coli* infections were more prevalent in the progression group.

Table 9. Clinical data related to evolution of kidney damage in 103 children with abnormal DMSA scan performed ≥90 days and with a follow-up scan >2 years after the index UTI .

	Regression n=20	**Unchanged n=63**	**Progression n=20**	**p-value**
Gender, Boys, n (%)	9 (45)	27 (43)	10 (50)	0.75
Age, months, median (range)	4.6 (0.5-22.0)	6.5 (0.3-21.4)	5.5 (1.3-16.0)	0.70
CRP highest, mg/L, median (range)	110 (5-210)	120 (5-300)	135 (23-430)	0.32
E.coli, n (%)	18 (90)	55 (87)	12 (60)	0.013

There was a significant relation between VUR grade and evolution of renal damage. In children without reflux or with VUR grade I-II 16 of 52 (31%) improved and 4 (8%) progressed, while in children with VUR grade III-V 3 of 48 (6%) improved and 16 (33%) progressed ($p<0.001$) (table 10, figure 14).

Renal damage at the index DMSA scan was minor in 53 children, moderate in 27 and pronounced in 23. There was a significant gender difference where minor damage was found in 20 of 46 (44%) boys, moderate in 10 (22%) and pronounced in 16 (35%), while for girls corresponding number were 33 of 57 (58%), 17 (30%) and 7 (12%), respectively ($p=0.02$).

In the 53 children with minor damage at index DMSA scan the damage improved in 11 (21%) and progressed in 5 (9%). Comparable numbers for the 50 children with moderate to pronounced damage were 9 (18%) and 15 (30%). This difference was not significant (p=0.06).
The proportion of focal damage was similar in boys and girls, 22 of 46 (48%) and 35 of 57 (61%), respectively (p=0.17). There was comparable ratio of focal to general damage in the three groups of damage evolution (p=0.21) (table 10)

Table 10. Results of index DMSA scan performed ≥90 days after index UTI and VUR grade related to evolution of renal damage at follow-up DMSA scan at > 2 years.

	Regression n=20	**Unchanged n=63**	**Progression n=20**	**p-value**
Abnormal index DMSA scan, n (%)				
minor	11 (55)	37 (59)	5 (25)	0.048
moderate	7 (35)	11 (17)	9 (35)	
pronounced	2 (10)	15 (24)	6 (30)	
focal	12 (60)	37 (59)	8 (40)	0.21
general	8 (40)	26 (41)	12 (60)	
VUR, n (%)				
no VUR	14 (70)	23 (37)	1 (5)	<0.001
VUR I-II	2 (10)	9 (14)	3 (15)	
VUR III-V	3 (15)	29 (46)	16 (80)	
VCUG not done	1	2		

Recurrent UTI occurred in 13 (28%) boys and 21 (37%) girls (p=0.4). Recurrence was significantly associated with evolution of renal damage; recurrence occurred in 2 of 20 (10%) in the regression group, in 19 of 63 (30%) in the unchanged group and in 13 of 20 (65%) in the progression group (p<0.001) (figure 15). In 7 children, 4 boys and 3 girls, the renal

damage progressed without documented recurrent UTI. Of those 1 did not have VUR, 2 had VUR grade III, 3 grade IV and 1 grade V. The damage at the index DMSA scan was mild in 3, moderate in 1 and pronounced in 3.

In univariable analysis comparing the progression group with the pooled regression and unchanged groups, progression was significantly associated with non-*E.coli* infection, moderate and pronounced renal damage at the index DMSA, VUR grade III-V and recurrent UTI (table 11).
In multivariable logistic regression analysis only VUR grade III-V and recurrent UTI remained significantly associated with progression of renal damage.

Table 11. Univariable logistic and multivariable stepwise logistic regression analysis of probable explaining factors for progression of kidney damage. In the analyses the progression group was compared with the pooled regression and unchanged groups.

	Univariable analysis		**Multivariable analysis**	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Bacteriology at index UTI				
E.coli (reference)	1.0			
Non-*E.coli*	4.9 (1.6-14.8)	0.005		
Abnormality at index DMSA scan				
Minor (reference)	1.0			
Moderate/pronounced	4.11 (1.4-12.4)	0.012		
VUR				
Grade 0-II (reference)	1.0			
Grade III-V	6.0 (1.8-19.6)	0.003	4.5 (1.3-15.3)	0.011
Recurrent UTI				
No (reference)	1.0			
Yes	5.5 (1.9-15.6)	0.001	3.8 (1.3-11.5)	0.00

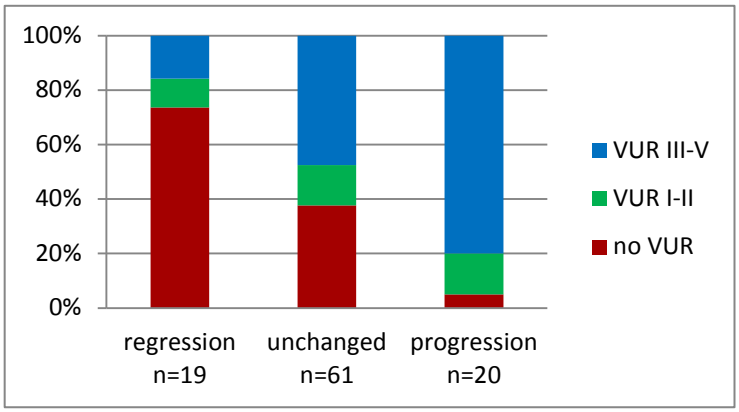

Figure 14. Grade of vesicoureteral reflux related to evolution of renal damage.

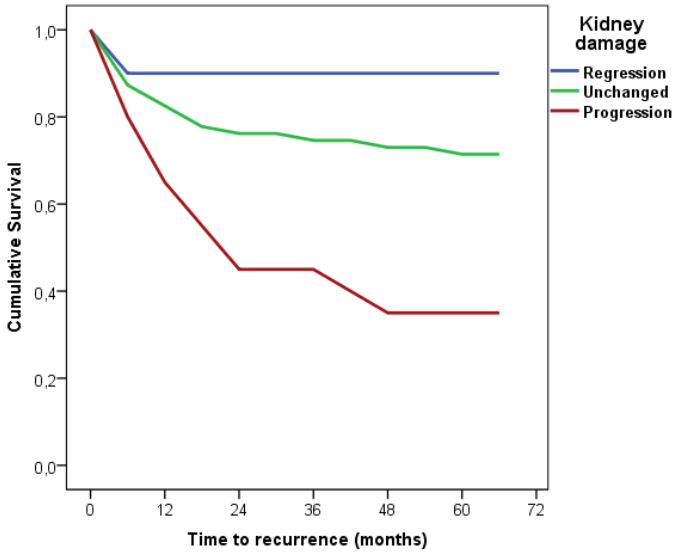

Figure 15. Survival chart of recurrent UTI related to evolution of renal damage.

6 DISCUSSION

6.1 Relation between UTI, VUR and renal damage

UTI is one of the most prevalent bacterial infections in infants and small children, found in 5-10% of children examined because of fever without apparent localizing signs.[98-100] Furthermore, among infants with bacteremia UTI has been reported in rates of up to 50%.[101] Besides the risk of a severe course of the acute infection, febrile UTI may lead to permanent renal damage. Proposed main risk factors for development of renal damage has been young age, vesicoureteral reflux, delay of treatment and recurrent UTI.[102-104] However, several studies could not confirm association between age and development of renal damage; on the contrary, some studies have reported renal damage to be more prevalent in children above 1 year than in infants.[17,104-106] Also, delayed treatment as a risk factor for renal damage has been questioned.[107,108]
An association between VUR and renal damage has been indicated in most studies. However, it is unclear to what extent VUR has a causative role or if it mainly is part of congenital dysplasia syndrome.[109] Still, children with VUR has an increased risk of pyelonephritis and renal scarring as do children with high grade (III-V) compared to low grade VUR (I-II).[85] Conversely, VUR is only found in around 50% of children with confirmed renal damage.[17,84]

Studies of the relation between recurrent UTI and renal damage have reported inconsistent results. While most have found renal damage to be associated with recurrent UTI, others could not confirm this association.[74,89,110-114] However, the evolution of new scars in previously normal kidneys has been related to breakthrough UTI.[93,104,115,116] Moreover, it is unlikely that a normal kidney develop new scars in the absence of recurrent UTI.[117]

In paper I permanent renal damage was associated with VUR grade II-V, recurrent febrile UTI, and temperature and CRP level at the first UTI. Furthermore, there were relationships between presence of VUR and recurrent febrile UTI and between grade of VUR and CRP at the first UTI.

DMSA scan 1 to 2 years after UTI was abnormal in 19% of the children without VUR. Compared to children without VUR, grade II and grade III had a modest but significant increased risk, odds ratio 2.2 and 2.5

respectively, while grade IV-V showed a high risk of renal damage, odds ratio 4.6. In logistic multivariable analysis of risk factors for renal damage, VUR was the only independent factor for boys, whereas both VUR and CRP were significant for girls.

The diagnosis of acquired renal damage by DMSA scan can only be made in the presence of serial DMSA scans. Accordingly, the causes of the observed relation between grade of VUR and renal damage cannot be stated. Speculatively, VUR involving the kidney, that is grade II or more, may imply a greater exposure of microbes to the renal parenchyma resulting in increased inflammatory response and risk of renal damage. Other explanations could be that high grade VUR may be part of a more general urinary tract malformation, including renal dysplasia, or that the reflux of urine has a direct effect on the growing kidney intrauterinaly. The genetic and embryological correlation between renal dysplasia and VUR is well established. Several human genetic syndromes are associated with VUR and renal dysplasia. Furthermore, mice models with knock-out of genes regulating urinary tract embryology have resulted in different malformations including the kidney and the distal ureter. In addition, animal studies have shown that urinary flow impairment can generate renal dysplasia.[118-120] The gender difference found may imply that the microbial exposure perhaps has greater impact in girls, explaining the relation between damage and CRP level at first UTI, whereas for boys VUR associated renal damage is mainly related to congenital dysplasia.

Reported risk factors for recurrent UTI have been female gender, age <6 months, bladder and bowel dysfunction, severe grade of VUR and renal damage.[114,121-123] In our study totally 47 episodes of recurrent febrile UTI was observed in 36 (12%) children, 15 (9%) boys and 21 (15%) girls. This gender difference was not significant (p=0.15). As all children were younger than 2 years at inclusion we do not have information about bladder function. Recurrent febrile UTI was related to the presence and severity of VUR, occurring in 22% of children with VUR grade III-V. This numbers is similar to other reports of febrile recurrences in children with VUR grade III-V.[122-124] In contrast, the total frequency of recurrences in children with high-grade VUR, also including non-febrile UTI, often is reported as high as 40%.[121,124,125]

6.2 Bacterial resistance

UTI, as other infections, is the result of microbes overcoming the defense mechanisms of the host. The microbes have numerous survival techniques to invade the host and overcome the defense, such as adherence by pili and flagella, toxin production, immune evasion by morphological changes and capsule, iron scavenging and biofilm formation.[43] In addition to these survival strategies, microbes have a great ability to develop resistance against the effects of antimicrobials. Since introduction of the first antimicrobial agents emerging resistance has been reported.[126] Drugs such as ampicillin and trimethoprim that used to be the first choice of treatment for UTI are no longer appropriate for empirical use. Furthermore, the increasing spread of multidrug-resistant organisms is an expanding threat against public health.[127]

In paper II the progression of *E.coli* resistance to oral antibiotics over a 10-year period, 1994 to 2003, was studied. An increased resistance to trimethoprim from 5% to 17% was observed, while the resistance to cefadroxil and nitrofurantoin remained at a low level below 1%. Furthermore, there was a correlation between *E.coli* resistance to trimethoprim and age, with an increase of resistance around 9 months of age. However, in multivariable analyses the relation between increased resistance, age and calendar year was only found in girls.

The increase of *E.coli* resistance to trimethoprim followed the trend of the general population in Sweden during the period, even though the rise of resistance appeared to occur earlier in the studied cohort. The resistance to trimethoprim increased significantly despite an impressive reduction in the prescription of antibiotics in Sweden, especially concerning young children.

The relation between antibacterial resistance and age has been addressed in a few studies. In a study of risk factors for trimethoprim resistant *E.coli*, antibiotic therapy within 4 weeks, age 2 to 6 years, urogenital abnormality and recent hospital admission were significantly associated with resistance.[128] In studies of ESBL-producing bacteria in children, age under 1 year has been shown to be one of the risk factors.[129,130] In a study of community fecal carriage of ESBL-producing *Enterobacteriaceae* in children 6-24 months of age, a higher carriage frequency was found in children above 12 months compared to younger children.[131] The reason for the increasing frequency of trimethoprim resistant *E.coli* found in children above 9 months is probably multifactorial. With growth infants have higher risk of picking up new bacteria from the surrounding as they get more mobile investigating the

environment and having more human contacts outside the closest family. During the end of the first year they also have a high frequency of upper respiratory tract infection with increased risk of antibiotic exposure, which is a recognized cause of colonization by resistant microbes.[128,131]
From published studies there is no clear gender difference in susceptibility of infections by resistant uropathogens. While some report infections by ESBL-producing *Enterobacteriaceae* to be more prevalent in boys others do not find such gender difference.[128-132]

Except for the relation between female gender and the frequency of trimethoprim resistant *E.coli* some other gender differences were found in our study. Boys were younger at the first UTI and had significantly more of non-*E-coli* infections, which could not be explained by a higher proportion of high grade VUR. This could be interpreted as the urinary tract in the infant boy in some way is compromised. On the other hand girls were older and had a more pronounced inflammation response.

It is well documented that there are gender differences in the immune response to different agents. Males have higher incidence of more severe infections, while females have a more pronounced immune response to both infections and autoantigens. These differences are partially attributed to different immune effects of sex hormones, but there is also evidence of the X chromosome having a genetic role in regulation of the immune system, among others concerning toll like receptors.[133-135]

6.3 The significance of low bacterial count

The diagnosis of UTI in children is based on growth of uropathogens in an adequately obtained urine specimen. As mentioned in the introduction recommendations of urine sampling methods as well cut-off levels for bacterial number varies in published guidelines. The cut-off levels are set to distinguish between true bacteriuria and contamination. The ideal sampling method is one with good discrimination, while in less appropriate methods there is varying degrees of overlap between bacteriuria and contamination. Urine sampling through suprapubic aspiration of the bladder is regarded as the method with the lowest risk of contamination.[14,25,26,136,137]

In paper III the significance of bacterial counts below 100,000 CFU/mL was analyzed in a population of 430 children below 1 years of age, where all samples were obtained by suprapubic aspiration.

Low bacterial count was found in 19% of the children. There was similar frequency of permanent renal damage at DMSA scan in the low and high bacterial group. Also the rate and severity of VUR was comparable in the groups. Recurrent UTI was more prevalent in the high bacterial group but the samples were small and the difference was not significant. On the other hand, the low bacterial group showed less inflammation response, measured as temperature, CRP and pyuria. Furthermore, non-E.coli infection was related to low bacterial count.

This portion of low bacterial count in UTI is coherent with several other studies and indicates a risk of misdiagnosing up to one fifth of children with UTI if adhering strictly to some of the guidelines.[25,30,38-40] Reasonably the results from urine collection by suprapubic aspiration should be relevant also for catheter or clean catch specimen.[138] Even if the incubation time may be shorter in urine collection by catheter or bladder aspiration, to be successful both methods demands a substantial urine volume of the bladder.

The similarities concerning the frequency of renal damage and VUR irrespectively of bacterial count implies that low bacterial UTI should be treated with the same caution as infection with higher bacterial number.

The finding of less intensive inflammation reaction related to low bacterial count was surprising and contrasting to the results of Kanellopoulos et al.[39] Therefor, as comparison, the material from the Swedish assurance project was reevaluated.[138] In all 808 children below 1 year of age, 474 boys and 334 girls, all with urine collection by suprapubic aspiration, were included. The results were similar to our study. Thus, 23% had bacterial count <100,000 CFU/mL. Comparing the high and low bacteria groups mean CRP was 69 mg/L and 43 mg/L, respectively ($p<0.0001$), and mean temperature 39.1 and 38.8 ($p=0.0035$).

Kunin et al studied low bacterial UTI in young women. They found an association between the magnitude of pyuria and bacterial count, but they could not explain low counts by dilution of urine or failure of bacteria to grow well in the patients´ urine. However, the frequency of voiding or the presence of residual urine was not analyzed. They concluded that UTI with low bacterial count may be an infection that had not become fully established.[29]

The lower inflammatory response may indicate that children with low bacterial count have some protective properties. In our study there were no

significant differences concerning gender, age, duration of fever before treatment or anatomy of the urinary tract. Unfortunately, information about bladder function is lacking. Speculatively, frequent emptying the bladder could have protective capacity resulting in lower bacterial number. Also, the efficiency of the immune system in protecting the urinary tract may be one of the factors involved.

6.4 Evolution of renal damage

The main goal in the handling of UTI in children, besides treating the acute disease, is to preserve renal function and prevent the evolution of renal damage. Probably the growing kidney is more vulnerable to infections than the adult kidney.[139] Therefore, it is important particularly during childhood to protect the kidneys from impairment. Several studies have shown that long-term antibiotic prophylaxis may significantly reduce the rate of recurrent UTI, but just one study -the Swedish reflux trial- has revealed prophylaxis to have an impact on the evolution of renal damage and this effect was only seen in girls.[93,140-142]

In paper IV the evolution of renal damage over time was analyzed. Progression of the renal damage was found in 19% of the children and an equal part showed regression. Progression was associated with VUR grade III-V, febrile recurrences, non-*E.coli* infection and severity of abnormality at index DMSA scan. In multivariable logistic regression analysis VUR grade III-V and febrile recurrences were the only significant factors. However, there was a gender difference, where VUR grade III-V was the single significant risk factor for progression in boys, while both recurrent febrile UTI and severity of initial renal damage was significant for girls.

The risk of progression was similar to the results from other studies, showing progression rates of 11% to 18%.[91-93] However, the studies are not directly comparable as they differ in included patient material. The cited studies were conducted on children with high-grade VUR, while our study comprised a cohort of children with renal damage.
The high resolution rate of 72% reported in the study by Parvex et al could not be confirmed.[94] Still, the regression frequency of 19% shown in our study is, except from the study by Parvex et al, higher than has been reported in previous studies.

In the study risk groups for deterioration of renal damage were found. Among 23 children with VUR grade III-IV and recurrent febrile UTI the renal

damage progressed in 10 children (43%). In boys with VUR grade III-V the renal damage deteriorated in 9 of 25 (36%) compared to only 1 of 20 (5%) without VUR or with VUR grade I-II. This means that 9 out of 10 boys with progression had VUR grade III-V. Similarly, in girls with recurrent febrile UTI 8 of 21 (38%) deteriorated compared to 2 of 36 (6%) without febrile recurrence, that is 8 of 10 girls with progression of renal damage had recurrent febrile UTI.

Even if recurrent UTI was a strong predictor for progression 7 children in the progression group did not have a documented febrile recurrence. Of those 6 had VUR grade III-V and 5 were boys. In the Swedish reflux trial 24 children showed deterioration of renal status, of which 9 did not have febrile recurrence.[93] Similarly, in the study by Sjöström et al 6 out of 19 children with renal deterioration did not show any breakthrough UTI.[92]

Progression was defined as a decrease in DRF of more than 3% in the injured kidney. However, it is not possible to determine if this decrease in DRF is actually a drop of function of the injured kidney or a compensatory growth of the contralateral kidney. It may be that for girls, where recurrent UTI seems more important, it actually is deterioration of the injured kidney that is important, while in boys the injured kidney more often has congenital origin with impaired capacity of growth. Nevertheless, it has not been recognized that the impact on long-term prognosis should be different in acquired and congenital forms of renal damage.

The long-term effect of a modest decrease in DRF over time is difficult to predict. Several studies of children with renal damage have shown excellent long-term results, with minor impact on kidney function, blood pressure or pregnancy.[88,90,143] Still, it is of importance to preserve as good renal status as possible during childhood. Historical data shows that kidney damage in childhood may have major influence on the health in adulthood.[88] Therefor it is of importance to continue to handle children with UTI with great care.

7 CONCLUSION

Significant relations were found between permanent renal damage, grade of VUR and recurrent UTI.

Between 1994 and 2003 the *E.coli* resistant rate to trimethoprim increased 3 fold, from 5% to 17%, while the resistance to cefadroxil and nitrofurantoin remained low.

One fifth of children with symptomatic UTI had bacterial count below 100,000 CFU/mL. UTI with low bacterial count was associated with similar frequency of renal damage and high-grade VUR as UTI with high bacterial number.

Renal damage remained unchanged over time in the majority of children. In one fifth of the children the renal damage progressed. Risk factors for progression were high grade VUR and recurrent UTI.

VUR and recurrent UTI were the main factors associated with the presence and progression of renal damage. Thus, imaging of the bladder still has its place in the work-up of children with UTI, especially in the situation of febrile recurrent infection. Furthermore, children with renal damage associated with high-grade reflux and recurrent febrile UTI have an increased risk of deterioration and in those long-term follow-up should be considered, whereas renal damage without high-grade reflux or recurrence has low risk of progression.

The substantial portion of UTIs with low bacterial count emphasizes that the UTI diagnosis in small children is not just a matter of a cut-off level for bacterial number. The entire clinical situation must be considered and the choice of method for obtaining urine specimen is of crucial importance. The most reliable methods in infants are through bladder aspiration or catheter.

The increasing antimicrobial resistance is a serious threat to many areas of modern medicine. Described is the emerging *E.coli* resistance to trimethoprim, most pronounced in children above 9 months of age and in females. This pattern may be similar in other forms of antimicrobial resistance appearing in the future. Thus, it is important to explore ways of protecting children from colonization by resistant microbes, especially during the first years of life. This is not just a task for the medical system but also

demands participation of other parts of the society. The most important issue is to reduce the world wide antimicrobial pressure from food processing, medical care and environment.

In the study gender differences were found concerning epidemiology, bacteriology, antibacterial resistance pattern, inflammation response, recurrences and prognosis. The reasons for these differences are only partially revealed and include variations in bladder function, bacterial colonization and regulation of the immune system. Further knowledge of these factors as well as of biomarkers for renal involvement could probably result in a more individualized and effective treatment of children with UTI.

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ERRATA

Paper I: Page 649, line 7.
“A new discrete focal scar was detected in a previously unscarred kidney in one child, and in another child there was progression of a preexisting scar.”

This sentence should be: “Among the patients with febrile recurrence during follow-up, a new discrete focal scar was detected in a previously unscarred kidney in one child, and in another child there was progression of a preexisting scar.”