

Urinary tract infection in infants

Studies on management and genetic susceptibility

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*"If we knew what it was we were doing,
it would not be called research, would it?"*

Albert Einstein

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ABSTRACT

Urinary tract infection (UTI) in infants is associated with kidney damage. Recommendations on investigations to identify patients at risk of recurrent UTIs and kidney related morbidity vary considerably. The objectives of this thesis were to evaluate current management of infants with their first UTI, including adherence to the Swedish pediatric UTI guidelines, and to investigate predictive clinical and genetic markers of UTI associated kidney damage.

Infants <1 year presenting with a first episode of UTI were included in a nationwide prospective study with one year follow-up. Patient characteristics, laboratory results and findings on imaging of the kidneys and urinary tract were described (Paper I). Performed investigations were related to guideline recommendations for adherence studies (Paper II). In a subgroup, blood sample DNA was used for genetic association analysis of permanent kidney damage identified by renal scintigraphy (Paper III). Anatomical abnormalities and kidney damage were identified in line with previous prevalence estimates at the expense of a high number of investigations. Diagnostic work-up and treatment were largely performed according to guidelines while adherence to recommended imaging was lower. A genetic profile could segregate infants with permanent kidney damage from those without persistent damage.

In conclusion, diagnostic and therapeutic management of infants with UTI is satisfying but the burden of radiation from imaging is high in relation to findings. A genetic susceptibility partly explains why some infants with kidney infection have persistent kidney defects while others heal without damage.

Keywords: Urinary tract infection, infant, clinical guidelines

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SAMMANFATTNING PÅ SVENSKA

Urinvägsinfektion (UVI) är en av de vanligaste bakteriella infektionerna i åldersgruppen 0-1 år och är ofta förknippad med infektion i en njure. En UVI kan orsaka en bestående njurskada och ibland är den kopplad till anatomiska avvikelser i urinvägarna. Därför görs ofta en efterföljande utredning med röntgen eller annan bilddiagnostik för att identifiera barn med risk för nya infektioner och för framtida sjuklighet till följd av njurskada. Tillförlitliga riskmarkörer saknas dock och det råder delade meningar om vilka utredningar som bör göras. Svenska och internationella vårdriktlinjer för UVI finns men rekommendationer skiljer sig avseende diagnostik, behandling och utredning.

Denna avhandling beskriver hur spädbarn med sin första UVI handläggs i Sverige där 29 barnkliniker har rapporterat uppgifter om 1357 barn. Diagnostik skedde till övervägande del enligt riktlinjerna där en viktig del är att säkra ett urinprov för bakterieodling. Antibiotikaresistensen hos *E. coli*, den vanligaste UVI-bakterien, har fortsatt att öka den senaste 20-årsperioden vilket är ett problem då det finns få antibiotika för små barn. Även behandling skedde till största del enligt riktlinjerna. Utrymme finns dock för minskad intravenös behandling på sjukhus och ökad behandling i öppenvård. I den uppföljande bilddiagnostiken identifierades barn med kvarstående njurskada i ungefär förväntad frekvens. Trots minskad utredningsintensitet jämfört med 1990-talet var utredningsbördan alltså hög och många barn med friska njurar utsattes för strålning i onödan. Av 836 barn som genomgick minst en njurskintigrafi påvisades njurskada i 126 fall. Följsamheten till rekommenderad bilddiagnostik var lägre, särskilt på mindre sjukhus, och orsakades oftare av överdriven utredning än av att man avstått från rekommenderad undersökning. De icke rekommenderade undersökningarna visade sällan något avvikande.

I en delstudie på en mindre grupp jämfördes ett stort antal gener på barn med njurskada efter UVI mot barn som inte fick njurskada. Förekomst av varianter i vissa gener hos barn med njurskada visade sig kunna särskilja grupperna, talande för en genetisk benägenhet för njurskada kopplad till UVI.

Slutsatser är att spädbarn med UVI sköts med stor noggrannhet på landets barnkliniker avseende diagnostik och behandling. De svenska vårdriktlinjerna bör dock ses över, främst vad gäller rekommenderad bilddiagnostik för att minska utredningsbördan. Det finns behov av nya riskmarkörer för njurskada där genetiska förklaringsmodeller kan bidra till sökandet efter sådana.

LIST OF PAPERS

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

- I. **Lindén M***, Rosenblad T*, Rosenborg K, Hansson S, Brandström P. Infant urinary tract infection in Sweden—A national study of current diagnostic procedures, imaging and treatment. *Pediatr Nephrol.* 2024; 39(11): 3251-3262. doi:10.1007/s00467-024-06415-4. *Shared first authors.
- II. **Lindén M**, Rosenblad T, Hansson S, Brandström P. Adherence to the Swedish paediatric guidelines for urinary tract infections. *Acta Paediatr.* 2024; 00: 1–9. doi:10.1111/apa.17554.
- III. Rosenblad T, **Lindén M**, Ambite I, Brandström P, Hansson S, Godaly G. Genetic determinants of renal scarring in children with febrile UTI. *Pediatr Nephrol.* 2024; 39(9): 2703–2715. doi:10.1007/s00467-024-06394-6.

PUBLICATIONS NOT INCLUDED IN THE THESIS

Brandström P, **Lindén M**. How Swedish guidelines on urinary tract infections in children compare to Canadian, American and European guidelines. *Acta Paediatr.* 2021; 110(6): 1759–1771.

Ambite I, Chao SM, Rosenblad T, Hopkins R, Storm P, Ng YH, Ganesan I, **Lindén M**, Haq F, Tran TH, Ahmadi S, Lee B, Chen S, Godaly G, Brandström P, Connolly JE, Svanborg C. Molecular analysis of acute pyelonephritis—excessive innate and attenuated adapted immunity. *Life Science Alliance.* 2025; 8(3): e202402926.

Hallander H, **Lindén M**, Lindqvist C, Olsson A, Larsson SM. Performance of enzymatic creatinine methods in the pediatric concentration range. *Scand J Clin Lab Invest.* 2025. Accepted for publication.

Ahmadi S, Rosenblad T, Sabari S, **Lindén M**, Brandström P, Chao SM, Svanborg C, Ambite I. Evidence of a cytokine storm in infants with febrile UTI—proteomic analysis of urine samples. Submitted.

CONTENTS

Abbreviations	vi
1 Introduction.....	1
1.1 Historical perspective.....	2
1.2 Epidemiology.....	2
1.3 Bacteriology and pathogenesis.....	4
1.4 Predisposing factors of UTI.....	6
1.4.1 Congenital anomalies.....	6
1.4.2 Bowel and bladder dysfunction.....	7
1.4.3 Genetic susceptibility.....	8
1.5 Renal scarring.....	9
1.5.1 Pathogenesis.....	9
1.5.2 Genetic influence.....	10
1.5.3 consequences of UTI and scarring.....	12
1.6 Management of UTI in infants.....	14
1.6.1 Diagnosis.....	14
1.6.2 Treatment.....	16
1.6.3 Imaging.....	17
1.7 UTI guidelines.....	19
1.8 Adherence to guidelines.....	23
2 Aims.....	25
3 Patients and Methods.....	26
3.1 Patient recruitment.....	26
3.2 Patient selection.....	27
3.3 Methods.....	28
4 Results.....	35
4.1 Paper I.....	35
4.2 Paper II.....	39
4.3 Paper III.....	42

5	Discussion	46
5.1	Paper I and paper II	46
5.2	Paper III.....	55
6	Conclusion.....	58
7	Future perspectives.....	60
	Acknowledgement.....	63
	References	65

ABBREVIATIONS

AAP	American Academy of Pediatrics
ABU	asymptomatic bacteriuria
ACE	angiotensin-converting enzyme
APD	anteroposterior diameter
APN	acute pyelonephritis
CAKUT	congenital anomalies of the kidneys and urinary tract
CFU	colony forming units
CI	confidence interval
CRF	case report form
CRP	C-reactive protein
DMSA	^{99m} Tc-dimercaptosuccinic acid
DNA	deoxyribonucleic acid
ESBL	extended spectrum beta-lactamase
ESKD	end stage kidney disease
IPA	ingenuity pathway analysis
IQR	interquartile range
IRF	interferon regulatory factors
KDIGO	Kidney Disease: Improving Global Outcomes
MAG3	^{99m} Tc-mercaptoacetyltriglycine
MRI	magnetic resonance imaging

NGAL	neutrophil gelatinase-associated lipocalin
NICE	National Institute for Health and Care Excellence
OECD	Organization for Economic Cooperation and Development
OR	odds ratio
PCA	principal component analysis
POCT	point of care test
RBUS	renal and bladder ultrasonography
RS	renal scarring
SNP	single nucleotide polymorphism
SPA	suprapubic aspiration
TGF	transforming growth factor
TLR	toll like receptor
U-Le	urine leukocyte esterase
UPEC	uropathogenic <i>E. coli</i>
UTI	urinary tract infection
VCUG	voiding cystourethrography
VUR	vesicoureteral reflux

1 INTRODUCTION

It is not a coincidence that urinary tract infections (UTIs) became the subject of my PhD project. My principal supervisor during my residency had an interest in pediatric nephrology and, from start of my training, introduced me to the principles of managing UTIs in young children. At that time, in the mid-1990s, all children less than two years of age who were diagnosed with a febrile UTI at our hospital were meticulously investigated with renal and bladder ultrasonography (RBUS), voiding cystourethrography (VCUG) and urography. Renal scintigraphy was about to be introduced to children at that time. They were then followed up in clinic at regular intervals with repeated urine cultures to monitor if bacteria reappeared. If backflow of urine from the bladder to the kidneys, vesicoureteral reflux (VUR), was detected, the child was prescribed long term prophylactic antibiotic treatment and a new VCUG planned for in a year or so to see if the reflux had resolved or diminished. For the doctor, this was very standardized and easy to perform and I learned how to motivate families to go through these procedures by explaining that new infections could harm the kidneys further, increasing the risk of future health problems like hypertension and chronic kidney failure.

Much research has been done since then and over the years recommendations on how to diagnose, treat and follow up children with UTI have changed. However, still no uniform internationally accepted guidance on how to best manage young children with UTI exists and personally, I have had more dubious thoughts about how to care for these children nowadays than in the beginning of my career. This led me to inquire among nephrology colleagues at Queen Silvia Children's Hospital in Gothenburg if there were any suitable UTI research projects for an ageing pediatrician to indulge in. There weren't but Per Brandström and Sverker Hansson, who later became my supervisors, inspired me to think about and conceptualize a new project including an evaluation of the Swedish clinical UTI guidelines. This was then expanded to become a joint venture with a research group in Lund with expertise in genetic and molecular research on bacteria, inflammation and UTI, using high end laboratory technology which made the project even more exciting. Thus, the research plans for me and my colleague PhD student Therese Rosenblad were born.

This thesis focuses on evaluating current management of UTIs in infants, including adherence to guideline recommendations, burden of investigations

and the yield of undertaken imaging of the kidneys and urinary tract. It also aims to address the influence of clinical and genetic characteristics on UTI related kidney damage.

1.1 HISTORICAL PERSPECTIVE

Literature from the previous century describes the association of febrile UTIs in children and severe nephropathy leading to progressive renal impairment and end stage renal disease [1, 2]. These children often had recurrent UTIs and a history of missed or late diagnosed infections. This is likely still an issue in developing countries related to socio-economic factors and limited access to healthcare although reliable research studies are scarce [3]. Thanks to pioneer work by Jan Winberg, Jean Smellie, Ulf Jodal and others during the 1960s to 1980s the spotlight was focused on recognizing and treating UTIs in children at an early stage which dramatically improved the management of childhood UTI. Furthermore, extensive imaging of the kidneys and urinary tract was recommended as follow-up to look for anomalies and in particular VUR. The presence of VUR was for many years considered the leading cause of UTIs and subsequent scarring of the kidneys. It was thought that curing the reflux would prevent further damage to the kidneys which inspired urologists to develop new surgical techniques that became regularly used to treat VUR. Another approach was to treat children with VUR with long term prophylactic antibiotics to prevent new infections and additional renal scarring. In the International Reflux study, children with dilating VUR (VUR grade 3–5) were randomized to either surgical correction or continuous antibiotic treatment and compared for renal growth and new renal scars. The study concluded that no significant difference between the groups could be seen at 5 and 10 years follow-up [4]. However, renal scarring can develop after UTIs in the absence of VUR [5]. Moreover, the kidney damage seen in children with VUR in many cases preceded the UTI and was, in fact, of congenital origin [6, 7]. This insight, and the improved attention to early recognition and treatment of UTIs, making severe kidney damage due to UTI a rare phenomenon in children, has changed the strategy of follow-up after UTIs with focus on decreasing the burden of radiation and unnecessary procedures.

1.2 EPIDEMIOLOGY

UTIs are more prevalent in boys the first 4–6 months of life but after that age it is more common in girls, depicted in Figure 1.

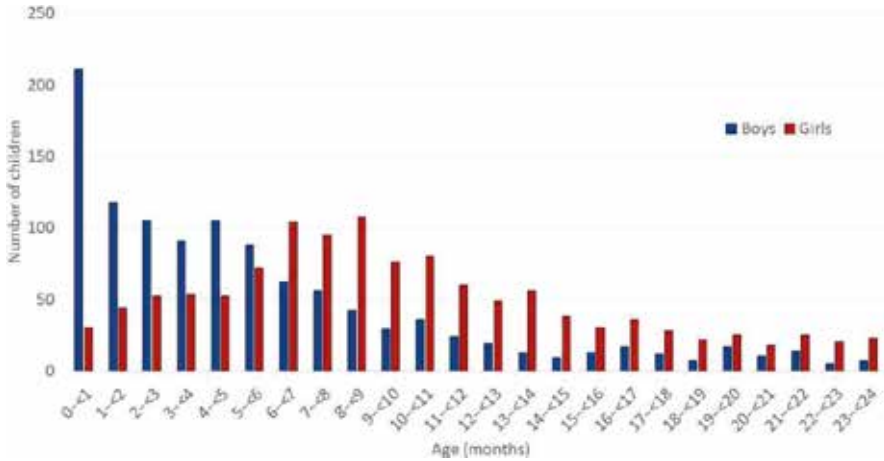


Figure 1 Age distribution of 1111 boys and 1198 girls 0–2 years of age at first UTI. Reprinted from *Pediatric Clinics of North America* 69(6). Brandström P, Hansson S. *Urinary tract infection in children, 1099–1114, (2022) with permission from Elsevier*

Uncircumcised boys are more likely to be affected than circumcised [8]. The high incidence of UTI during infancy coincides with a vulnerable phase in life when symptoms of bacterial infections are unspecific and can easily be missed or mistaken for more benign conditions if not properly investigated for the possibility of UTI. Particularly in newborns, bacteria causing UTI as well as other bacterial infections, can evade the immature immune system and cause sepsis and meningitis despite few preceding signs of serious infection [9, 10]. Older infants may have a variable disease course ranging from mild self-limiting illness to a life-threatening condition.

UTIs can be classified in three different categories or levels: upper UTI/acute pyelonephritis (APN), lower UTI/cystitis and asymptomatic bacteriuria (ABU) [11, 12]. In upper UTI, the infection affects the kidney and causes parenchymal inflammation. The leading symptom in infants is fever, sometimes accompanied with irritability, vomiting and poor feeding. In cystitis, the infection is restricted to the bladder and urethra causing dysuria and pain of the bladder. These symptoms, of course, are difficult to interpret in preverbal children. In ABU, the bladder is colonized by bacteria causing foul-smelling urine but no symptoms or harm. A meta-analysis estimated the prevalence of ABU in boys <1 year to be 0.66% but only 0.08% in boys >1 year [13]. In contrast, the prevalence of ABU in girls <2 years of age was 0.22% but was higher after this age, 0.52% in this study. The highest prevalence of ABU during childhood is thought to be in uncircumcised boys during early infancy:

1.6% in boys <2 months [14]. Pyelonephritis is the predominating UTI in infants while cystitis is rarely diagnosed in this age group. In addition to having the potential to cause acute serious infections and being a nuisance for the child, pyelonephritis has been associated with kidney injury [15]. This has been the main focus of research in childhood UTI since the 1950s. This thesis focuses on UTI in infants <1 year motivated by the high incidence in this age group and by a long tradition in Sweden to manage these patients in specialist pediatric care which facilitates clinical research.

1.3 BACTERIOLOGY AND PATHOGENESIS

The pathogenesis of UTI is complex. Bacteria from the intestinal tract can enter the urinary tract through the urethra to the bladder and then ascend to the kidneys via the ureters. Less commonly, they can invade the kidneys directly through the blood stream. The predominating bacteria causing UTI in young children is *E. coli* with a prevalence of about 90% in population-based studies but lower in children with complicated UTIs [16-19]. Other bacteria causing UTI, as *Klebsiella*, *Enterobacter*, *Proteus*, *Enterococcus* and *Pseudomonas* species, hereafter named non-*E. coli*, are associated with a higher risk of anomalies of the kidneys and urinary tract [20-22]. A European survey among pediatric nephrologists in 10 countries studied positive urine cultures from children under 24 months of age from hospital-patients and outpatients between 2010 and 2012 [23]. The survey confirmed that *E. coli* was the most common bacterium, however, in cultures sampled in 10 of 16 hospitals and in 6 of 15 outpatient clinics, *E. coli* was isolated in less than 50%. Most centers participating in this survey were referral hospitals for pediatric nephrology patients which certainly inferred a selection bias but the study emphasizes the importance of being aware of a more varied bacterial spectrum causing UTIs in certain populations.

Among *E. coli*, some strains possess important virulence factors that can facilitate their invasive properties while other *E. coli* will settle in the bladder and cause no harm to the host [13, 24]. Important virulence factors among uropathogenic *E. coli* (UPEC) include type 1 pili and adhesion molecules, such as FimH, for adhering to the uroepithelium, P-fimbriae for ascending the urinary tract, toxin secretion that disrupts the mucosal barrier, iron uptake ability for survival and mechanisms to evade the host's immune response by entering epithelial cells and producing intracellular bacterial communities or

biofilm-like structures [25-29]. The host, on the other hand, has defense mechanisms to protect the urinary tract from infection, illustrated in Figure 2.

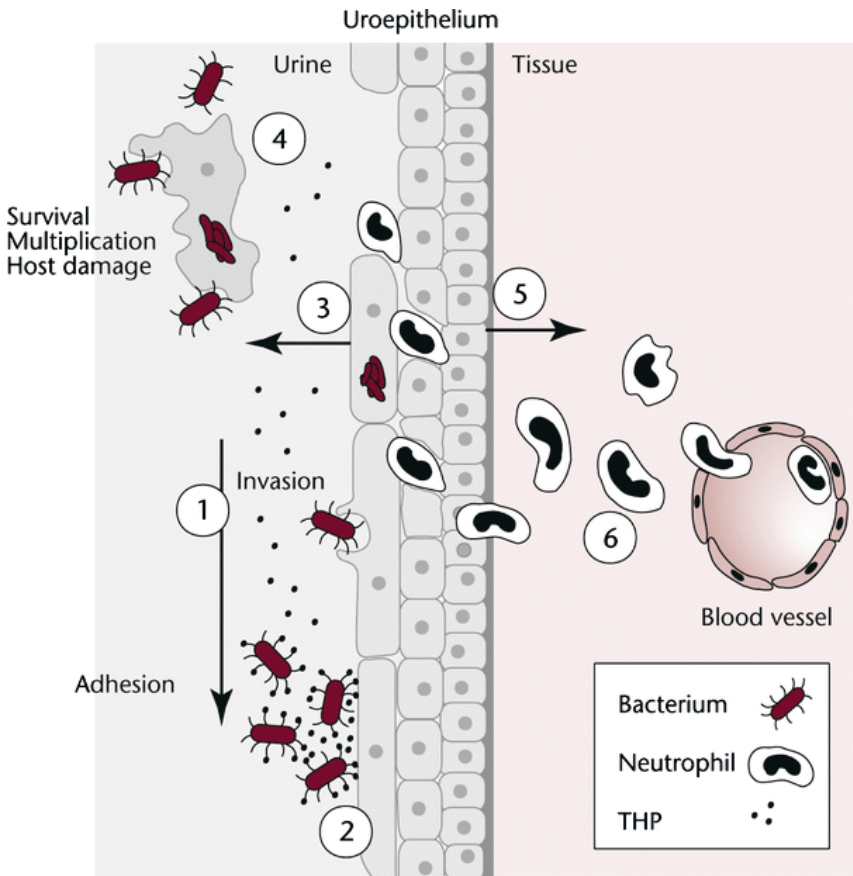


Figure 2 Protective host factors: 1 Urine flow, 2 antiadherence factors (e.g. THP=Tamm-Horsfall protein), 3 antimicrobial factors, 4 cell-exfoliation, 5 production of chemokines and cytokines, 6 neutrophils. Chromek M, Brauner A. *J Mol Med.* 2008;86:36–47. With permission from Springer Nature.

To further complicate the understanding of the evolution of UTI, it has been shown that the UPEC genome can mutate and adapt to the defense mechanisms met in the new environment in the host by losing or gaining genetic material [30, 31].

Other important properties of bacteria, crucial for the treatment of patients with UTI, are different resistance mechanisms to antibiotics. In a global perspective,

this is an increasing problem with high resistance rates in *E. coli* and other uropathogens to commonly available antibiotics, particularly in developing countries. In a meta-analysis of antimicrobial resistance in children 0–5 years with UTI caused by *E. coli*, the resistance rate to trimethoprim-sulfamethoxazole in OECD (Organization for Economic Cooperation and Development) countries was 30% and in non-OECD countries 71%. The corresponding figures for amoxicillin-clavulanic acid were 10% and 72%, respectively [32]. Resistance patterns in Sweden and neighboring countries are still more favorable but extended spectrum beta-lactamase production (ESBL) is continually increasing, although from a low level [33-35]. Hence, due to antimicrobial resistance, the use of broad-spectrum antibiotics is often required, known to drive resistance even further. Increasing antimicrobial resistance is also a concern for the choice of first line antibiotics used for empirical treatment of UTIs as delayed effective treatment might imply a higher risk of kidney damage.

1.4 PREDISPOSING FACTORS OF UTI

1.4.1 CONGENITAL ANOMALIES

Infants with congenital anomalies of the kidneys and urinary tract (CAKUT) have an increased risk of UTI during early life [12, 36, 37]. Ureteropelvic junction obstruction, ureteric stenosis and posterior urethral valves will obstruct the normal flow of urine which facilitates for bacteria to persist and multiply proximal to the obstruction, sometimes causing complicated infections refractory to antibiotic treatment and the need of surgical drainage. Likewise, an ectopic ureter terminating distal to the bladder can be the cause of UTIs ascending to the kidney. With improved ultrasonography technique and antenatal screening programs it has become more common to identify major organ abnormalities in the fetus, sometimes even leading to interventions in utero. However, not all anomalies in the urinary tract are detected on antenatal screening and a UTI can be the presenting symptom of CAKUT identified on imaging performed as a consequence of the UTI.

VESICoureTERAL REFLUX

As mentioned above, VUR is a major predisposing factor for upper UTIs and is associated with recurrent UTIs [38, 39]. The prevalence of VUR in the population is not known but has been estimated at 1–2% [40]. This, however, has been questioned by several researchers and in infants, is likely to be much

higher [41, 42]. Moreover, the grade of reflux, defined by the grading system (Figure 3) proposed by Lebowitz et al. is of importance for the risk of UTIs [43]. Dilating VUR, where the flow of urine from the bladder reaches up to the kidney with increasing dilatation of the ureter and renal pelvis for each grade, has a higher risk of APN, increasing with VUR grade [44]. VUR, however, has a high chance of spontaneous resolution, in particular the lower grades [45]. In a population based Swedish study on 2309 children <2 years presenting with their first UTI, 1953 children were examined by VCUg of whom 30% had VUR [46]. The rate of dilating VUR among those who were examined by VCUg in this study was 18% in girls and 13% in boys. Spontaneous resolution or regression to non-dilating VUR (grade 1–2) was seen in more than 50% of the children, more often in boys than in girls, within a follow-up period of 4–6 years [44]. In this study, girls also had a higher frequency of recurrent febrile UTIs (54%) compared to boys (33%). This, in combination with the higher persistence rate of VUR, could make girls more vulnerable for new infections ascending to the kidneys.

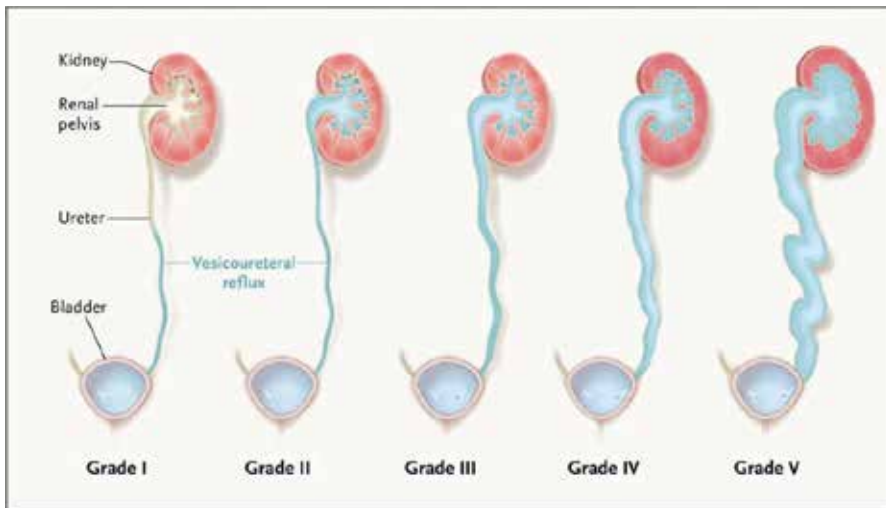


Figure 3 International classification of vesicoureteral reflux. Reproduced with permission from Montini G, Tullus K. N Engl J Med. 2011;365(3), Copyright Massachusetts Medical Society.

1.4.2 BOWEL AND BLADDER DYSFUNCTION

Bowel and bladder dysfunction has been shown to increase the risk of recurrent UTIs in older children [38, 47-49]. Dysfunctional bladder emptying is also associated with renal damage and persistent VUR [50]. Whether UTIs are

associated with abnormal bladder function also in infants is not entirely clear. In a study by Sjöström et al., infants with dilating VUR were examined with videocystometry at a mean age of 6 months and repeated at 20 and 40 months [51]. The majority of these infants had pyelonephritis as presenting symptom and half of them experienced a recurrent UTI during the follow-up period. At 6 months, the urodynamic pattern couldn't be distinguished from the normal pattern in infants of that age but at 20 months, the pattern was abnormal with primarily increased bladder capacity and residual volume. High residual volume at the 6-month investigation was a predictor of dilated bladder dysfunction at the 20 months follow-up. Abnormal bladder emptying is usually not evident for caregivers of infants and non-toilet trained children but for the doctor, asking about bowel function may be helpful since also infants can have constipation interfering with bladder emptying.

1.4.3 GENETIC SUSCEPTIBILITY

In recent years, it has become increasingly evident that a genetic predisposition plays an important role for UTI susceptibility. Family pedigree studies have shown that APN was significantly more common in relatives to children who had been treated for APN compared to family members of control subjects [52]. The interactions between the invading bacteria and the defense mechanisms of the host are central in the pathogenesis of UTI and for the severity of infection. The immune response that is triggered by bacterial invasion includes the production of chemokines by epithelial cells in the urinary tract, enhancing recruitment of neutrophils [53]. Toll like receptors (TLRs) play a crucial role in the recognition of uropathogenic bacteria and the initiation of innate immune response. TLR4 signaling triggered by bacterial recognition and upregulation of chemokines and receptors like CXCL8 (IL-8) and CXCR1, elicits a strong innate immune response with massive neutrophil recruitment that will contribute to bacterial clearance [54, 55]. Variations and polymorphisms in the coding genes for these important players in immune activation will affect the susceptibility and the course of the infection. Depending on how genetic variations affect the signaling pathways, innate immune dysregulation can either inhibit immune activation, favoring a state of ABU, or elicit an exaggerated immune response with destructive inflammation [56]. Polymorphisms in genes regulating transcription factors as the IRF3-IRF7 complex has been shown to dramatically alter the regulation of the innate immune response [57]. In murine models, knock-out mice with absence of functional IRF3-signaling developed severe urosepsis and kidney pathology with abscess formation and tissue damage during UPEC infection [58]. IRF3

promoter sequence variations affecting IRF3 expression was also linked to APN susceptibility in humans followed-up about 30 years after their first UTI in childhood [58]. Thus, it is apparent that APN susceptibility is not only influenced by anatomical abnormalities and bacterial virulence factors but also by genetic predisposition in the host.

Furthermore, inheritance patterns of VUR indicate that genetic components are important also for this condition which, in turn, is predisposing for UTI. The prevalence of VUR has been estimated at around 30% in siblings to patients with VUR and 35% in offspring to parents with VUR [59, 60]. Many of these children, however, will have low grade VUR with a high chance of resolution and most of them will not be affected by a UTI. There is growing evidence that embryonic developmental disturbances of the urinary tract, including VUR, have a genetic background [61-63]. Although many candidate genes associated with VUR have been investigated, no single gene locus has been identified and most researchers have concluded that VUR is a genetically heterogenous condition possibly influenced by environmental factors [40, 64, 65].

1.5 RENAL SCARRING

1.5.1 PATHOGENESIS

A major concern in young children with APN is the risk of developing permanent damage of the affected kidney. Numerous investigations have shown that new parenchymal defects can evolve following an episode of APN [2, 66-68]. Experimental animal studies and studies in children presenting with APN have shown that areas of inflammation identified by acute renal cortical scintigraphy in some individuals resolve completely at follow-up but in some develop into permanently scarred tissue at the site of the acute infection [5, 69, 70]. The frequency of permanent kidney damage identified by ^{99m}Tc-dimercaptosuccinic acid scintigraphy (DMSA) following the first UTI in young children is reported in several studies to be around 10-26%, but in some populations as high as 49% [66, 71-73]. Furthermore, several studies have shown that recurrent UTIs are associated with new renal damage, particularly in girls [74-78]. However, many of the studies reporting the prevalence of kidney damage detected on investigations performed after an initial UTI cannot differentiate between acquired renal scarring caused by the infection and preexisting kidney defects of congenital origin. Since renal scintigraphy is rarely performed in healthy children, unless prenatal ultrasound screening or

imaging of the kidneys for other reason have shown anomalies, the prevalence of congenital kidney damage is not known.

High grade VUR is associated with congenital kidney abnormalities including hypoplasia but also with focal dysplasia [40, 60]. The exact pathogenetic mechanisms behind reflux associated dysplasia are not fully understood but the phenomenon of congenital focal dysplasia has an important clinical implication. While dysplasia is a histopathological diagnosis, focal uptake defects on DMSA scintigraphy caused by infection can be indistinguishable from the picture seen in focal dysplasia, making many clinical studies difficult to interpret [74, 79, 80]. Moreover, there is a gender difference with boys more often being identified with high grade VUR and congenital kidney abnormalities during early infancy while girls have a stronger tendency to acquired kidney damage following UTIs later in childhood [6, 72, 75]. Nevertheless, pyelonephritis can result in new kidney scarring regardless of VUR or preexisting kidney defects and should therefore be promptly diagnosed and treated [81].

1.5.2 GENETIC INFLUENCE

It is reasonable to believe that the severity of infection and extent of inflammation in the acute infection is decisive for the evolution of renal scarring following APN. Yet, in most individuals, the inflammation subsides without apparent residual injury, while others develop persistent scarring and sometimes experience progression over time [68]. The genetic influence on the scarring process following APN has been studied but is far from completely understood. Furthermore, the interplay between congenital and acquired scarring makes genetic studies on patient cohorts with heterogeneous phenotypes complex and difficult to interpret [82]. Specifically, genes involved in the inflammatory response, tissue repair, and fibrosis, such as TLR4, angiotensin-converting enzyme (ACE) and transforming growth factor-beta 1 (TGF- β 1), have been suggested to play important roles in the scarring process [83-87]. Children with variations in TLR4 signaling have an increased risk of severe UTIs and prolonged inflammation, which could enhance the risk of renal scarring [88]. Insertion/deletion polymorphisms that influence ACE activity have been linked to increased risk of kidney damage and fibrosis, thought to be mediated by vasoconstriction and increased intraglomerular pressure inducing glomerular sclerosis [89]. TGF- β 1 regulates tissue repair and plays a central role in promoting extracellular matrix deposition, such as collagens, activating myofibroblasts which contribute to fibrosis [87]. None of

the regulating genes alone has been shown to explain the development of renal scarring after UTI but it is plausible that an individual genetic profile has an influence on the susceptibility of scarring.

In a recent study, a cohort of children in Singapore and a subset of infants from the Swedish infant UTI study were investigated regarding the molecular and genetic basis of disease severity in APN [90]. Gene expression analysis showed an immune imbalance with hyper-activation of innate immunity, in particular neutrophil degranulation pathway genes, and attenuation of the adaptive immune system in children with renal involvement on DMSA scintigraphy in the acute phase of infection. Furthermore, deoxyribonucleic acid (DNA) analysis showed distinct genetic profiles separating children with renal involvement on acute DMSA scans from children with febrile UTIs without DMSA abnormalities. When comparing gene expression profiles with outcome on follow-up DMSA scans, genes regulated in the acute phase, were not associated with renal scarring, nor was there evidence of a persisting hyper-inflammatory response in patients who developed renal scarring [90]. However, DNA analysis identified gene profiles specifically associated with renal scarring that could discriminate children with permanent kidney damage from those with resolved defects on the late DMSA scan with a near 100% accuracy. The DNA profile associated with acute renal involvement differed from the profile linked to renal scarring and there was no evidence that the most elevated acute response parameters predicted renal scarring. These findings suggest that there are other mechanisms than exaggerated acute inflammation or lasting inflammation that influence the evolution of scarring after pyelonephritis. It is also possible that genetic predisposition and environmental factors interact and could affect multiple processes as embryonic development of the urinary tract, immune response in the acute phase of infection and tissue repair [83]. Further research in this field is essential as many aspects of genetic influence on renal scarring are still unexplored. Identifying children with high-risk genetic profiles could help in the early detection of those at risk for scarring, allowing for more targeted interventions and follow-up to improve long-term kidney health. The different components affecting renal scarring are illustrated in Figure 4.

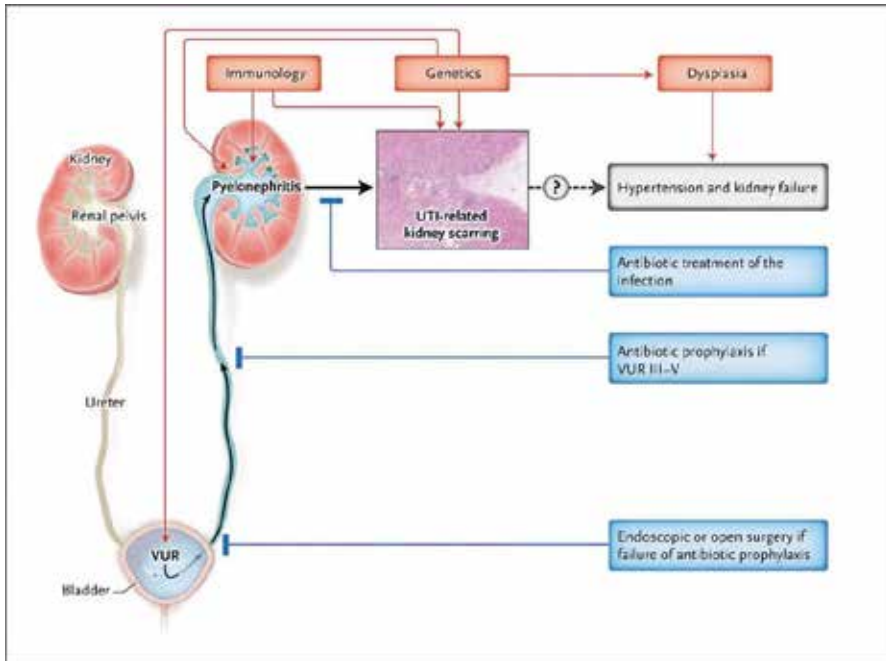


Figure 4 Illustration of components affecting UTI-related renal scarring. Reproduced with permission from Montini G, Tullus K. *N Engl J Med.* 2011;365(3), Copyright Massachusetts Medical Society.

1.5.3 CONSEQUENCES OF UTI AND SCARRING

In the short term, the consequences of a UTI are mostly related to the clinical condition and age of the child at infection. Children presenting with signs of sepsis, dehydration, vomiting or other symptoms of severe infection need inpatient care with close surveillance, intravenous fluid and antibiotic therapy and monitoring with repeated blood sampling. Potential serious complications include fluid and electrolyte disturbances, bacteremia and meningitis, particularly in the very young infants [9, 91-93]. Children who are unresponsive to antibiotic treatment with continuing fever should be evaluated for obstruction in the urinary tract and might need acute surgical drainage although these children are few [94, 95]. The majority of children with UTI, however, are in good clinical condition and respond to antibiotic treatment within a few days [95].

The pathogenetic mechanisms potentially inducing progressive damage in scarred or hypoplastic kidneys is a slow process thought to be caused by

hyperfiltration in overloaded remaining nephrons accompanied by structural lesions, a theory supported by experimental studies by Brenner et al. [96]. This process continues for decades making long-term consequences of kidney injury in infancy difficult to explore. Acknowledging that APN is sometimes followed by permanent kidney damage and that congenital kidney damage is sometimes identified on investigations conducted as part of a UTI work-up, this can have implications for kidney health later in life. The main concerns have been impaired kidney function, hypertension and complications during pregnancy [12]. There are few long-term investigations addressing these important issues and some reflect the outcome in children treated for UTI in a different era when missed infections or delayed treatment was more common than nowadays. Furthermore, renal scarring diagnosed by urography does not equate to scarring seen on DMSA scintigraphy which will detect also minor defects.

A Swedish study by Wennerström et al. on children diagnosed with renal scarring by urography after their first UTI in the 1970s and followed up 16-26 years later concluded that the kidney function was generally well preserved but children with bilateral scarring had a worse prognosis and among those with unilateral scarring, a significant deterioration in function of the affected kidney was seen [97]. Another Swedish cohort comparing females with and without renal scarring following their first childhood UTI was studied at a median age of 27 years by Martinell et al. and again at 41 years by Gebäck et al. [98, 99]. These studies also confirmed that kidney function, even in those with severe scarring, generally was well maintained but women with bilateral kidney damage had a significant decrease in glomerular filtration rate from 93 to 81 mL/min/1.73 m² between the two timepoints of follow-up. A large Israeli registry study investigating 1.5 million persons examined for military service found a risk of 1.4% for end stage kidney disease (ESKD) among those with a history of pyelonephritis compared to 0.2% in those without pyelonephritis and that renal scarring and decreased kidney function at enrolment were predictors of worse outcome [100]. The authors emphasized that ESKD only represents a small fraction of chronic kidney disease, suggesting that children with pyelonephritis could benefit from evaluation of kidney scarring.

In the cohort followed by Wennerström et al., no increased risk of hypertension was seen when compared to matched controls without renal scarring, [101] whereas women with renal scarring in the Martinell and Gebäck cohort had an increased risk of hypertension (38%) compared to those without scarring (14%) at the later follow-up [102]. A literature review by Toffolo et al. on

clinical consequences following childhood UTI summarized that the risk of hypertension later in life is low and usually associated with kidney damage at start [103].

The risk of complications during pregnancy is increased in women with pronounced renal scarring and decreased kidney function or poor blood pressure control [104, 105]. However, the risks are low in women with minor or moderate scarring with preserved kidney function and normal blood pressure, comprising the majority of women in these cohorts. This was also the conclusion in the review by Toffolo et al. [103]. On the other hand, a meta-analysis of reports and case series estimating the risk of pregnancy-related morbidity in women with reflux related nephropathy found a tenfold increased risk of hypertensive disorders including late maternal preeclampsia [106].

To summarize, most authors agree that the risk for serious sequelae caused by acquired kidney scarring following UTI is low in countries with advanced healthcare, although it is important to identify signs of chronic kidney disease in order to prevent further disease progression in this patient group.

1.6 MANAGEMENT OF UTI IN INFANTS

1.6.1 DIAGNOSIS

Important questions to include when evaluating young children with a suspicion of UTI are antenatally diagnosed kidney abnormalities, a family history of VUR or kidney disease, constipation and poor urine flow. In the preverbal child, symptoms like dysuria and loin pain are not expressed. In infants, the presenting symptom of UTIs is usually fever without localized symptoms or signs. The physical examination should include inspection of the genitals and back to look for overt malformations and palpation of the abdomen to identify an enlarged bladder or abdominal mass. However, the history and physical examination in infants usually only provide clues indicating the possibility of a UTI and the diagnosis relies heavily on laboratory investigations.

Definitions of UTI and required diagnostic procedures for a UTI diagnosis vary between different authors and guidelines. All guidelines discourage the use of nappy pads or perineal plastic bags as urine collection method for culture due to a high contamination risk of up to 50–60% [107]. Most guidelines accept urine collection by clean-catch as an approved method acknowledging a

contamination risk of 16-26% [108, 109]. The National Institute for Health and Care Excellence (NICE) guidelines, however, accept the use of nappy pads if clean-catch is not possible while the American Academy of Pediatrics (AAP) guidelines require urine sampling by suprapubic aspiration (SPA) or catheter to establish a diagnosis of UTI [110, 111]. Blood culture and urine sampling by SPA or catheter are often motivated for children in poor clinical condition where the initiation of antibiotics is urgent and intravenous therapy is warranted.

The presence of leukocytes in urine, pyuria, is supportive of UTI, but unspecific, and is not uncommon in febrile infections other than UTI [112]. Pyuria can also be present in other inflammatory conditions in the kidneys than infection. Pyuria is usually assessed by urine dipstick tests for leukocyte esterase (U-Le) in Swedish pediatric services. The sensitivity and specificity for a U-Le test to identify bacteriuria is estimated at 83% and 78% respectively [113]. Urine dipsticks have been shown to be an adequate screening test for UTI also in the very young infants [114, 115]. The urine nitrite test has a specificity of close to 100% for the presence of bacteria but the sensitivity is low at about 25–50% in children, making it useful to diagnose bacteriuria when the test is positive but less useful as a screening tool for UTI [111, 113, 116].

Different guidelines propose divergent thresholds for bacterial colony counts necessary for a UTI diagnosis. The AAP guidelines are the most rigorous, requiring both a positive leukocyte test and the presence of at least 5×10^4 colony forming units (CFU)/mL of a single uropathogenic bacterium in urine collected by SPA or catheter [111]. Different cut-off levels of colony numbers for different urine sampling methods are proposed in various guidelines but these are based on contamination risks rather than biological circumstances making such requirements unsuitable for diagnostic decision-making in clinical practice [117]. Several studies have challenged the requirement of a high bacterial number needed for a diagnosis of UTI, particularly in infants who empty the bladder at random intervals, thus impeding the assessment of bladder incubation time [18, 118, 119]. The requirement of pyuria for establishing a UTI diagnosis has also been disputed and notably, the absence of leukocytes is associated with UTIs caused by non-*E. coli* bacteria [17, 120, 121]. When diagnosing a UTI, the clinician must also be aware of the possibility of a child having ABU presenting with fever caused by another infection, usually virus.

Inflammatory markers in blood are used to help differentiating between upper and lower UTI but are non-specific and have a limited role in the diagnostic process. A Cochrane review on this matter concluded that a low C-reactive protein (CRP), <20 mg/dL, is helpful in ruling out pyelonephritis while procalcitonin seems to be a better marker for identifying pyelonephritis [122]. CRP has also been studied as a risk factor in prediction models to identify children with increased risk of permanent kidney damage [20, 72, 123]. Plasma creatinine is not a diagnostic marker of UTI but can be useful for identifying children with severe infection, concurrent significant dehydration, or preexisting renal impairment suggesting a more meticulous management.

1.6.2 TREATMENT

The local antibiotic sensitivity patterns of common bacteria, in particular for *E. coli*, should be considered in the initial empiric treatment of UTIs. Most guidelines recommend 7–10 days of antibiotic treatment for febrile UTIs and point out that clinical improvement is usually seen within 2–3 days. Initial intravenous antibiotic treatment is often recommended for the youngest infants, below two months of age, due to an increased risk of sepsis and meningitis in this age group and a higher probability of undiagnosed malformations in the urinary tract. If clinical improvement after a few days of parenteral treatment is seen as expected and the antibiogram report shows sensitivity to an appropriate oral alternative, the course can generally be completed by the oral route. It has been proposed that children aged one month or more in good clinical condition and able to retain oral medication can be treated with empiric oral antibiotics from start and that this is equally safe and effective as initial intravenous treatment [124-126].

Antibiotic treatment should be initiated without delay, preferably within 72 hours from the onset of fever, after collecting urine for culture when pyelonephritis is suspected. Several studies have shown that the risk of renal scarring increases when effective therapy is delayed [127-129]. Other investigators, however, could not find an association between delayed treatment and renal scarring [130, 131]. Nonetheless, early initiation of therapy will relieve symptoms of infection and shorten the time of malady for the child.

The value of prophylactic antibiotic treatment is more controversial and conclusions from clinical studies are conflicting. A Cochrane review from 2019 concluded that continuous antibiotic prophylaxis (CAP) may reduce the risk of recurrent UTIs but the benefit seems small [132]. This review also pointed out an increased risk of antimicrobial resistance with the use of CAP.

Another comprehensive review found insufficient evidence of prophylaxis to prevent new kidney scarring in children with any grade of VUR and does not recommend a routine use of CAP [133]. The Swedish Reflux Trial, however, showed a significant protective effect on new scarring in girls with VUR grade 3–4 when comparing CAP to a surveillance group [75].

1.6.3 IMAGING

ULTRASONOGRAPHY

Recommendations on imaging after a first episode of UTI in infants and young children vary considerably among international guidelines. Most guidelines recommend RBUS examinations in all infants with febrile UTIs to identify major anatomical abnormalities of the kidneys and urinary tract. Ultrasonography has the advantages of being easily available, relatively inexpensive, non-invasive and causes no radiation exposure to the child. RBUS has a reasonable ability to detect significant renal hypoplasia, obstructive uropathies, kidney abscesses, duplication of the collecting system and bladder anomalies [134]. It is also useful for acute imaging in children who fail to improve on antibiotic treatment to identify conditions that might require surgical drainage. RBUS, however, has poor ability to predict renal parenchymal damage [135, 136], and a normal kidney on ultrasonography does not exclude high grade VUR [71, 137, 138]. The utility of a routine RBUS examination after the first UTI has been questioned due to a lack of evidence showing that the detection of structural abnormalities will alter the management or lead to long-term benefits for the patient [135, 139]. A recent systematic review concluded that 1 in 4 to 5 children examined with RBUS after the first UTI will have an abnormality detected and only 1 in 32 will have an abnormality that changes the clinical management [94]. This, however, is contradicted by other researchers illustrating the lack of solid evidence [140]. In particular, the utility of routine RBUS investigation in children with a first UTI has been challenged since many abnormalities are detected on prenatal ultrasonography [141]. Furthermore, concerns regarding overdiagnosis of minor abnormalities leading to unnecessary investigations have been raised [142].

VCUG

The major relevance of VCUG is to detect VUR and classify VUR grade. It is also used to diagnose posterior urethral valves in boys and can identify, for example, ureteroceles and bladder anomalies. Most guidelines recommend

VCUG only in infants and young children considered at increased risk of having significant abnormalities in the urinary tract. These risk factors vary between guidelines. The AAP guidelines recommend VCUG in children 2–24 months if RBUS reveals hydronephrosis or other major abnormalities and in the event of recurrent febrile UTIs while the NICE guidelines propose VCUG for infants younger than 6 months with atypical UTI (i.e. poor urine flow, sepsis, non- *E. coli* infection) or recurrent UTIs and to be considered for children up to 3 years of age with atypical UTI [110, 111]. The Italian guidelines suggest VCUG for children 2–36 months of age with first time UTI caused by non-*E. coli* bacteria or with recurrent febrile UTIs and provide a list of abnormalities on RBUS, other than isolated dilatation of the renal pelvis, that should warrant imaging with VCUG [143]. Important drawbacks of a VCUG are that it is invasive and unpleasant for the child, commonly requires sedation, impose radiation and has a risk of inducing an infection. Moreover, with the limited benefits of surgical procedures and prophylactic antibiotic treatment to prevent further kidney damage, VUR management has changed and the rationale for identifying VUR has diminished [144]. The dynamic nature of reflux and interobserver variability among radiologists in the grading of VUR must also be considered when making clinical decisions on VCUG findings.

DMSA SCINTIGRAPHY

DMSA scintigraphy is the gold standard for detecting acute kidney involvement in UTIs and, if performed 4–6 months or more after the UTI, for identifying residual kidney defects [144, 145]. The timing of the scan in relation to the UTI is crucial for the interpretation of the result since some uptake defects will change and resolve over time. Differentiating between defects related to acute inflammation, preexisting defects and acquired scarring is difficult and sometimes impossible [146-148]. An acute DMSA scan can be helpful in febrile children with ambiguous signs of UTI or when a confirmative UTI diagnose is impossible due to the administration of antibiotics before collecting urine for culture. A late DMSA scan, performed to identify children with permanent bilateral kidney defects or severe unilateral scarring, can be useful for deciding on whom to offer long term follow-up while the value of detecting mild and moderate defects is more questionable. However, the precise risk of long term morbidity related to degree of kidney damage on DMSA scintigraphy is not known. Figure 5 shows examples of different grades of unilateral damage.

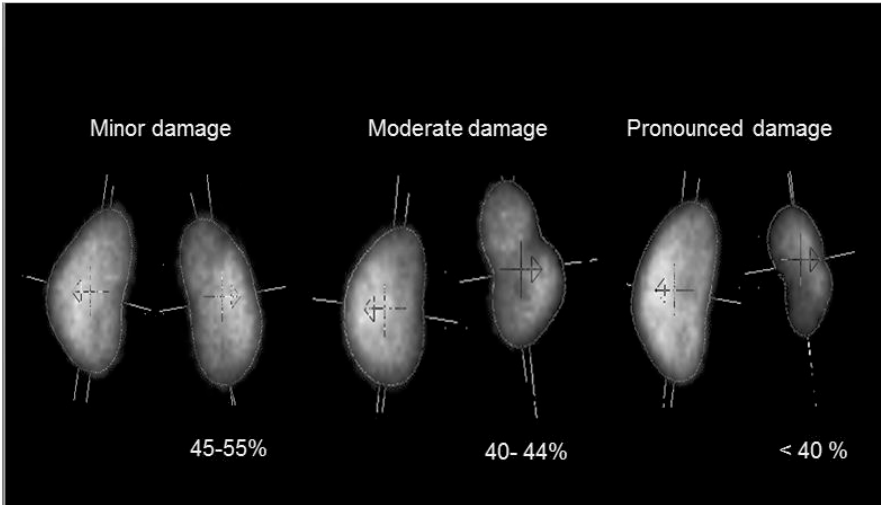


Figure 5 Example of grades of kidney damage on DMSA scintigraphy. With permission from Swerkersson S. (2016). Urinary tract infection in small children: aspects of bacteriology, vesicoureteral reflux and renal damage. PhD thesis. University of Gothenburg.

The radiation dose of a DMSA scan is approximately 0.7–1 mSv and it requires intravenous access and sometimes sedation [149]. It is also a relatively expensive and time-consuming procedure and the availability of a nuclear medicine service might be a limiting factor for its use. Opinions on the purpose and value of DMSA scintigraphy as part of the follow-up after a first UTI are divergent. Several guidelines propose to perform a late DMSA scan in selected patients, such as children with high grade VUR, atypical infection, recurrent febrile UTIs, decreased renal function or other imaging indicating major kidney abnormalities [95, 110, 134, 143, 150, 151]. In contrast, DMSA scintigraphy is not considered as part of the evaluation of children with first time UTI in the AAP guidelines.

1.7 UTI GUIDELINES

There are several published pediatric UTI guidelines around the world but so far, no universal recommendations on the management and follow-up have been accepted [95, 110, 111, 143, 152]. Differences exist in recommended diagnostic evaluation, treatment, as well as follow-up investigations indicating some missing evidence for best practice, but disparities can also partly be explained by differences in healthcare systems and populations. The Swedish

guidelines were launched via the Swedish Society for Pediatric Nephrology website in 2013 and were published in 2021 along with a mini review comparing them to Canadian, American and European guidelines [153]. After this review, several updated and additional guidelines have been published [95, 134, 150, 151, 154]. Common for all guidelines published during the past two decades is the ambition to ease the burden of invasive procedures for children with UTI and restrict imaging with ionizing radiation. This was also a major purpose when the Swedish guidelines were introduced along with the aim to harmonize UTI management in the country [153].

THE SWEDISH UTI GUIDELINES

The Swedish UTI guidelines for children <2 years of age propose that urine for culture is collected by clean-catch technique, catheter or SPA (preferred method in infants <1 year), when a UTI is suspected. Recommended laboratory tests are urine dipstick tests for nitrite and U-Le and blood tests for CRP and creatinine, both used for risk assessment. A positive U-Le test is supportive but not designative of UTI while a negative test makes UTI less likely. No specific limit for bacterial numbers in urine culture is stated for a diagnose of UTI.

Empiric antibiotic treatment with intravenous cefotaxime is recommended for children in poor general condition or unable to tolerate oral medication. An aminoglycoside can be added in severely ill patients. Oral empiric treatment with a third-generation cephalosporine is recommended for children in good clinical condition. This may be switched to trimethoprim-sulfamethoxazole when the antibiotic susceptibility report is received. Indications for prophylactic antibiotic treatment is also provided in the guidelines. The main diagnostic and treatment recommendations are listed in Table 1.

Table 1 Summary of diagnostic and treatment recommendations in the Swedish UTI guidelines for children <2 years of age.

Guideline recommendations	
Diagnostic evaluation	Record body temperature
	Urine dipstick test for leukocytes and nitrite
	Urine culture
	Urine collection by SPA, catheter or clean catch
	Plasma CRP for risk stratification
	Plasma creatinine for risk stratification
Treatment	Blood culture if intravenous antibiotics are planned
	Intravenous cefotaxime in critically ill children or unable to retain oral fluid (empiric treatment)
	Oral 3 rd generation cephalosporine in well-appearing children (empiric treatment)
	Oral trimethoprim/sulfamethoxazole option after bacterial sensitivity report
	Antimicrobial prophylaxis indications: -VUR grade 3–5 -recurrent febrile UTIs -to be considered if dilatation on RBUS while awaiting VCUG

The imaging guideline for children <2 years of age recommends RBUS examination in all patients with UTI. The algorithm for further imaging represents a top-down approach using DMSA scintigraphy to identify renal involvement in the acute phase of infection in a selected group of patients considered at increased risk of severe infection or having anomalies of the urinary tract, shown in Figure 6. The risk factors include CRP ≥ 70 mg/L, non-*E. coli* infection and decreased renal function. VCUG is restricted to those with dilatation of the renal pelvis on RBUS or when a DMSA scan shows a relative function <45%. In children without risk factors but who experience a recurrent febrile UTI, an early DMSA scan is recommended in the same manner. A follow-up DMSA scan 6–12 months after the UTI is recommended to patients with moderate or severe defects on the initial scan and to patients with dilating VUR to identify permanent kidney damage.

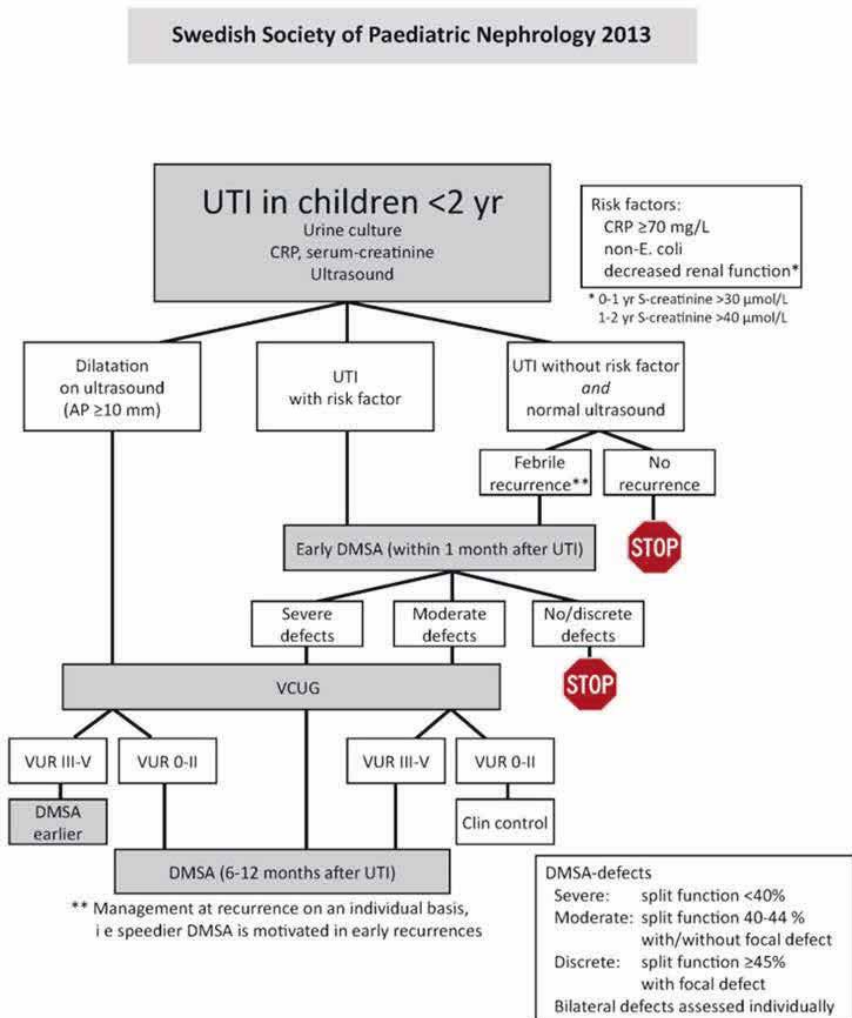


Figure 6 Swedish UTI guideline algorithm depicting imaging recommendations for children <2 years of age. Brandström P, Lindén M. Acta Paediatr. 2021;110(6). Open access.

This imaging strategy is based on studies showing that children with a normal DMSA scan are unlikely to have dilating VUR, thus making the investigation by VCUg unnecessary [155, 156]. This has also been confirmed by others [157, 158]. However, other investigators have found that this strategy could miss about a third of children with dilating VUR [159, 160].

The Swedish guidelines also include an alternative imaging algorithm for centers with limited access to DMSA scintigraphy which recommends only a late DMSA scan in children with risk factors. This algorithm reflects a bottom-up approach where children with risk factors first are investigated with VCUg to identify dilating VUR.

1.8 ADHERENCE TO GUIDELINES

Clinical practice guidelines can be defined as “systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances” [161]. In order to optimize health outcome and add value for the patient, guidelines have to be based on scientific evidence or at least firmly established clinical experience. Furthermore, the guidelines have to be implemented thoroughly in the health care systems they are intended to support in order to achieve a reasonable level of adherence to the recommendations among practitioners. At worst, however, clinical guidelines can cause more harm than good since solid scientific evidence is often lacking and expert opinions can be ineffective or even harmful [162]. Moreover, the generalizability of actions and interventions that have proved to be useful in research settings may be poor when it comes to implementation in every day clinical practice. The barriers of implementation of clinical guidelines are numerous and include financial issues, lack of learning culture, lack of skills, complexity of the guidelines, organizational resistance and lack of motivation among practitioners [163-165]. Some proposed strategies to overcome these barriers of implementation are educational meetings, training programs, repeated reminders, keeping the guidelines simple and providing easy access [164, 166]. However, there are no universal strategies for a successful implementation, thus it is important to audit practice patterns and adherence to recommendations after the implementation of clinical guidelines to evaluate their effectiveness [166, 167].

There are relatively few published studies on adherence to pediatric UTI guidelines and most of these have shown significant shortcomings. A Dutch single center study evaluating adherence to diagnostic recommendations for UTI before and after the implementation of a UTI guideline showed significant improvement after the implementation, yet the adherence rate was only 46.7% after the introduction of the guidelines [167]. In an American survey, with mainly pediatricians among responders, regarding which urine collection method to use for diagnosis of UTI in non-toilet trained children <2 years of age, 28% answered that they preferred bag specimens, contrary to the AAP

recommendations [168]. An Italian survey among 121 emergency units showed that almost 80% of the units used perineal bag specimens for urine culture in children <3 years of age which is not recommended in their guidelines [169]. In a large study in the USA on children <2 years of age treated for UTI, 32% did not have urinalysis or urine culture performed before initiating treatment, and a retrospective study in the UK showed that only 28% of children <3 years of age with unexplained fever had a urine sample tested according to guideline recommendations [170, 171]. In the latter study, adherence to performing renal investigations on children with confirmed UTI in accordance with the NICE guidelines was only 45%.

Similarly, adherence to guideline recommendations on imaging work-up following first time UTI studied at a teaching hospital in the USA showed an adherence rate of 61% [172]. In the aforementioned Italian survey, 60% of the units answered that they routinely performed RBUS after a first episode of febrile UTI according to the guidelines [169]. These studies indicate poor adherence to UTI guidelines in several aspects, however, there are no established optimal levels of adherence and the recommendations are not intended to be used as mandatory instructions. Recommendations that are evidence based on a group level may be inappropriate for the individual patient motivating non-adherence in some circumstances [162]. Furthermore, patients' preferences and needs must be considered in shared decision-making on the management and these might interfere with guideline recommendations.

The Swedish pediatric UTI guidelines were introduced in 2013 and adopted at all pediatric hospitals in the country with only one hospital applying minor adaptations of the guidelines. Neither management of UTIs nor adherence to the guidelines have been studied and evaluated after the implementation. Several international UTI guidelines have been published or updated after 2013, of which the majority have further reduced imaging recommendations after the first UTI compared to the Swedish guidelines. Therefore, it has become essential to audit current UTI management and adherence to the guidelines in Sweden. We have chosen to do this in infants <1 year of age as these patients are almost exclusively managed in pediatric specialist care. In the evaluation of current management of UTIs, a quality assurance project on UTIs in children <2 years of age in the mid-1990s was available for comparison [46].

2 AIMS

The overall aim of this thesis was to evaluate current management of infants with their first UTI, adherence to the Swedish UTI guidelines, and to investigate predictive clinical and genetic markers of permanent kidney damage associated with UTI.

Specific aims

Paper I

To evaluate current management of first time UTIs in infants by investigating the basis of diagnosis, bacterial findings and antimicrobial resistance, choice of empiric antibiotic treatment, use of antimicrobial prophylaxis and performed renal imaging during follow-up. Additional aims were to determine the detection rates of dilating VUR and permanent kidney damage in current practice and compare these with corresponding rates in previous studies.

Paper II

To evaluate adherence to the Swedish pediatric UTI guidelines in a population-based cohort of infants presenting with their first UTI with respect to key recommendations on diagnostic procedures, treatment and imaging. Secondary aims were to compare outcome on VCUG and renal scintigraphy performed according to recommendations with findings on imaging performed without guideline indication and to identify predictors of non-adherence.

Paper III

To identify genetic host factors associated with renal scarring defined by persisting kidney defects on DMSA scintigraphy 6 months or more after the first febrile UTI in infants. Additional aims were to assess genetic markers predisposing to dilating VUR and recurrent UTIs.

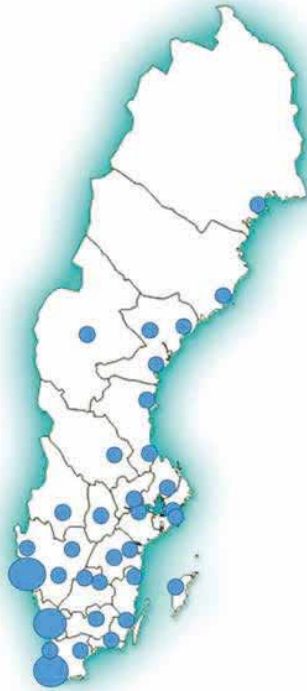
3 PATIENTS AND METHODS

3.1 PATIENT RECRUITEMENT

In a broad population based collaborating project between clinicians and scientists, we aimed at recruiting a large cohort suitable for both clinical and molecular studies on UTI. With the foundation of an existing nationwide collaborating network of pediatricians and members of the Swedish pediatric nephrology association we invited all pediatric hospital departments in Sweden to participate in patient recruitment for studies on infants with a first episode of UTI. Of 33 invited centers, 29 accepted participation covering all geographic areas of the country. A local collaborating investigator was appointed at each center. They attended meetings with the principal investigators and a coordinating study nurse on two occasions before study start to discuss the study protocol and coordinate uniform management in the inclusion process and data reporting within the study. This group also met on several occasions during the inclusion period to discuss issues that arose on the way.

Participating centers:

Borås, Eskilstuna, Falun, Gävle, Göteborg,
Halmstad, Helsingborg, Hudiksvall,
Jönköping, Kalmar, Karlskrona, Karlstad,
Kristianstad, Luleå, Lund/Malmö, Skövde,
Sollefteå, Stockholm/Karolinska sjukhuset,
Stockholm/Sachska Barnsjukhuset,
Sundsvall, Trollhättan, Umeå, Uppsala,
Visby, Västervik, Västerås, Växjö, Örebro
and Östersund.



3.2 PATIENT SELECTION

Infants below one year of age were enrolled in the study at pediatric hospitals and emergency units when antibiotic treatment for a suspected first episode of UTI was initiated. No other predetermined diagnostic criteria were required for inclusion, however, specific inclusion criteria were applied in the different studies as described below. Exclusion criteria were previous UTIs, myelomeningocele, overt urogenital malformations and ongoing bladder catheter therapy. If the initial clinical diagnosis of UTI was abandoned, the case was excluded. The nationwide inclusion was conducted from March 20, 2017 to September 30, 2019, comprising a cohort of 1357 infants studied in **Paper I** and **Paper II**. As part of a sub-study on genetic and molecular aspects of UTI, the three principal investigating centers, Göteborg, Halmstad and Malmö/Lund, continued patient recruitment until February 28, 2021, adding 57 infants eligible for inclusion in the study reported in **Paper III**.

In **Paper I** 1306 infants with UTI and growth of a single pathogen in urine culture were included independent of urine sampling method and bacterial numbers. Fifty-one infants with a clinical diagnosis of UTI but in whom the urine culture was missing, negative or showed mixed growth were analyzed separately for comparison with the study group. In **Paper II**, describing adherence to the clinical UTI guidelines, all 1357 infants in the nationwide cohort were included. A flow diagram of the nationwide inclusion process and study populations in **Paper I** and **Paper II** is shown in Figure 7.

In **Paper III**, additional inclusion criteria were applied including temperature $\geq 38.0^{\circ}\text{C}$ at the acute infection and a positive urine culture defined as any growth in urine from suprapubic aspiration, $\geq 10,000$ CFU/mL in catheterized urine or $\geq 100,000$ CFU/mL in a clean catch specimen. Furthermore, a blood sample for DNA analysis was required. These criteria were fulfilled in 1087 infants.

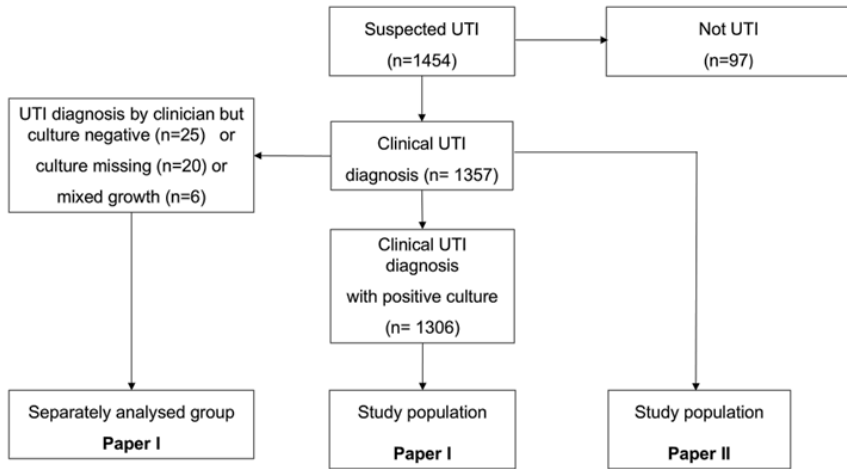


Figure 7 Flow diagram of study populations in *Paper I* and *Paper II*.

3.3 METHODS

In a survey before starting patient recruitment, the local investigators were asked about whether their center applied the national Swedish guidelines in the management of children with UTI or if other guidelines or local routines were used. They were instructed to continue their usual practice in the management of individuals included in the study. At inclusion, the caretakers were asked about consent to reporting pseudonymized clinical and laboratory data to a database and a separate consent for drawing a blood sample for DNA extraction and genetic analyses.

DATA COLLECTION

A case report form (CRF) was used to report clinical and laboratory data, antibiotic treatment and findings on any imaging of the kidneys and urinary tract. At inclusion, a first report was filed including data on maximum recorded temperature during the acute phase of infection, duration of fever (days) prior to treatment, circumcision status in boys, urine collection method, initial in- or outpatient care, initial empiric antibiotic treatment, administration mode, duration of treatment (days), and any change in antibiotic treatment. Furthermore, laboratory data at diagnosis were reported including maximum plasma CRP, plasma creatinine, urine dipstick tests for U-Le and nitrite,

bacterial species in urine cultures, number of colony forming units and any resistance to relevant antibiotics reported on the antibiogram. Findings on imaging of the kidneys and urinary tract by RBUS, VCUG, DMSA scintigraphy and ^{99m}Tc-mercaptoacetyltriglycine (MAG3) renography performed as part of initial investigations were also reported on the first CRF. The reports were compiled by the local investigator at each center and sent to the principal study center in Gothenburg.

One year after the initial UTI, a second report was completed by the local investigator with follow-up data including information on the use and duration of prophylactic antibiotics, recurrence of febrile UTIs and surgical interventions. Any additional imaging of the kidneys and urinary tract performed after the initial investigations were also registered in the second report. All CRFs were reviewed by the study nurse and, if questions arose, discussed with the investigator before data was transferred to the computer database.

LABORATORY TESTS

Urine dipstick tests, plasma CRP and creatinine were analyzed at the local laboratories. The U-Le dipstick test was graded 0–4 and pyuria defined as $\geq 1+$, corresponding to ≥ 15 cells/ μ L. Urine cultures and antibiotic susceptibility testing were performed by the local microbiology laboratories and the bacterial numbers in urine culture reported in intervals of $<10,000$, $10,000$ to $<100,000$ and $\geq 100,000$ CFU/mL.

In **Paper III**, heparinized peripheral blood was used for DNA extraction by the QIAamp DNA Blood mini kit and exome genotyping was performed by Illumina Infinium Exome bead chip technology.

IMAGING

Imaging investigations were performed at the local hospitals and assessed by radiologists at each center. Information in the radiology reports was transferred to the CRFs by the local investigators.

Renal and bladder ultrasonography

The purpose of ultrasound examination of the kidneys and urinary tract was mainly to identify major malformations and specifically to look for dilatation of the renal pelvis and/or ureters. The CRF included a check box for dilatation,

yes or no, and the anteroposterior diameter (APD) of the renal pelvis for right and left side, respectively. Other important findings were optional to report in a free text box on the CRF. Due to poor reporting of the APD, only absence or presence of dilatation was used in the analysis in **Paper I–II**.

Voiding cystourethrography

VCUG was conducted in selected patients, mainly infants with dilatation on RBUS or abnormalities identified on renal scintigraphy. The main purpose of a VCUG was to identify infants with VUR but also to diagnose posterior urethral valves in boys and anomalies of the bladder or collecting systems. VUR was graded 1–5 as proposed by the study group of the International Reflux Study in Children [43]. The maximum VUR grade was used in infants who underwent more than one VCUG during the study period. When bilateral VUR was detected, the side with the highest grade was used to characterize the patient. Other findings, such as urethral valves and duplicated collecting systems, were reported in free text.

Renal scintigraphy

DMSA scans were performed in patients with risk factors according to the Swedish UTI guidelines or on decision by the managing pediatrician. The performance and interpretation of DMSA scans at the participating hospitals were based on recommendations in the European Association of Nuclear Medicine guidelines [145]. In the acute phase of the UTI, DMSA scans were performed to detect kidney involvement of the infection and/or preexisting congenital anomalies of the kidneys. A follow-up scan performed six months or more after the index UTI was used to identify permanent kidney damage. The scans were assessed for uptake defects in the kidneys and for relative split function between the right and left sides. DMSA scans were defined as abnormal when a focal uptake defect was observed or when the relative function of a kidney was below 45%. In cases with bilateral uptake defects, the kidney with the lowest relative function was used to characterize the patient. Infants without abnormalities on a first DMSA scan and with no recurrence of febrile UTI were assumed to have normal kidneys during the follow-up period [71].

In **Paper I** and **Paper II**, some infants underwent a MAG3 scan instead of a DMSA scan and these were assessed using the same criteria as described

above, aware of the inferior image resolution on MAG3 and performance in detecting focal defects.

STUDY DESIGN

In **Paper I**, we described patient characteristics of the study population and the management of infants with their first UTI, at diagnosis and during the following year. The basis of diagnosis was described by presenting results from blood and urine tests, urine sampling methods, bacterial species and colony counts in urine cultures. Treatment management was depicted by the choice of initial empiric antibiotic treatment, administration mode, any change of antibiotics, treatment length and the use of prophylactic antibiotics. Findings on imaging of the urinary tract performed at initial investigations and during the follow-up period were presented and compared with data from previous studies. Recurrent febrile UTIs and any surgical interventions during follow-up were also reported. Group comparisons were performed between boys and girls, age groups, and for *E. coli* vs. non-*E.coli* infections. In a separate analysis, 51 infants without confirmative urine culture were compared to the study group.

In **Paper II**, adherence to guideline recommendations were analyzed in three domains: diagnostic evaluation, treatment and imaging. Dichotomous variables for each of the studied recommendations were created and defined as either adherent or non-adherent. Overall adherence rates to the diagnostic and imaging domains were calculated and defined as fulfillment of all underlying criteria in the respective domain. The variables used in the assessment of the diagnostic and treatment domains are listed in Table 2.

Table 2 Key guideline recommendations for diagnostic evaluation and treatment used to assess adherence in these domains.

Domain	Guideline recommendations
Diagnostic evaluation	Body temperature recorded
	Correct urine sampling method (SPA, catheter or clean catch)
	Urine dipstick test performed for U-Le and U-nitrite
	Urine culture performed
	CRP performed
	Creatinine performed
Treatment	Use of recommended antibiotics for initial empiric treatment: <ul style="list-style-type: none"> ▪ Cefotaxime for intravenously treated patients ▪ Third-generation oral cephalosporine for orally treated
	Antimicrobial prophylaxis in accordance with guideline recommendations: <ul style="list-style-type: none"> ▪ VUR grade 3–5 ▪ Recurrent febrile UTI ▪ To be considered in infants with dilatation on RBUS while awaiting VCUG

For the imaging domain, adherence to the recommendations in the four initial pathways of the guideline imaging algorithm were studied. Two additional recommendations downstream in the imaging algorithm were also analyzed but not included in the overall adherence in this domain, see Figure 8.

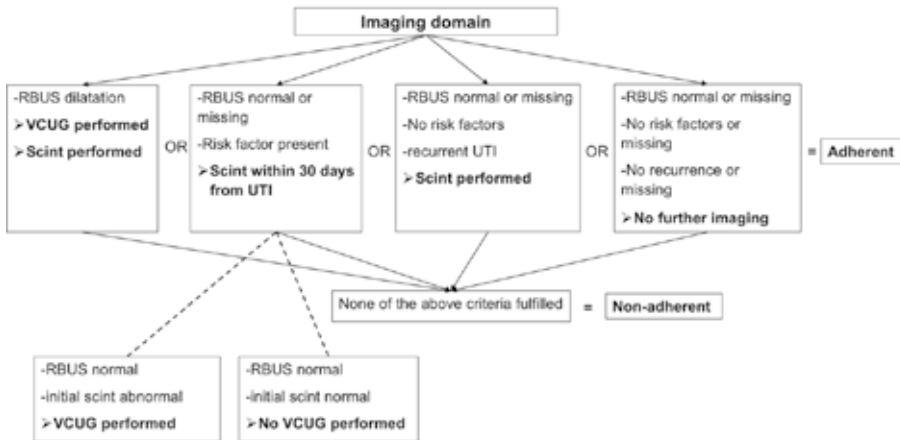


Figure 8 Model of the guideline algorithm, used to calculate overall adherence to the imaging domain by adherence to any one of four initial pathways. Arrows represent the recommended action if the above conditions are fulfilled.

To compare findings on imaging performed according to recommendations with imaging performed without guideline recommendation, we analyzed the frequency of dilating VUR on VCUGs and proportion of kidney damage detected by scintigraphy. The variables used to study predictors of non-adherence were gender, age at infection, outpatient management only and hospital category (university or county hospital).

In **Paper III**, APN was defined as focal uptake defects on DMSA scans performed within seven days from the UTI diagnosis. In this paper, the term renal scarring (RS) was used to define kidney damage detected on DMSA scans performed ≥ 6 months from the UTI episode as described above.

To study genetic susceptibility factors associated with RS, we performed gene association analyses comparing (1) infants with RS to infants with resolved APN at the follow-up DMSA scan and (2) infants with RS to infants with febrile UTI but no kidney involvement on the acute scan. The secondary aims, to explore genetic factors associated with dilating VUR and recurrent febrile UTIs, were assessed by gene association analyses comparing infants with (3) no VUR or VUR grade 1–2 to VUR grade 3–5 and (4) recurrent UTIs to no recurrences.

STATISTICAL ANALYSIS

In all papers, quantitative variables were presented using means and standard deviations for normally distributed variables and medians and interquartile range (IQR) for skewed variables. Categorical variables were presented with frequency and percent. In comparison between groups, the Fisher's exact test was used for dichotomous variables, the Chi²-test for non-ordered categorical variables and Mantel Haenszel Chi²-test for ordered categorical variables. For continuous variables with normal distribution, a two-sample t-test was used in comparison between groups and a Mann-Whitney U-test for skewed continuous variables.

In **Paper I**, risk factors for recurrent UTI were assessed using univariable and multivariable logistic regression. Univariable logistic regression was also used in **Paper II** when analyzing predictors of non-adherence to guideline recommendations. In these analyses, odds ratios (OR) with 95% CI and associated *p*-values were presented. All tests were 2-tailed and *p*-values < 0.05 were considered statistically significant.

In **Paper III**, the Kruskal-Wallis test was used for continuous or ordered categorical variables in comparison between more than two groups.

Gene association analysis for group comparisons was performed using allelic frequency test and OR calculations. For each single nucleotide polymorphism (SNP), OR-values and p -values were calculated using Fisher's exact test and variants with p -values <0.01 were considered significant. The SNP with the lowest p -value for each gene was selected as the representative SNP for this gene. Heatmaps were generated for visualization of the genes with a representative SNP with p -value <0.005 . Samples from each patient group were clustered-based on DMSA results (positive vs. negative), VUR status (no VUR/non-dilating VUR vs. dilating VUR) or UTI recurrence status (yes vs. no), respectively. Heatmaps were visualized by ordering the SNPs by increasing OR values in the columns with the highest OR on top. Principal component analysis (PCA) plots were used to show clusters of samples based on their similarity, illustrating the genetic closeness within a patient group and differences between the groups.

Ingenuity pathway analysis (IPA), with genes from the IPA Knowledge database as background reference, was used to explore presumably affected cell signaling pathways, major regulators and disease and toxicity functions associated with genes with significant SNPs. As a control, allele frequency in our study population was compared to the estimated prevalence of the SNPs in the general Swedish population retrieved from the SweGen database.

4 RESULTS

4.1 PAPER I

Patient characteristics were in line with previous population-based studies indicating a representative study group. Of 1306 infants, 601 (46%) were boys with a median age of 2.7 months and 705 (54%) were girls with a median age of 5.6 months. The age and gender distributions are shown in Figure 9.

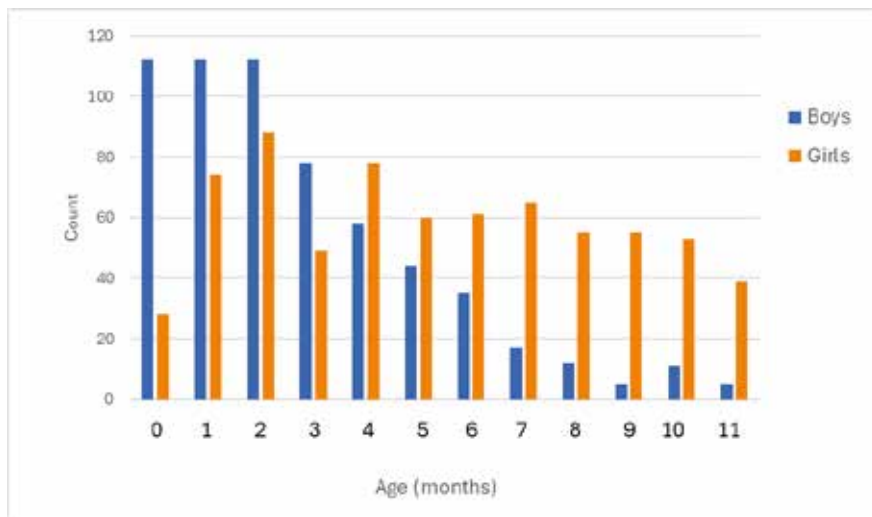


Figure 9 Age and gender distribution of 1306 infants at the time of their first UTI.

DIAGNOSIS

In the diagnostic evaluation, urine sampling was performed by clean catch technique in 92.5% of the cases, SPA in 5%, catheter in 1.6% and perineal collecting bags in less than 1%. The spectrum of bacterial species in urine cultures was in accordance with previous studies with *E. coli* in 90% of the isolates. Non-*E. coli* infections had lower rates of pyuria and positive U-nitrite compared to *E. coli* and were associated with lower CRP, higher rates of elevated creatinine and abnormalities on RBUS.

Resistance rates in *E. coli* isolates to commonly available antibiotics for oral treatment of UTI in this age group were for trimethoprim 22%, amoxicillin 8% and cefibuten 2%. ESBL was seen in 4% of the isolates.

TREATMENT

Intravenous antibiotic treatment was initiated in 37% of the infants and of these, cefotaxime was used in 97% for empiric treatment. The proportion of initial oral antibiotic treatment differed widely between centers; 82% and 84% of patients at the two largest centers and 54% (range 0–90%) in remaining centers. Among infants treated with oral antibiotics from start, ceftibuten or cefixime was prescribed to 87% while trimethoprim-sulfamethoxazole was used in 9% and amoxicillin-clavulanic acid in 1%. A change of antibiotic due to antimicrobial resistance was made in 2% of the cases. Among infants <3 months of age, 4% had a fever duration of 4 days or more before treatment was initiated while this was reported in 36% of infants 6– <12 months. There was no association between permanent kidney damage and fever duration prior to treatment, nor was there a difference in the rate of permanent kidney damage between infants with initial intravenous treatment and those with oral treatment from start.

IMAGING

RBUS was performed in 1300 of 1306 infants and revealed dilatation of the collecting system in 14% of the patients, more often in boys (19%) than in girls (9%). Other abnormalities and malformations of the kidneys were identified in 31 infants.

VCUG, performed in 390 (30%) of the infants, identified VUR in 147 (38%); VUR grade 1–2 in 37 (9%) and VUR grade 3–5 in 110 (28%). Notably, seven of 23 infants (30%) with VUR grade 5 had no dilatation on RBUS. Posterior urethral valves were found in seven infants.

DMSA scintigraphy was performed in 774 infants and a MAG3 scan in 62. At least one additional nuclear scan was performed in 213 infants during follow-up. Permanent kidney damage six months or more after the initial UTI was evaluated in 648 infants of which 126 (19%) were abnormal, representing a minimum rate of 10% in the total study population. Young age was associated with more severe kidney defects: 20 of 23 infants with relative function <30% were below 6 months of age at the time of infection. Boys had more severe kidney damage than girls (Figure 10).

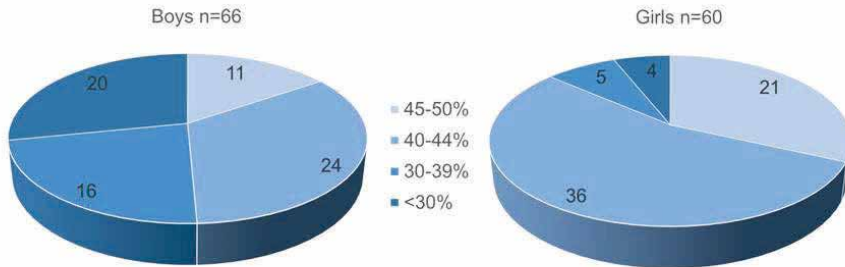


Figure 10 Relative function of the most affected kidney in 126 infants with permanent kidney damage. Bilateral damage was seen in 5 cases. Adapted from Lindén M, Rosenblad T et al. *Pediatr Nephrol.* 2024;39(11):3251–3262. Open access.

FOLLOW-UP

Eighteen percent of the infants experienced at least one recurrent febrile UTI during follow-up. Female gender and dilatation on RBUS were identified as independent risk factors for recurrence. Long-term (≥ 2 months) antimicrobial prophylaxis was prescribed to 16% of the study group. Surgical interventions by urologists were reported in 60 patients.

INFLUENCE OF AGE

The study population was dichotomized in two groups: 140 infants < 1 month and 1166 infants ≥ 1 month of age, to compare the influence of age. The younger group differed from the older with a marked preponderance of boys, less fever, lower rate of pyuria, higher rate of non-*E. coli* infection, higher creatinine and a higher rate of dilation on RBUS. Furthermore, 72% of infants in the younger group were initially treated intravenously compared to 35% in the older age group. A comparison between these age groups are shown in Table 3.

Table 3 Clinical, laboratory and imaging findings in 140 infants <1 month compared to 1166 infants ≥1 month of age.

	<1 month n=140	≥1 month n=1166	p-value
Male (%)	112 (80.0%)	489 (41.9%)	<0.001
Temperature ≥38°C	120/138 (87.0%)	1070/1132 (95.5%)	0.002
Urine sampling method			
Clean catch	106 (82.8%)	1044 (93.6%)	
SPA	15 (11.7%)	47 (4.2%)	
Catheter	3 (2.3%)	17 (1.5%)	
Bag	4 (3.1%)	7 (0.6%)	
	n=128	n=1115	
Nitrite positive	58/139 (41.7%)	441/1156 (38.1%)	0.408
U-Le positive (≥1+)	130 (92.9%)	1133/1157 (97.9%)	0.002
Non- <i>E. coli</i>	22 (15.7%)	115 (9.9%)	0.040
Bacterial count ≥100,000 CFU/mL	119/138 (86.2%)	939/1163 (80.7%)	0.133
CRP (mg/mL) median (IQR)	59.5 (24-102)	63 (28-110) n=1154	0.236
Creatinine >30 µmol/L	34/137 (24.8%)	45/1119 (4.0%)	<0.001
Intravenous antibiotics	101 (72.1%)	405/1165 (34.8%)	<0.001
Dilatation on RBUS	30 (21.4%)	150/1160 (12.9%)	<0.001
VUR grade 3–5	17/57 (29.8%)	93/333 (27.9%)	0.752
Abnormal scintigraphy 6 months after initial UTI	18/84 (21.4%)	108/564 (19.1%)	0.658

INFANTS WITHOUT CONFIRMATIVE URINE CULTURE

Twenty-five infants with negative urine cultures were, nevertheless, managed as UTI. These were severely ill and in most cases were given antibiotics before the urine culture was performed. They had a higher rate of positive U-nitrite (50%) compared to the study population (39%), indicating a high probability of bacteriuria. The culture negative group were younger, had a higher rate of elevated creatinine and dilatation on RBUS compared to the group with confirmed bacterial growth. Four of these infants underwent surgical interventions during follow-up. No significant differences were seen between the study population and 26 infants with missing urine culture or mixed bacterial growth yet managed as UTIs.

4.2 PAPER II

DIAGNOSTIC EVALUATION

Overall adherence to diagnostic recommendations was 86.1%. Urine sampling methods were applied in accordance with the guidelines in 99.0% of patients although SPA, which is recommended as the preferred method in infants <1 year, was performed in only 5%. Adherence rates to the other diagnostic recommendations, recording body temperature, urine dipstick tests for U-Le and U-nitrite, urine culture, plasma CRP and creatinine, were all above 96%.

TREATMENT

Adherence to initial empiric treatment with recommended antibiotics was 96.5% for infants initially treated intravenously and was 87.7% for infants initially treated with oral antibiotics. Trimethoprim-sulfamethoxazole was used as empiric treatment in 9.1% of infants with initial oral treatment, contrary to the recommendation to use this only when antibiotic susceptibility had been reported. Long term prophylactic antibiotics, used in 255 infants during follow-up, was initiated in accordance with guideline recommendations in 96.1% of these.

IMAGING

RBUS was performed in all but six infants (99.6%) as recommended by the guidelines. Six centers announced the use of an alternative imaging algorithm to the main guideline algorithm. Hence, 47 infants were excluded, leaving 1310 infants for further analysis in the imaging domain. Adherence rates to the four initial pathways of the imaging algorithm are shown in Figure 11. The recommendation to perform DMSA scintigraphy within one month from the first UTI in infants with risk factors but no dilatation on RBUS had the lowest adherence rate in this domain, 47.4%, and was lower in county hospitals, 41.9%, than in university hospitals, 58.3% ($p < 0.001$). The overall adherence rate to the imaging recommendations was 64.2%.

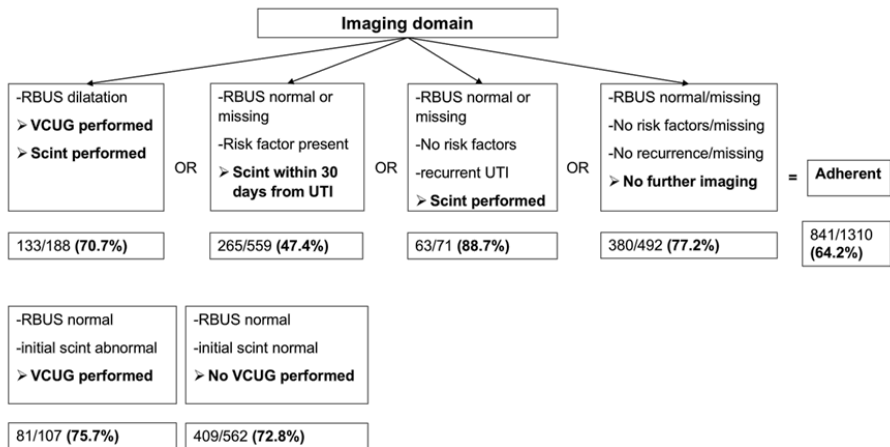


Figure 11 Overall adherence rate (64.2%) in the imaging domain and adherence rates to recommendations in the four initial pathways of the guideline algorithm. Adherence rates are also shown for two downstream recommendations.

IMAGING WITH AND WITHOUT GUIDELINE RECOMMENDATION

Among 396 infants investigated by VCUG, 178 underwent this procedure without guideline recommendation. Furthermore, of 492 infants without guideline indication for a DMSA scan, 107 (21.7%) were still investigated by renal scintigraphy (104 DMSA scans and 3 MAG3 scans). Data on permanent kidney injury 6 months or more after the first UTI was available in 95 of the 107 infants who were exposed to scintigraphy without guideline indication. The outcome of these investigations compared to findings on imaging performed according to recommendations in the guidelines are presented in Table 4. None of the kidneys examined without guideline recommendation had permanent defects with a relative function <40% while this was seen in 39 of 117 (33%) infants with scarred kidneys that were investigated according to guidelines.

Regardless of whether VCUG was performed with or without guideline recommendation, VUR grade 3–5 was more prevalent among infants who experienced a UTI recurrence (30 of 90) than in those without UTI recurrence (7 of 88, $p < 0.001$).

Table 4 VUR status (n=396) and findings on renal scintigraphy (n=842) performed with and without indication according to the UTI guidelines. Scintigraphy data for evaluation of permanent kidney damage was available in 639 infants. Adapted from Lindén M et al. Acta Paediatr. 2024. Online ahead of print. Open access.

	With guideline recommendation	Without recommendation
VUR grade 3–5 on VCUG	78/218 (35.8%) ^a	37/178 (20.8%) ^b
Abnormal first scintigraphy	282/735 (38.4%)	20/107 (18.7%)
Permanent kidney damage on renal scintigraphy	117/544 (21.5%) ^c	5/95 5/95 (5.3%) ^d

^a 19 VUR grade 3 (24%), 35 grade 4 (45%) and 24 grade 5 (31%)

^b 16 VUR grade 3 (43%), 18 grade 4 (49%) and 3 grade 5 (8%)

^c 26 discrete (relative function $\geq 45\%$), 52 moderate (relative function 40–44%), 20 pronounced (relative function 30–39%) and 19 severe (relative function $< 30\%$)

^d 4 discrete and 1 moderate

PREDICTORS OF NON-ADHERENCE

In the analysis of predictors of non-adherence we found that county hospitals were more likely to be non-adherent to diagnostic recommendations while non-adherence to treatment recommendations was associated with higher age and exclusive outpatient management. In the imaging domain, non-adherence was associated with initial inpatient care and county hospital care. Non-adherence was more often due to excessive imaging than recommended imaging being withheld.

4.3 PAPER III

Among 1087 infants eligible for the genetic studies, 624 were investigated with an initial DMSA scan of whom 201 (32%) showed kidney involvement. Of these, 129 infants were evaluated with a second DMSA scan ≥ 6 months later, showing RS in 63 (49%) and resolved APN in 66 (51%). Gene association analysis was carried out in a subset of 137 infants (65 boys and 72 girls) who had been investigated with an initial DMSA scan. To ascertain that patients selected for DNA analysis were representative of the group of infants examined with DMSA scintigraphy, we compared the group that was not genetically evaluated with the group included in genetic evaluation. No significant differences were seen in gender distribution, age, maximum temperature or maximum CRP levels.

Infants evaluated with a follow-up DMSA scan who were also examined by VCUG (n=104) were analyzed for association of RS and the presence or absence of dilating VUR (Figure 12A). Similarly, RS in relation to recurrent UTI or no recurrence among the 129 infants with end-point data on DMSA scintigraphy is depicted in Figure 12B. As anticipated, due to the selection of infants subjected to DMSA scan, RS was more prevalent in infants with dilating VUR and in infants with recurrent UTIs.

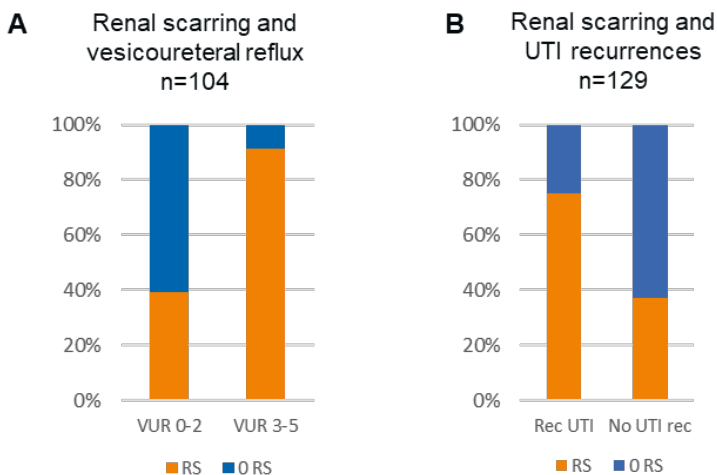


Figure 12 Prevalence of renal scarring in relation to (A) reflux status in 104 infants investigated with VCUG and (B) UTI recurrence status in 129 infants. Rosenblad T et al. *Pediatr Nephrol.* 2024;39(9):2703–2715. Adapted with permission from Springer Nature.

GENE ASSOCIATION TO RENAL SCARRING

Exome genotyping was performed in 56 infants who were examined with DMSA scintigraphy within 7 days from the UTI diagnosis, 32 with APN and 24 without kidney involvement. Among the 32 patients with APN, a heatmap comparing 7 infants with RS to 25 infants with resolved APN showed a distinct separation with alternative SNP variants being more prevalent in the RS group. This group separation was visualized further in a PCA plot (Figure 13).

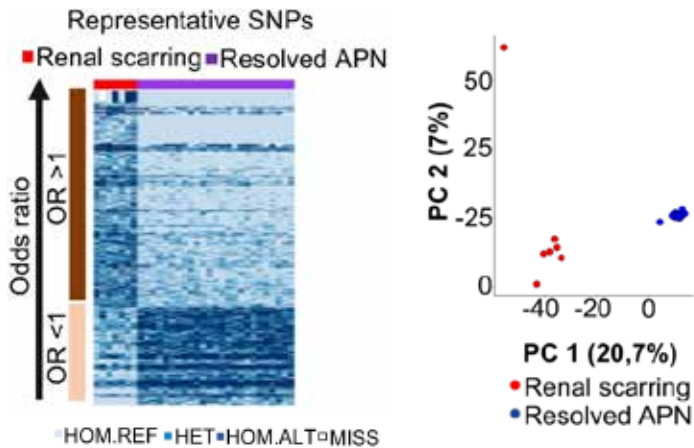


Figure 13 Heatmap (left panel) and PCA-plot (right panel) showing group separation between 7 infants with renal scarring and 25 infants with resolved APN. Rosenblad T et al. *Pediatr Nephrol.* 2024;39(9):2703–2715. Reproduced with permission from Springer Nature.

Gene association analysis found 582 SNPs significantly more prevalent in RS compared to resolved APN. Of the 25 most significant variants, 22 were associated with mitochondrial function, in particular, the mitochondrial respiratory system. A similar differentiation of the genetic profiles was seen when comparing infants with RS to infants with no kidney involvement on the acute DMSA scan. Furthermore, canonical pathway analysis, using IPA, identified ten signaling pathways associated with polymorphic RS genes with genes causing mitochondrial dysfunction showing the strongest association.

GENE ASSOCIATION TO VUR

Of 104 infants investigated with both a second DMSA scan and VCUG, 54 infants with no VUR or VUR grade 1–2 and 28 infants with VUR grade 3–5, were included for genetic evaluation. Similar to the gene association analysis

in RS, the heatmap and PCA plot from the VUR analysis showed a clear separation between infants with dilating VUR and infants with no VUR or non-dilating VUR, illustrated in Figure 14. Gene association analysis found 893 SNPs significantly associated with dilating VUR compared to no or non-dilating VUR. Of the 50 most significant variants, 22 were related to mitochondrial function and polymorphisms in genes previously associated with CAKUT, like *GRIP1*, were also linked to dilating VUR.

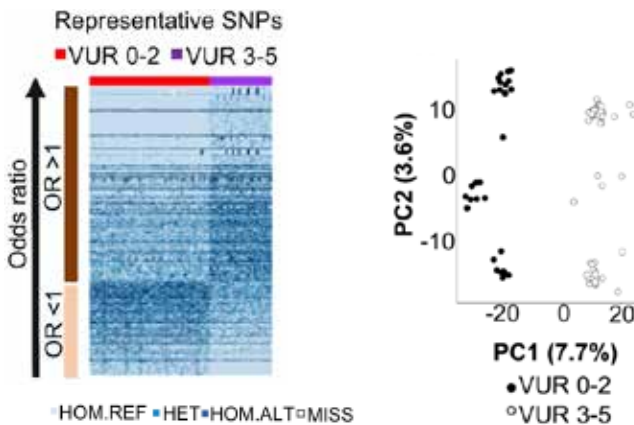


Figure 14 Heatmap (left panel) and PCA-plot (right panel) showing group separation between 54 infants with no VUR or VUR grade 1–2 and 28 infants with VUR grade 3–5. (Reference: see Figure 13).

GENE ASSOCIATION TO UTI RECURRENCE

A gene association analysis comparing 36 infants with recurrent febrile UTI to 77 infants without UTI recurrences identified 814 significant SNPs. This analysis displayed heterogeneity with no dominant immunity genes. Again, similar group separations on the heatmap and PCA-plot were seen (Figure 15).

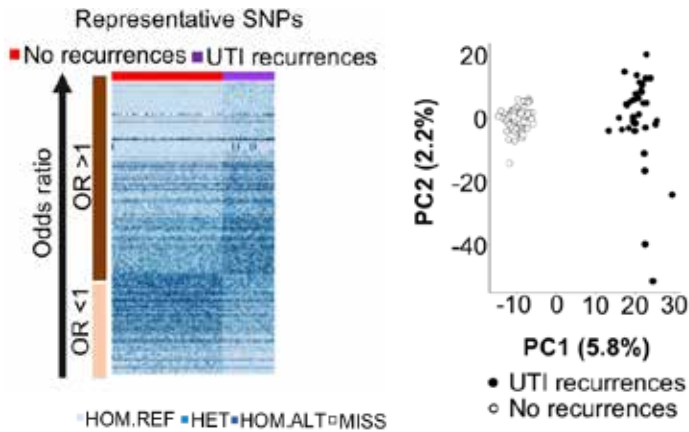


Figure 15 Heatmap (left panel) and PCA-plot (right panel) showing group separation between 36 infants with recurrent febrile UTIs and 77 infants without recurrence. (Reference: see Figure 13).

In a search for shared risk genes we identified 36 common genes significantly associated with RS, dilating VUR and recurrent UTI. Twenty-three of these were related to mitochondrial dysfunction, suggesting this as a common risk factor for these conditions.

5 DISCUSSION

5.1 PAPER I AND PAPER II

For the clinical parts of the project, evaluating current management of infant UTI and adherence to the clinical guidelines, there was no quality registry or database in Sweden covering children with UTI that could be used to retrieve relevant data. We chose a prospective method for the studies rather than trying to identify patients retrospectively and collecting data through hospital records. Being part of a study setting might have influenced co-workers who included infants in the study to be more meticulous in the management of patients resulting in a skewed picture of true UTI management but we believe that the strengths of a prospective study outweigh this drawback. Furthermore, the multicenter nationwide participation and large size of the study group was likely to provide a representative sample of infants with a first episode of UTI. From expected UTI incidence in infants and demographic data we estimate that there were about 5000 infants eligible for inclusion during the study period. Hence, it is possible that there was a selection bias in the inclusion process although no indications of such have been identified.

Since one of the objectives with the project was to evaluate current clinical management, all children who were treated for a clinical diagnosis of UTI were eligible for inclusion. More specific diagnostic criteria were then applied for the individual studies. In **Paper I**, we chose to include 42 infants with growth of a single uropathogen even though the bacterial count was <10,000 CFU/mL as the guidelines do not specify a limit for a UTI diagnosis. Some of these infants might have been falsely classified as UTI due to contaminated urine. However, they accounted for only 3% of the total study population and apart from showing a higher rate of non-*E. coli* isolates they did not differ from infants with higher colony counts in rate of RBUS dilatation, dilating VUR or permanent kidney damage. Thus, it is unlikely that these cases with <10,000 CFU/mL have influenced the results. In **Paper II**, we included all infants treated for a clinical diagnosis of UTI regardless of bacterial growth as the objective in this study was to evaluate adherence to guidelines.

A limitation with all the studies was that imaging and laboratory investigations were assessed and reported at the local hospitals with the risk of individual variations and errors. A central assessment of VCUGs and DMSA scans would

have led to a more uniform interpretation but this was hampered by the large number of investigations.

DIAGNOSTIC EVALUATION

In **Paper I**, we showed that perineal bags as urine collection method are rarely used in current practice while clean catch is by far the predominant sampling method, even among the youngest infants. This was in sharp contrast to practice in the 1990s when bag samples were used in 43% and SPA in 50% of infants <1 year treated for UTI [46]. The Swedish UTI guidelines state that SPA is the preferred urine sampling method in infants <1 year but include the alternatives of clean catch or catheter. Practitioners obviously prefer a non-invasive method despite a higher contamination risk which might lead to a false diagnosis of UTI and unnecessary invasive investigations. The reasons for this are likely multifactorial. SPA requires trained staff and resources and preferably, access to an ultrasound device. Yet, in “real-life” clinical practice, even with guidance of ultrasound, the success rate of SPA might be as low as about 50% [173]. Parents preference should also be considered in the management of infants which further restricts the use of SPA since this method is painful, more so than catheterization, which has similar challenges and drawbacks [174, 175]. Urine collection by clean catch, on the other hand, can be performed with a low contamination risk of 5% if carried out in a standardized manor and with proper cleaning of the perineum [176-178]. Apart from the contamination risk, clean catch can be time consuming and thus impractical in the emergency unit setting and in critically ill patients. Nevertheless, our study confirmed that clean catch technique is now widely used and seems to be a functional method for infants in pediatric emergency hospital use in Sweden. Its place as a recommended urine sampling method in guidelines is motivated for well appearing infants but maintaining skills among pediatricians and staff for invasive urine collection is important for patients with need of urgent care. The risk of over-investigating infants falsely diagnosed with UTI on clean catch specimens might also counterbalance the disadvantages of invasive urine sampling if recommendations for imaging are further reduced.

Adherence to perform urine dipstick tests in the diagnostic evaluation was excellent, almost 99%. In **Paper I**, we reported that pyuria was present in 97% of the infants. This was significantly higher compared to the pyuria rate of 87% found by Shaikh et al. in a similar age group with UTI posing the question whether some infants were not included in our study due to the absence of

pyuria [121]. However, we found a similar pattern of lower prevalence of pyuria in infants with non-*E. coli* infection compared to those with *E. coli* as in the aforementioned study which is also documented by others [18, 179]. Moreover, we found a distinct correlation with decreasing grade of pyuria with lower bacterial colony counts. This emphasizes the importance of not disregarding patients with absent or low grade pyuria when there is a clinical suspicion of UTI. It also highlights the need of better rapid diagnostic tests for UTI in children.

The usefulness of defining UTIs by colony counts in urine culture has been extensively discussed but so far, there is no consensus on this matter and guidelines differ widely [18, 119, 180, 181]. While it might be important in a study setting to minimize the risk of including falsely diagnosed UTIs by requiring a high colony count, this is not necessarily applicable in clinical practice [117]. Our study did not address this question specifically but the majority of patients (81%) had colony counts $\geq 100,000$ CFU/mL and of the isolates with lower counts, 15.6% were non-*E. coli*, known to be associated with a higher risk of urinary tract abnormalities. Disregarding infants with lower bacterial counts from UTI in the diagnostic evaluation would most likely have caused some patients with true UTI not receiving proper treatment. This has to be weighed against the risk of over-treatment and unnecessary investigations. Again, if recommendations on invasive imaging are further reduced, over-investigating a few infants with a false diagnosis of UTI based on low bacterial counts could be preferable to withholding treatment to others with true infection.

Adherence to perform blood tests for CRP and creatinine in the diagnostic work-up were satisfactorily high as these tests are used for risk stratification in the guidelines. As diagnostic markers of UTI, however, these tests are unspecific and not very useful. CRP is sometimes used in the diagnostic evaluation to discriminate upper UTI from lower UTI and as a predictor of serious bacterial infection [182]. Several international guidelines, however, do not recommend CRP for identifying renal involvement [110, 111, 143]. Procalcitonin has been proposed as better and more specific marker than CRP for both APN and as risk factor for renal scarring after UTI in children [183, 184]. While CRP is a cheap and readily available test in pediatric emergency units in Sweden, procalcitonin has been introduced more recently in many hospitals and was not included in our studies. Low volume capillary blood tests for procalcitonin with relatively speedy analysis are now available and further

studies of its performance as a predictor of renal involvement in infants with UTI would be of interest.

In **Paper I**, we showed that creatinine was elevated in 15.3% of infants with non-*E. coli* infection and in 24.8% of infants <1 month. In the latter group, the cause of raised creatinine in some infants may have been maternal creatinine since the median age in this group was only 19 days. Another reason could have been dehydration related to the infection. We were unable to assess permanent kidney impairment in these infants as follow-up values of creatinine were not available but we believe this is unlikely since very few cases of bilateral kidney scarring and no solitary kidney with prominent abnormality were seen on scintigraphy. However, in a recent study on children with a median age of 6 months who were hospitalized for febrile UTI, acute kidney injury as defined by the creatinine criteria of Kidney Disease: Improving Global Outcomes (KDIGO), was seen in 14.6% and, if CAKUT was present, in 30% [185]. Moreover, acute kidney injury was considered a significant indicator of underlying VUR. Although not useful in the diagnosis of UTI, creatinine can be valuable in the management of infants with UTI, in particular in severely ill patients or those not responding promptly to antibiotic treatment and in the very youngest infants.

TREATMENT

In **Paper II**, we reported high adherence rates to guideline recommendations on empiric antibiotic treatment, 87.7% in orally treated and 96.5% in intravenously treated patients. The Swedish guidelines include only one first line option for oral use and one for intravenous treatment, both cephalosporines. In the assessment of bacterial isolates and antibiotic susceptibility, 3rd generation cephalosporines proved to be very suitable for empiric treatment with only 2% of *E. coli* isolates being resistant to ceftibuten. The paucity of antibiotics for empiric use, however, is problematic, partly due to a limited access to oral cephalosporines in Sweden and partly because one-sided use of antibiotics can promote bacteria to become resistant. In **Paper I**, we present current resistance rates to relevant oral antibiotics in *E. coli*. These data are unique in that they are derived from UTI patients in a population based study in this particular age group and differ somewhat from compiled data from microbiology laboratories with unknown clinical context [33]. The resistance rate to amoxicillin-clavulanic acid was reported at 8% in our study, similar to that in Norway and Denmark (both 7.2%) where this drug is commonly used for empiric treatment of children with UTI [34, 35]. Furthermore, amoxicillin-

clavulanic acid has been shown to be effective in clinical trials and in a Cochrane analysis on UTI treatment [125, 186]. In addition, the European Committee on Antibiotic Susceptibility Testing has revised the minimal inhibitory concentration breakpoints for aminopenicillins in UTI treatment and states that high dose amoxicillin-clavulanic acid is adequate for treating *E. coli*. It should therefore be considered as an alternative empirical treatment option in an update of the guidelines. Resistance to trimethoprim in *E. coli* was 22%, considerably higher than the average resistance rate of 14% reported in children <2 years of age over a ten-year period 1994–2003 [187]. This supports the guideline recommendation that trimethoprim-sulfamethoxazole should be used only after antibiotic susceptibility testing.

Antimicrobial resistance rates in Sweden are favorable in a global perspective but increasing, and we already encounter children with UTI caused by multi-resistant bacteria in our practice. Antimicrobial resistance is identified as one of the current major health threats in the world [188]. It is likely to be a growing problem also in Sweden, not least in specialized units caring for children with preexisting anomalies and kidney pathology. With increasing resistance, it is likely that more UTIs will require intravenous treatment and might lead to an increase in kidney damage due to delayed or ineffective treatment. Therefore, it is of great concern that we keep high vigilance in diagnosing UTIs in children and continue to monitor local resistance patterns.

In the analysis on influence of age in **Paper I**, 72.1% of infants <1 month were treated with intravenous antibiotics compared to 34.8% of older infants. The Swedish guidelines do not provide any specific recommendations on treatment of the youngest infants while many international guidelines recommend that infants <2 months of age are initially treated intravenously. Of 39 infants <1 month treated exclusively with oral antibiotics in our study, 17 were still initially hospitalized. The reason for this is not known but could have been for close monitoring or due to failure to establish intravenous access. In a review article on management of infants aged 0–2 months with UTI, the authors propose that infants <1 month are hospitalized and treated with intravenous antibiotics for 2–3 days while awaiting blood culture and that well-appearing infants 1–2 months can be treated all-orally [189]. Clearly, despite the lack of guideline recommendations, this age group is managed differently and more intensively than older infants which deserves to be commented in a future revision of the guidelines.

The use of short term antimicrobial prophylaxis (<2 months) had dropped dramatically to 2% in our cohort compared to 79% in the 1990s while long term use (≥ 2 months) remained at a similar rate as in the 1990s-study, 16% and 20 % respectively [46]. In the adherence analysis we found that the long term prescription of prophylaxis was in accordance with guideline indications in 96% of these. The benefits of prophylaxis, however, have been questioned during the past decades [190-192]. A meta-analysis on children with VUR showed that patients with long term antibiotic prophylaxis were more likely to encounter recurrent UTIs with multidrug-resistant bacteria compared to no treatment or placebo [193]. The authors concluded that one multidrug-resistant UTI would develop for every 21 prophylaxis treated patients. The Swedish guidelines recommend antibiotic prophylaxis to all infants with dilating VUR, for boys until one year of age and for girls during one year from the first UTI. Other major UTI guidelines are more restrictive and do not recommend the routine use of prophylaxis after the first UTI [110, 111, 143]. In light of this and the increasing antimicrobial resistance, the Swedish recommendations on prophylaxis might need to be revised. Waiting to see if any UTI recurrence occurs could be one option.

IMAGING AND FOLLOW-UP

RBUS

Adherence to performing RBUS examination after the first UTI was close to 100% in our study. We asked for information only on dilatation of the renal pelvis and the APD while other abnormalities were optional to report in free text. The APD, unfortunately, was often not reported, forcing us to use only dilatation as a parameter for analysis of imaging investigations in **Paper II**. Pelvicalyceal dilatation is a subjective estimation by the ultrasonographer with a risk of individual variations. The APD, however, is not a static measurement but can fluctuate with the natural dynamics of the urinary tract, urine production and flow of urine. In comparison with a previous Swedish UTI study on infants applying a standardized RBUS protocol, the rate of dilatation in our study correlated very well [137]. Yet this does not rule out the possibility that our adherence estimates of imaging further down in the guideline algorithm could have been affected.

Most other major guidelines also recommend RBUS in infants after the first UTI although voices have been raised questioning the value of this investigation [135]. One suggestion is to perform RBUS only in those with

non-*E. coli* infection and those who experience recurrent UTIs [139]. As previously mentioned, RBUS has limited prediction value for dilating VUR which also was confirmed in **Paper I** where 56% of infants with VUR grade 3–5 had no dilatation on RBUS. The other leading argument against routine RBUS examination, that most infants with significant kidney abnormalities are detected at prenatal ultrasound screening, is dependent on when and by whom this screening is performed. In most areas in Sweden, prenatal ultrasound including organ screening is offered at gestational week 18–19. This may be different in other parts of the world and, if screening is performed in the third trimester, chances of detecting anomalies are increased [194]. An example from our study illustrating this aspect is an infant with no remarks on prenatal screening but where RBUS following the first UTI revealed a solitary kidney. Therefore, the local antenatal screening program has to be considered in UTI guideline recommendations on imaging with RBUS.

In **Paper I**, we reported 31 (2.4%) kidney anomalies other than dilatation identified on RBUS performed as a consequence of the first UTI. This is a minimum figure since these findings were optional to report. An observation from our study is that there is room for improvement in the RBUS evaluation by the ultrasonographer. Including APD, kidney length and information on swelling could add important information for the clinician [195, 196].

VCUG

We reported a substantial decrease of VCUG investigations compared to the 1990s which was expected. While only few cases of low grade VUR were reported, VUR grade 4–5 was identified in 6% of the total study population. This matches the rate reported in similar cohorts (4-5%) where all children were examined by VCUG, suggesting that the currently used imaging strategy is functional in identifying high grade VUR which was a goal with the 2013 guidelines [156, 160]. Yet, 390 of 1306 infants in our study were exposed to VCUG of which 243 (62%) were normal. Considering the limited benefit of prophylactic antibiotic treatment for dilating VUR the value of identifying these infants after their first UTI must be questioned, in particular in light of the burden carried by the infants without VUR yet exposed to a VCUG [197].

In the adherence study we found 37 infants with VUR grade 3–5 investigated by VCUG that was not indicated by the guideline imaging algorithm. These represent about 1/3 of the infants with dilating VUR, indicating that the algorithm in fact misses to identify a significant proportion of these. There

might have been alternative reasons for performing a VCUG, such as abnormalities on RBUS other than dilatation, but these are not included in the guidelines. Furthermore, 30 of the 37 infants subjected to VCUG without recommendation had recurrent UTIs which also could have been the reason for VCUG.

Reported in **Paper II**, there were 178 infants investigated with VCUG without guideline indication. The detection rate of dilating VUR was significantly lower among these compared to those performed with indication. In particular, among infants without guideline indication and without recurrent UTI only 7 of 88 infants had dilating VUR, none with grade 5. Our data did not provide information on the reasons for these investigations but clearly, a remarkable number of patients were unnecessarily exposed to VCUG. Waiting for the second UTI before considering a VCUG or applying other indications for VCUG that would reduce the number of investigations should be discussed in a guideline revision.

DMSA SCINTIGRAPHY

One advantage with performing a DMSA scan shortly after the UTI is that children with a normal scan can be dismissed from further investigations at an early stage, reassured that the kidneys are in good health [156]. Compared to performing VCUG in all infants after the first UTI, this strategy decreases the number of VCUGs significantly but many children with abnormal early DMSA scans will not benefit since the majority of findings on the subsequent VCUG will not change the clinical management [198].

In the 1990s study about half of the patients were investigated with either urography or DMSA scintigraphy after their first UTI, mainly to identify those with permanent renal damage. In our study (**Paper I**), 774 (59%) of the infants were investigated with at least one DMSA scintigraphy and 180 of these were exposed to a second DMSA scan to diagnose permanent kidney damage. In addition, at least 62 MAG3 scans were performed during the study period. This heavy burden of renal imaging reflects the strategy of a top down imaging model. The guideline algorithm was designed to detect significant permanent renal damage, motivating the second DMSA scan in patients with defects on the initial scan. In this sense, the model is successful with a 10% detection rate of permanent kidney damage which is roughly in line with the expected rate. However, the burden of radiation and costs for health services are worrying and have to be weighed against the benefits. A study comparing the yield,

radiation dose and costs in different imaging protocols concluded that the top down approach was superior in detecting dilating VUR and kidney damage but at a high radiation and financial cost with questionable benefit [199].

In the adherence analysis it was evident that performing a speedy DMSA scan according to the guideline recommendation was problematic, in particular at county hospitals where only 41.9% achieved to perform this within the one-month time limit. Many scans were performed between 1 and 3 months from the UTI, a time when some acute uptake defects have resolved and some are still visible but will resolve with time. Thereby, DMSA scans performed in this time interval are not suited for the intended purpose of the imaging algorithm.

Similar to the VCUGs, many infants (about 8% of the study group) were investigated with scintigraphy without apparent guideline indication. Again, credible reasons for this might have been findings on RBUS but the yield of these investigations was low: only 5 infants with permanent kidney damage were identified, none with severe kidney defects. In light of these observations, the utility and benefits of the guideline imaging model must be questioned also regarding DMSA scintigraphy. Since an acute scan, primarily aimed to identify renal involvement evidently is difficult to achieve, this imaging strategy seems less useful in everyday clinical practice. Performing only a late DMSA scan for detection of kidney damage in selected patients would considerably reduce the number of scintigraphies. Other guidelines have toned down the benefits of identifying kidney defects with the argument that few children will develop complications [110, 143]. The long term risks, however, need to be further investigated to back up this position with stronger scientific evidence. The risk of missing significant kidney damage with a more relaxed investigation strategy in combination with an increasing proportion of UTIs being caused by resistant bacteria must also be considered.

FOLLOW-UP

At least one recurrent febrile UTI was reported in 18% of our cohort. There are few large studies reporting UTI recurrence rates and most include also older children and only children with VUR. An Australian study on children <5 years of age including children with and without VUR, reported a 12% recurrence rate within one year from the first UTI [39]. As previously mentioned, recurrent UTIs are much more prevalent in girls than in boys after one year of age. We showed that recurrences occurred more frequently in girls (21%) than

in boys (14%) also in this well-defined group of young infants where dilating VUR was more prevalent among the boys.

Among 1306 infants, 60 (4.6%) were subjected to surgical interventions by pediatric urologists. The most common procedure was endoscopic treatment of VUR, performed in 24 children. The Swedish UTI guidelines give advice on indications for referral to urologists but the decision to perform an intervention is made on individual basis in joint consultation with the caretakers. Therefore, it was not possible to assess adherence to recommendations on surgical treatment of VUR. The following two most frequently performed procedures were posterior urethral valve ablation in seven and pyeloplasty of ureteropelvic junction obstruction in six.

5.2 PAPER III

For the genetic studies, a protocol including strict UTI criteria and time points for kidney imaging would have been preferred but this would have interfered with the clinical aims of the project. We therefore reasoned that selecting a subgroup from the entire cohort by applying relevant criteria for the genetic studies would be sufficient for this part of the project. We had anticipated a higher number of DMSA scans being performed within the 7-day time limit from UTI applied in this study to identify renal involvement in the acute phase of infection. Moreover, our endeavor to include more patients in this study by extending the inclusion period at the three principal study sites coincided with the Covid 19 pandemic which virtually stopped the inclusion process and substantially reduced the study group eligible for genetic analysis. To overcome this, we applied a stricter significance threshold to limit the selection of single nucleotide polymorphisms for gene association. The small sample size of the study groups in **Paper III**, however, is a limiting factor making these studies more of explorative nature.

Infants who underwent DMSA scintigraphy were selected by the guidelines' risk criteria forming a high-risk group for kidney involvement. Thus, some infants not investigated by DMSA scintigraphy might have had kidney involvement but were excluded from the genetic evaluation, which might have influenced the results of DNA analyses. However, among infants investigated by DMSA scintigraphy, those selected for DNA analysis did not differ from those in whom no DNA analysis was performed. The scarring rate of 22% among infants with kidney involvement on the first DMSA scan and included in DNA analysis was in accordance with previous studies [66, 67].

Furthermore, the DMSA investigations were assessed and reported at the local hospitals. A central reading of the DMSA scans would have added strength, in particular to the genetic association analysis.

GENE ASSOCIATION ANALYSIS

The clear separation of infants with renal scarring from those with resolved APN seen in the DNA association analysis indicate a genetic susceptibility for scarring. This is further supported by the finding of a similar group separation by genetic profile when comparing the scarring group with infants who did not have kidney involvement on the initial DMSA scan. Interestingly, the gene profile linked to renal scarring was dominated by genes involved in mitochondrial function which, to our knowledge, has not previously been reported in UTI-related kidney injury. Mitochondrial dysfunction and mitochondrial DNA mutations, however, have been linked to both acute kidney injury and several chronic kidney diseases such as tubulopathies and focal segmental glomerulosclerosis [200, 201]. Mitochondria are essential for cell function by being the main generator of chemical energy needed to drive virtually all biochemical processes in the cell. Inheritance of mitochondrial genes and the biological consequences of mitochondrial gene variants are complex and make environmental factors crucial in the phenotype manifestations of mitochondrial dysfunction [201, 202]. The kidney is a mitochondria rich and energy-dependent organ and therefore sensitive to disturbances in energy production. Mitochondrial dysfunction not only may induce cell injury but also can play a role in tissue repair, thus potentially inhibiting the healing of an acute insult [203]. It is therefore intriguing to hypothesize that the polymorphisms in mitochondrial genes, found to be associated with renal scarring in our study, may play an important role in the scarring process.

A similar genetic profile that clearly discriminated children with renal scarring from those with resolved APN in a cohort from Singapore further supports the hypothesis of genetic scarring susceptibility [90]. It would be interesting to compare the most significant gene variants in this cohort with the Swedish cohort to identify shared gene polymorphisms and, in particular those involved in mitochondrial function.

Group separation was also demonstrated when comparing infants with no VUR or VUR grade 1–2 to VUR grade 3–5 by gene association analysis. Again, a mitochondrial gene profile was linked to dilating VUR although the most

significant gene variants were not identical to those in the renal scarring group. Since renal scarring is associated with the presence of dilating VUR, it is possible that these conditions, to some extent, have a common genetic background. However, in contrast to the results in our study, no association was seen between VUR status and genetic profile in the previously mentioned Singapore cohort [90].

The heterogeneity of gene associations with renal scarring in our study and in previous studies makes the prospect of a simple gene test for risk assessment of scarring after pyelonephritis still distant. However, the novel association with mitochondrial function might add to our understanding of genetic susceptibility and the molecular mechanisms of renal scarring. The search for better prediction markers of UTI related kidney injury must go on since a clinically useful marker would significantly change the investigation strategy with less burden for infants with UTI.

6 CONCLUSION

The studies on management show a notable shift over time in urine collection method used for bacterial culture with clean catch now being the predominant method while SPA is rarely used. There is no indication in the reported clinical data that this has entailed a worrying number of false UTI diagnoses due to contamination. Adherence to the diagnostic procedures recommended in the Swedish UTI guidelines is overall very high, although non-adherence is more common in county hospitals compared to university hospitals.

The spectrum of causative bacteria in infants is consistent with previous studies with *E. coli* accounting for 90% of UTIs. The resistance pattern in *E. coli* isolates is yet advantageous in an international perspective but the trend with increasing resistance to trimethoprim continues, now reaching 22%, which confirms the policy of not using it for empiric treatment of UTIs. The resistance rate for amoxicillin-clavulanic acid in *E. coli* is more favorable, 8%, suggesting this as an option for empiric treatment of UTIs in children to moderate the current one-sided use of cephalosporines.

Adherence to treatment recommendations is satisfactory at about 90% although lower in outpatient care compared to inpatient care, most likely reflecting a limited availability of oral cephalosporines during the study period. There is also a considerable discrepancy between university hospitals and county hospitals in initial oral treatment with room for county hospitals to use this more often, especially in older infants. On the other hand, infants <1 month of age differ in several ways from older infants with a male predominance, less pyuria, higher prevalence of non-*E. coli* infection, elevated creatinine and kidney abnormalities on RBUS, motivating special consideration of this age group in future guidelines.

Dilating VUR and permanent kidney damage are identified in expected frequency in routine clinical practice using the current model of investigating only a selected group of infants after their first UTI if risk factors are present. However, the burden of imaging investigations is still high, implying discomfort and ionizing radiation to a considerable number of otherwise healthy children as well as workload and financial stress to health care services. Furthermore, obvious difficulties in performing an early DMSA scan as recommended after the first UTI, in particular at county hospitals, challenges the usefulness of the current guideline algorithm for imaging. Non-adherence to imaging recommendations is commonly due to excessive imaging with low

yield of significant findings that would alter the management or follow-up. This needs to be addressed in an update of the guidelines.

There is growing evidence that renal scarring is genetically influenced shown by the ability of a genetic profile to clearly separate infants with renal scarring after UTIs from those with resolved APN. In particular, variations in genes affecting mitochondrial function seem to play an important role in the susceptibility of UTI associated kidney injury. Since mitochondrial gene mutations and mitochondrial dysfunction has been linked to other kidney diseases, this opens for new intriguing questions regarding the pathogenesis of renal scarring in children with UTI. Although not currently applicable for risk stratification in clinical practice, this novel finding can be valuable in the search for better prediction markers of renal scarring needed for a more individual and targeted assessment of children with UTI.

7 FUTURE PERSPECTIVES

The wide discrepancy among guidelines and experts on how to diagnose, treat and follow-up UTIs in young children highlights the need of better diagnostic and prognostic tools for clinical practice. Blood or urine biomarkers that could discriminate UTIs from other febrile infections and separate APN from UTI without kidney involvement would be of great benefit in the diagnostic assessment. In particular, if this was a point of care test (POCT), it would be helpful in preventing unnecessary antibiotic treatment and imaging. As mentioned, procalcitonin has shown promising abilities in diagnosing APN and predicting renal scarring but would need to be validated further, especially in infants. A urine biomarker would be even better. Neutrophil gelatinase-associated lipocalin (NGAL) has been pointed out as a potential marker for clinical use in the near future [204]. A rapid POCT for urine NGAL is already available.

Furthermore, the genetic approach for risk assessment of severe infection or renal scarring, as described in this thesis, will for sure continue to be explored. Hopefully, future genetic studies will contribute to the development of new markers to be used as prognostic tools in clinic.

Traditional urine culture is slow and labor intensive making quicker methods for the identification of bacteria called for. Several new techniques are being developed such as polymerase chain reaction gene chips, ELISA assays and antibody-based tests [205, 206]. A new innovative point of care digital format assay using an ultra-quick culture technique is being tested for rapid detection of five of the most common uropathogens [207].

Few new antibiotics have been developed the past two decades and focus is now on other methods to combat the expanding problem with multi-resistant bacteria. Some examples of experimental techniques are synthetic or modified antimicrobial peptides, antibacterial antibodies to block bacterial virulence factors, oligonucleotides that can penetrate into bacteria to inhibit bacterial ribonucleic acid translation and bacteriophage virus therapy for lysis of bacteria [208]. Vaccines might be another option. As pointed out in this thesis, antimicrobial resistance is already a major global health issue which needs to be counteracted promptly to prevent us from being pushed back to a situation similar to that in the pre-antibiotic era.

Imaging techniques are continuously being improved, including the reduction of radiation. Ultrasound has the potential to provide more information than is reported routinely today but has the disadvantage of being user dependent which limits this potential in everyday clinical practice. Magnetic resonance imaging (MRI) is an appealing alternative imaging modality since it causes no radiation exposure and can produce detailed anatomical images of the kidneys and urinary tract. Functional imaging is being developed but not ready to replace DMSA scintigraphy. Drawbacks with MRI are that it is relatively expensive, time consuming and often needs sedation of young children which currently limits its use.

While waiting for some of these new methods to be further developed and implemented in clinical care, it is time to update and rewrite the Swedish UTI guidelines. Two of the papers in this thesis will hopefully be useful as part in this work. The Swedish Pediatric Society has recently requested that the Choosing wisely campaign is considered when developing clinical guidelines. This campaign aims at reducing unnecessary tests and procedures of doubtful or no benefit for the patient, minimize harmful interventions and managing health care resources wisely [209, 210]. In this regard, we have identified some issues with the current guidelines that should be addressed, in particular the burden of imaging and radiation.

A possible way to further reduce the number of VCUGs is to perform this only in children with abnormalities on RBUS (which need to be defined), non-*E. coli* infection or if a febrile UTI recurrence occurs. Moreover, early scintigraphy could be limited to children with poor response to treatment, if surgical interventions are considered or if other reasons for detecting or ruling out APN are present. Using DMSA scintigraphy for the detection of kidney damage by a late scan only in children with RBUS abnormalities or when a VCUG has shown dilating VUR would significantly reduce the number of investigations and radiation burden in the initial evaluation. However, RBUS has low sensitivity for predicting kidney damage on late DMSA scans, thus this strategy would miss to identify a significant proportion of children with renal scarring [211, 212]. Adding non-*E. coli* infection or raised procalcitonin as additional indication for scintigraphy would increase the sensitivity but this needs to be further evaluated and balanced against the number of scintigraphies this would imply on children with normal kidneys.

Lastly, it is crucial that we bring the high awareness for UTIs with us into the future. For this purpose, the unique and tight network of pediatric nephrologists

and general pediatricians with interest in nephrology that has been built up over half a century in Sweden, is a good platform to keep building on.

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