# New Perspectives on Imaging of Urinary Tract Infections in Infants

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Cover illustration: Fusion image of diffusion and T2-weighted MRI acquisitions of a one-month old infant with bilateral pyelonephritis.

Trycksak
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*"You can observe a lot by just watching"*

*Yogi Berra*

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## ABSTRACT

*Background:* Urinary tract infection (UTI) is a common disease in infants that may lead to renal damage with an increased risk of long term complications. The diagnostic imaging aims to identify risk factors as underlying urinary tract abnormalities and renal involvement of the infection for prevention of long term adverse outcome. There is a need for alternative methods to the ones presently used for investigation and follow-up of this patient group without the use of invasive procedures, contrast agents or ionizing radiation.

*The aim* of this thesis was to evaluate the potential of ultrasound (US), diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) in the initial evaluation of the urinary tract in infants with their first UTI.

*Methods:* Infants with their first symptomatic UTI were included in four prospective studies. The infants were examined with US, magnetic resonance imaging (MRI) including DWI and DTI, and 99mTc-dimercaptosuccinic acid (DMSA) scintigraphy during the acute phase of the infection. Inflammatory parameters, C-reactive protein and body temperature, were registered. Follow-up examinations included US after 1 month and scintigraphy after one year.

*Results:* Renal size measured at early US determined renal swelling in infants with a UTI. The renal swelling correlated with inflammatory parameters and was associated with renal damage at acute and follow-up DMSA scintigraphy. There was an agreement between DWI and DMSA scintigraphy in the detection of pyelonephritis. With the use of DTI, differences were found in quantitative and qualitative parameters in lesions compared to normal tissue and further lesion characterization patterns were recognised.

*Conclusion:* The results show that US, DWI and DTI are valuable non-invasive, non-radiating tools in the initial evaluation of infants with their first UTI. Renal length US measurements adds value to the early US examination by helping to identify patients at risk for renal damage even though it cannot replace DMSA scintigraphy. DWI and DTI have the potential to be advantageous alternatives to DMSA scintigraphy. However, studies of larger cohorts are needed to verify the results.

# SAMMANFATTNING PÅ SVENSKA

Urinvägsinfektion är vanligt förekommande hos barn upp till 1 års ålder. Utredning som idag innefattar såväl ultraljud, DMSA-scintigrafi (njurfunktionsundersökning) samt eventuellt blåsröntgen syftar till att identifiera barn med ökad risk att utveckla njurskada som t.ex. barn med urinvägsmissbildning eller infektion i njuren (pyelonefrit). En nackdel med såväl DMSA-scintigrafi samt blåsröntgen är att dessa undersökningar är invasiva och strålbelastande. De senaste åren har det pågått en debatt om omfattningen av utredningen av spädbarn med förstagångs-urinvägsinfektion. Det finns ett behov av att studera alternativa metoder för förenklad samt förbättrad bilddiagnostik för denna patientgrupp. Denna avhandling utvärderar möjligheterna vid användning av ultraljud samt magnetkameraundersökningar som icke strålbelastande eller invasiva tekniker vid utredning av spädbarn (< 1 år) med urinvägsinfektion.

I delarbete I samt II studerades njursvullnad, uppmätt som längd samt volym, med ultraljud på barn < 1 år med förstagångs-urinvägsinfektion. Resultaten visade på en signifikant njursvullnad vid insjuknande som minskade vid uppföljande kontroll efter fyra veckor. Graden av svullnad korrelerade både med graden av feber samt CRP (inflammationsmarkör i blod) vid insjuknandet. Våra resultat har även påvisat ett samband mellan njursvullnad mätt med ultraljud och njurskadeutveckling på DMSA-scintigrafi. Njurstorlekmätning är enkelt att utföra med ultraljud och bedömningen av njursvullnad kan vara av värde i riskbedömningen, även om den inte kan ersätta DMSA-scintigafi i uppföljningen av spädbarn med urinvägsinfektion.

I delarbete III o IV undersöktes barn < 6 månader med förstagångs-urinvägsinfektion med MR-kamera inklusive konventionell DWI (diffusionsviktad avbildning) samt DTI (riktningskänslig DWI). I delarbete III påvisade vi en samstämmighet mellan DWI och DMSA-scintigrafi avseende möjligheten att påvisa pyelonfritförändringar i njurarna. Delarbete IV visade på möjligheten att med DTI få ytterligare information beskrivande förändringarna i njurarna med möjlighet att kunna särskilja typiska pyelonfritförändringar från ex medfödda förändringar i njurparenkymet. DWI samt DTI har således potentialen att ersätta DMSA-scintigrafi och är således ett attraktivt alternativ för spädbarn. Resultaten från delarbetena behöver dock bekräftas i studier med större patientgrupper

# LIST OF STUDIES

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

I.     Simrén Y, Stokland E, Lagerstrand KM, Valdimarsson S, Hansson S. *Ultrasound is an effective and noninvasive method of evaluating renal swelling in infants with their first urinary tract infection.* Acta Paediatr. 2017;106(11):1868-74.

II.    Simrén Y, Valdimarsson S, Stokland E, Lagerstrand KM, Sixt R, Hansson S. *Renal swelling indicates renal damage in infants with their first urinary tract infection.* Acta Paediatr. 2018;107(11):2004-10.

III.   Simrén Y, Stokland E, Hansson S, Sixt R, Svensson PA, Lagerstrand KM. *Diffusion weighted imaging is a promising method to detect acute pyelonephritis in non-sedated free breathing infants*. J Pediatr Urol. 2020;16(3):320-5.

IV.    Simrén Y, Stokland E, Hansson S, Hebelka H, Svensson PA, Lagerstrand KM. *Diffusion tensor imaging based multiparametric characterization of renal lesions in infants with urinary tract infections: an explorative study*. Submitted.

# CONTENT

# ABBREVIATIONS

| | |
|---|---|
| ADC | Apparent diffusion coefficient |
| AP | Anterior-posterior |
| CAKUT | Congenital anomalies of the kidney and urinary tract |
| CEUS | Contrast enhanced ultrasound |
| CRP | C-reactive protein |
| CT | Computed tomography |
| CV | Coefficient of variation |
| DMSA | $^{99m}$Tc-dimercaptosuccinic acid |
| DTI | Diffusion tensor imaging |
| DWI | Diffusion weighted imaging |
| FA | Fractional anisotropy |
| K | Kappa |
| MRI | Magnetic resonance imaging |
| ROC | Receiver operating characteristic |
| ROI | Region of interest |
| SD | Standard deviation |
| SDS | Standard deviation score |
| SNR | Signal-to-noise ratio |
| US | Ultrasound |
| UTI | Urinary tract infection |
| VCUG | Voiding cystourethrography |
| VUR | Vesicoureteral reflux |
| 2D | Two-dimensional |
| 3D | Three-dimensional |

# 1  PURPOSE OF THIS THESIS

The second most common infection at the pediatric emergency ward is urinary tract infections (UTI). This is reflected in our pediatric radiology department, where I found that it was rare to have a day program of ultrasound (US) examinations that did not include follow-up of infants with a UTI. Despite the knowledge that only a few of these infants will have long-term complications after their infection, almost all patients undergo a follow-up program including imaging that is invasive, time-consuming, exposes the infant to ionizing radiation, and may be distressing for the infants and parents. However, the imaging program is needed to identify predictors of renal damage, such as underlying urinary tract abnormalities and renal involvement of the infection. The use of a more limited imaging protocol that would reduce the number of examinations for the large group of patients would risk missing the few patients at risk. It is important to identify the patients at risk at an early stage as they are in need of further treatment and follow-up. Since most of the patients have a low risk profile, it is also important to use methods that are easy to perform, fast and well tolerated by the infants and their parents. However, despite extensive research, there is no simple way to find these patients at an early stage. In radiology, there is a rapid advancement in technology with development of new and existing methods. US is currently widely used in the evaluation of infants with UTI, but the value in the detection of renal damage has been questioned. However, earlier reports may not be applicable today, as the technique has improved substantially with higher resolution, better contrast and fewer artefacts. In addition, since US is available in most centers, the full potential of the method needs to be further explored. We have noticed that there is renal swelling in infants with a UTI, and this finding might bring useful additional information from the US. Magnetic resonance imaging (MRI) is currently not a routine method in UTI assessment. However, when performing MRI on infants for evaluation of complex renal malformations, we noted that high quality images could be produced using fast free breathing diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) protocols. As these methods are known to be highly sensitive to early pathology, we saw the potential of these methods in the evaluation of children with UTI.

Thus, the general aim of this thesis was to evaluate the potential of US, DWI and DTI as noninvasive, non-radiating imaging methods in the initial evaluation of the urinary tract in infants with their first urinary tract infection.

# 2 INTRODUCTION/BACKGROUND

## 2.1 Urinary tract infection

UTI is defined as colonization of a pathogen occurring anywhere along the urinary tract: kidney, ureter, bladder, and urethra. UTI is often classified by the site of infection, i.e. involving the lower urinary tract as the bladder (cystitis) or including the upper urinary tract as the ureters or the kidney (pyelonephritis).

The urinary tract consists of the organs and passageways involved in the production and excretion of urine from the kidneys to the urinary meatus. The main morphological structures of the urinary tract are shown in Figure 1. The kidney can be divided into three units; the cortex, the medulla, and the hilum. The renal cortex is made up of the renal corpuscles, proximal convoluted tubule and interlobular vessels. The medulla includes the Loop of Henle and collecting ducts together with interlobar arteries, all oriented in a radial pattern. The renal sinus consists of the pelvicalyceal system, renal and segmental vessels, as well as fat. When the blood enters the kidney from branches of the renal artery it is first filtrated in the corpuscle entering the tubules. The urine is produced by the filtrate traveling along the tubules where selective reabsorption and excretion takes place. The urine produced enters the collecting system through the collecting ducts at the papillary tips. The structures identified in the pelvicalyceal system are the calyces surrounding the papillary tips and the renal pelvis. The pelvis drains the urine trough the pelvo-ureteral junction into the ureter. The ureteral orifices enter the bladder in the lateral trigonal corners. They run obliquely through the muscular portion of the bladder wall and end in a submucosal tunnel. The urine finally leaves the body by bladder contractions through the urethra.

In the evaluation of the urinary tract in children it is important to be aware of differences in morphology as well as physiology across the different age groups. The human kidney begins to develop in the 4-5th gestational week and starts to produce urine between the 10th and 12th week of gestation. At birth the nephrogenesis is completed, so that a term neonate is born with all its nephrons, but the glomerular and tubular functions are immature with a low glomerular filtration rate (1). The glomerular filtration rate rapidly improves during the first months and reaches the level of adults related to body mass at about two years of age. The renal immaturity in infants also has implications in the evaluation of the morphology of the kidneys, for example in infants the cortex is considerably thinner compared with adults.

1. Renal corpuscle   5. Collecting duct
2. Proximal tubule   6. Cortex
3. Loop of Henle     7. Medulla
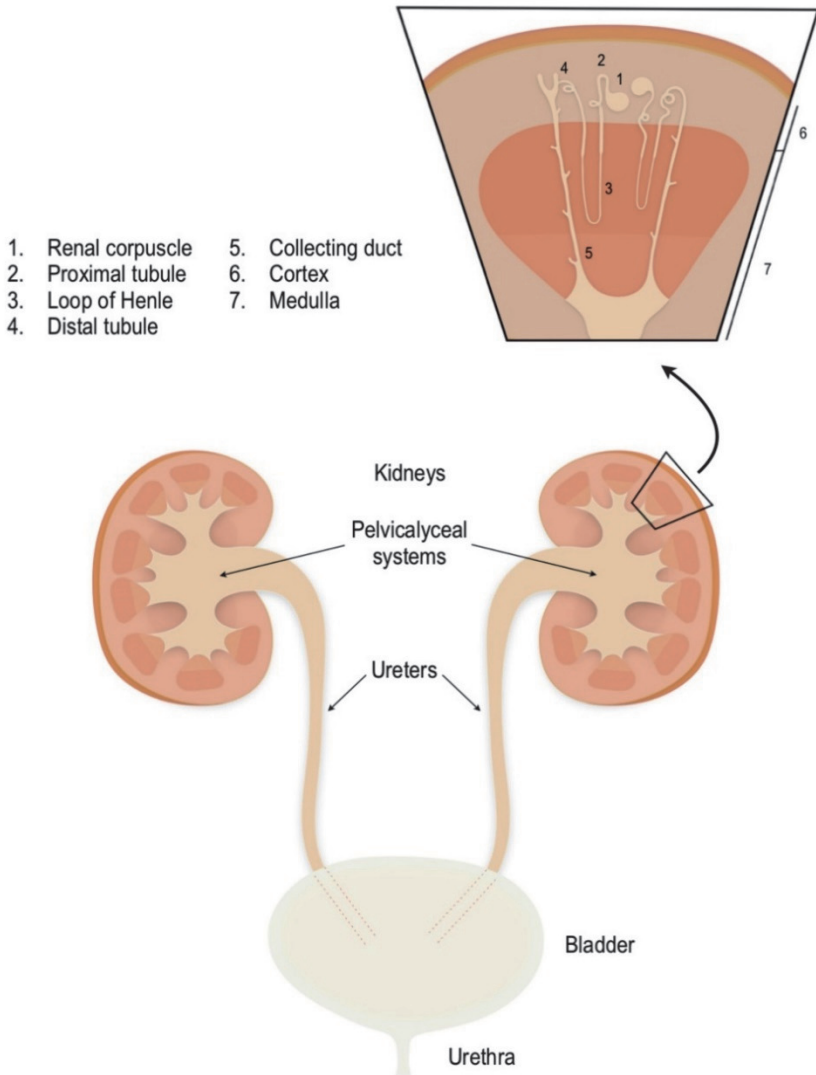4. Distal tubule

*Figure 1. Cross section illustration of the urinary tract. A magnification showing a detailed section of the nephron with the renal corpuscles and tubules. Figure design Nora Odqvist.*

## 2.1.1 Pathophysiology

The urinary tract is normally sterile. *E. coli* is the most frequently documented uropathogen. *E. coli* with fimbriae adheres to the mucosa but also bacteria without adherence causes infection, especially where there is an obstruction or other congenital malformation. UTI is a retrograde ascending infection, from the perineal area into the bladder which may ascend into the ureter and the kidneys. Bacterial invasion of the kidney causes inflammation in the parenchyma with an inflammatory response.

## 2.1.2 Epidemiology

Estimates of the incidence of UTI varies depending mainly on age and sex of the cohort, diagnostic criteria, and the rate of circumcision. The reported incidence of UTI in children is 3-8% for girls and 1-2% for boys (2, 3). In a Swedish study the estimated minimum cumulative incidence in children 0-2 years of age was to 2.2% for boys and 2.1% for girls (Figure 2) (4). Another study in a Swedish population reported a 6% cumulative risk of symptomatic UTI during the first six years of life in girls (5). Occurrence of first-time, symptomatic UTI is highest in boys and girls during the first year of life and markedly decreases after that.
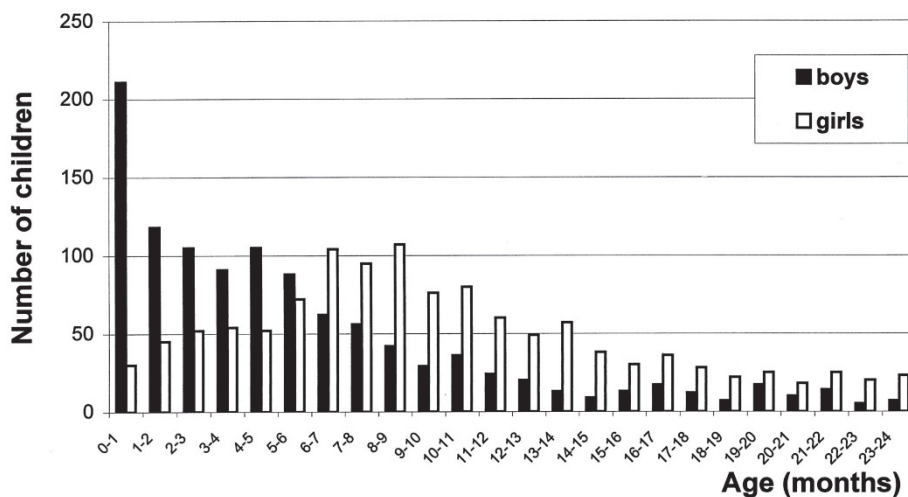


*Figure 2. Age distribution of first UTI in boys and girls, 0-2 years of age from Minimal Incidence and Diagnostic Rate of First Urinary tract infection. Jacobsson et al. Reproduced with permission from Pediatrics, Vol. 104, Page 223, Copyright © 1999 by the AAP.*

## 2.1.3  Symptoms

The clinical presentation of UTI in children varies substantially, ranging from absence of specific symptoms to fulminant urosepsis. Symptoms of a UTI also vary with age. Older children often present with dysuria, urgency, and abdominal or back pain similar to the adult population. The diagnosis of UTI in infants that are not able to describe their discomforts is more challenging. The most common symptom is fever, but a UTI should also be suspected in infants with poor weight gain, irritability or vomiting.

## 2.1.4  Diagnostic criteria

Traditionally, the diagnosis of a UTI has been based on symptoms and bacteriuria but this approach may be challenging, especially in infants since the only symptom of a UTI might be fever, which is a nonspecific sign. The final diagnosis of bacteriuria is made on the basis of urine culture (6). Any single bacterial growth in the urine specimen obtained from suprapubic aspiration is considered pathological. For other methods of urine collection the number of colony forming counts required varies but a cut-off level of 100,000 colony forming units/ml is often used (7). Suprapubic aspiration is considered the gold standard for accurately identifying bacteria within the bladder. Urethral catheterization in young children is a more commonly used method outside Sweden. Clean-catch midstream urine specimen is a useful alternative but requires cooperation of the parents of the infants.

The diagnosis of pyelonephritis indicates that the kidneys are affected by the infection. In addition to urine culture, increased C-reactive protein (CRP) together with elevated body temperature, are indicators for renal parenchymal involvement, but these have low specificity and cannot rule out other causes of infection. As it is important with a correct diagnosis of a UTI for more restricted and targeted approach to treatment, imaging can be used to evaluate the renal involvement of the infection.

## 2.2   Renal damage

UTI with involvement of the renal parenchyma, with subsequent inflammatory reaction, may lead to permanent renal damage (Figure 3) with risk of long-term complications. The frequency of renal damage in infants is around 20%, but there is a great variability of the reported renal scarring rate attributed to both heterogeneous study populations and the methods used. The earlier reported renal scarring rate has been affected by the change of method to diagnose renal damage, from urography to the more sensitive $^{99m}$Tc-dimercaptosuccinic acid  (DMSA) scintigraphy that is currently commonly used (8). The use of prenatal US has revealed that part of the findings that we earlier reported as scars, actually were congenital as the differentiation is difficult at the time-point of their first UTI (9). There are also sex differences with boys more often having congenital renal damage, while girls have more UTI-related focal damage (10). In a review by Sheik et al.in 2010, the risk of permanent renal damage was estimated to 15% (11), and in a population based study from Gothenburg published in 2011, 26% of the children had permanent renal damage, evaluated by DMSA scintigraphy, after their first UTI (12). Thus, most febrile children with a UTI do not develop renal damage. Furthermore, progression of renal damage has been shown to occur in 20% (13).
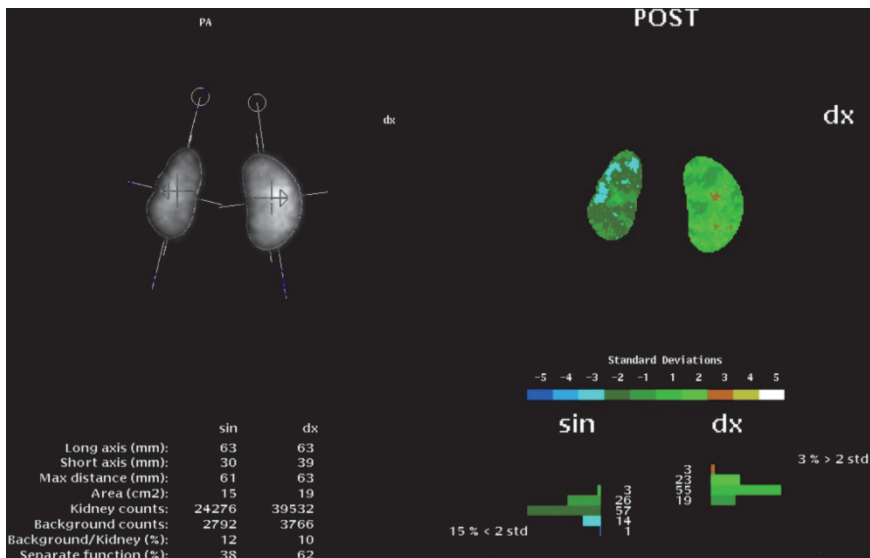


*Figure 3. Findings of renal damage of the left kidney on a one year follow-up DMSA scintigraphy of a girl with a first UTI at the age of one month.*

## 2.2.1 Risk factors for renal damage

It has been shown that delayed treatment of acute infections (14), number of pyelonephritic attacks, congenital anomalies, such as dilated vesicoureteral reflux (VUR) and severe inflammation are risk factors for renal scarring, i.e. permanent renal damage (15, 16). However, despite extensive research we still do not fully know how to best identify patients at risk at an early stage of the disease. Most of the studies published on predictors are based on a selected population, often with inpatients from tertiary centers. Several of the studies also use temperature or CRP in the selection of patients, which limits the possibility to study inflammatory dependent parameters as predictors. In a meta-analyses from 2014, Shaikh et al. found that findings of dilating VUR, abnormal ultrasonography findings, elevated CRP or body temperature, *non-E.coli* infections, and increased polymorphonuclear cell count were strong predictors of renal scarring (16). Findings of congenital abnormalities, indicators of severe inflammation and renal scarring on DMSA, are considered as indicators of a kidney with increased risk of developing end stage renal disease (9).

Congenital anomalies of the kidney and the urinary tract (CAKUT) is a broad spectrum of malformations, including agenesis, hypo-/dysplasia, duplicated collecting system, ureteropelvic junction obstruction, VUR and posterior urethral valves. CAKUT occur in about 1 of 500 births. The most common urinary tract abnormality in infants is VUR. VUR, which is the pathological retrograde flow of urine from the bladder into one or both ureters and the renal pelvis. Although its exact prevalence is unknown, it is estimated to be found in about one third of children with a UTI (17, 18). In a Swedish population based study in 1999 Hansson et al. found VUR in 30% of children <2 years of age with a UTI (7). Preda et al. found VUR in 18% (9% grade III-V) of 290 children <1 year of age, investigated after their first UTI (19). Many clinical programs managing UTIs in children have focused on VUR detection and treatment, since it has been believed to be the primary cause of renal scarring. However, VUR is neither necessary nor sufficient for the development of renal scarring and its importance has been questioned (16, 17, 20-23). Although the focus have changed from VUR to detection of renal damage, dilating VUR (grade III-V) is still considered a significant predictor for renal damage in children with a UTI (12, 16, 24-27). On the other hand, non-dilating low grade VUR (grade I-II ) is a weak predictor of renal damage (28).

The role of CAKUT as a predictor of renal damage is well described, but the optimal choice of modality and time-point of imaging is debated (16, 19, 29). In a study by Preda et al., structural abnormalities in infants <1 year of age from a catchment area of 0.7 million residents were recorded. Of 324 children screened following a UTI, 40 infants had important structural abnormalities (including VUR gr III-IV) in addition to 28 detected abnormalities outside the UTI study, of which 15 were diagnosed at prenatal screening (19). The imaging method that detected the highest number of structural abnormalities was DMSA scintigraphy, which detected 37 out of 40 malformations compared to 30 out of 40 by US.

Scarring as a result of the inflammatory response to the bacterial infection has been described in early experimental studies (30). There has recently been an increasing interest in the inflammatory response to renal infection as a risk factor for subsequent renal damage. Several authors have reported on the use of indicators of inflammation as risk factors for renal damage (12, 31-34). Extensive renal inflammation on early DMSA scans has been described to increase the risk of renal damage (8, 12, 15, 35). There are several nonspecific indicators of the severity of renal inflammation, such as elevated CRP, procalcitonin, white cell counts and high body temperature. In addition, renal swelling has also been suggested as an indicator of extensive renal inflammation (19, 24, 31, 36).

To conclude, normal renal function and normal kidneys at the initial evaluation of a UTI suggests low risk whereas high inflammatory markers, congenital abnormalities (especially dilating VUR) and the finding of acute renal damage on early DMSA scintigraphy indicates high risk for development of severe renal damage.

## 2.2.2  Renal damage and long term complications

The current disagreement about management protocol for UTI is to a great extent caused by the lack of knowledge about the long term risk of late complication of renal damage. The long-term consequences of renal scarring are considered to be impaired renal function, hypertension and pregnancy related complications but the reported frequencies vary greatly (37). In a meta-analyses by Toffolo et al., only 0.4% of children with a febrile UTI, normal renal function and normal kidney at start showed a reduced renal function at follow up (37). The prevalence of hypertension varied between 1-35% and pregnancy related events could be found in 12% of the pregnancies. Gebäck et

al. recently published two population based 35-year follow-up studies of 86 women with their first UTI in childhood (38, 39). They found significant decrease in kidney function and increased frequency of hypertension in those with bilateral and severe renal scarring. To summarize, the published reports indicate that the risks of late complications are low and thus justifies questioning of general extensive follow-up protocols after uncomplicated first UTI in infants.

## 2.3 Imaging

The role of imaging in infants with their first UTI is to identify cases with increased risk for long-term sequelae by diagnosing risk factors, such as obstructive malformation, dilating VUR, renal inflammation and congenital or acquired scarring (28, 40). Imaging is also used in the diagnosis of UTIs in equivocal cases, and for detection and follow-up of complications as abscesses. The most commonly used methods are US, DMSA scintigraphy, and voiding cystourethrography (VCUG). The US technique continuously improves with higher resolution, better contrast and reduced artefacts. MRI is also a rapidly developing technique with great potential in the field, but its use as a standard procedure has so far been limited by availability, cost and the need for sedation in many children. Computed tomography (CT) is not routinely used in the primary investigation of a UTI in order to limit the exposure of ionizing radiation to the pediatric population.

### 2.3.1 Ultrasound

The commercial use of US in medicine dates back to 1963 when B mode (brightness mode) devices were constructed, enabling the examiner to visualize a two-dimensional image. In 1980s the real-time US started to appear, followed by significantly improved image resolution and the introduction of contrast-enhanced ultrasound (CEUS). In the 1990s, the field went one step further with three-dimensional (3D) and even four-dimensional images. US is presently a widespread technology based on the advantage of being noninvasive and lacking ionizing radiation. Thus, the method has advantages that are of great value to the pediatric population. In addition, the lower body mass of children compared with adults is optimal in order to achieve high resolution images. It is often possible to perform real-time US examinations without sedation even in children that are not cooperating. In clinical practice

US is currently the most commonly used imaging method for assessing the kidneys and the urinary tract in children. The disadvantage with the method is that it is investigator-dependent and requires thorough validation for both clinical practice and research.

The standard examination of the urinary tract includes evaluation of the kidneys, pelvicalyceal systems and the bladder. The mid part of the ureters can normally not be visualized if not dilated. The normal appearance of the kidneys in infants vary with age. Renal size increases with age and is related to body size (41). In younger infants the cortex is thinner and hyper/iso-echogenic relative to the liver in contrast to what is seen in older children (Figure 4). The neonatal kidney can also show persisting fetal lobulation with marked hypo-echogenic medulla. Evaluation of the bladder is dependent on the degree of filling of the bladder, which is not controllable in infants without catherization.
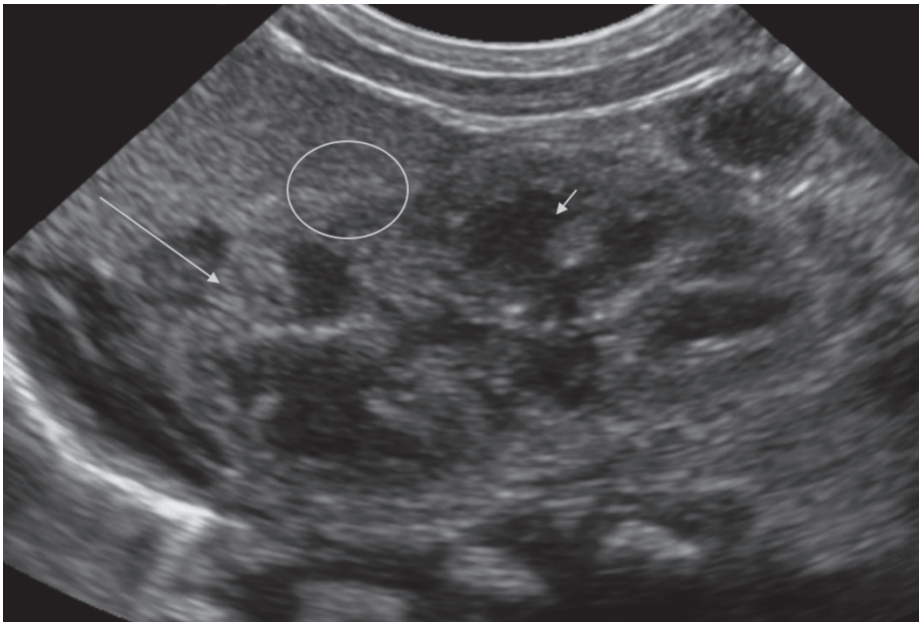


*Figure 4. US appearance of a one-day old infant showing normal findings for the age with iso-echogenic renal cortex relative to the liver (circle) hypo-echogenic medulla (arrowhead) and persistent fetal lobulation (arrow).*

**Value of early US in UTI**

The value of an early US in imaging at the first UTI is contentious. The need for an US to detect congenital malformations at time-point of a first UTI has decreased, as many of the malformations are currently detected at prenatal screening (42-45). There are conflicting reports on the impact of the use of an early US on management or care in settings with prenatal screening (19, 21, 24, 44-49). US has been shown to have a low sensitivity in detecting VUR (45, 49, 50). However, the importance of VUR has been downplayed as the focus has changed to detection of renal damage. The ability of US to detect acute and permanent renal damage is limited (29, 51-53) and the ability of US to predict renal damage is shown to be low (43, 45, 54). However, the results are more promising if used together with additional indicators of renal damage, such as for example CRP or procalcitonin (12, 32).

**US findings in UTI**

The role of US in the evaluation of the urinary tract is well established regarding the detection of congenital malformations. Findings indicating malformation at the initial US are changes in renal size, position, shape or dilation of the urinary tract. Investigation of the bladder can reveal increased bladder thickness and ureteroceles. The sensitivity and specificity of an early US to detect acute pyelonephritis has been proven to be low (29, 52, 53). However, there are several findings that can be seen on US during the acute phase of the infection (Figure 5) (55, 56). The ureters and renal pelvis can be slightly widened with or without thickened urothelium. There might also be thickening of the bladder wall with thickened urothelium. In some cases, floating particles can be seen in the bladder. The kidney changes may include altered echogenicity with loss of cortico-medullary differentiation, changes in power-doppler signal or increased renal size due to swelling. The dilatation of the pelvicalyceal system is preferably described both for the calyces as well for the renal pelvis at the level of the renal hilum in the anterior-posterior view (AP diameter). Renal size can be evaluated by renal measurements of length and width, with calculation of renal volume with the ellipsoid formula if a 3D technique is not used.
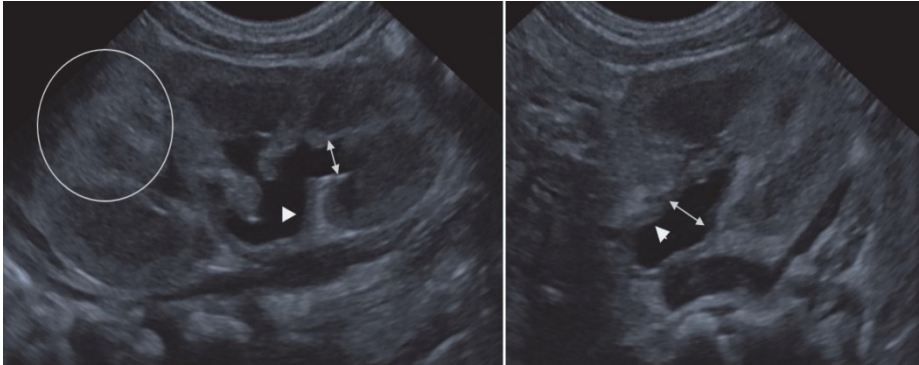
*Figure 5. US findings in a one-month old infant with acute pyelonephritis. There is reduced cortico-medullary differentiation (circle) apically, thickening of the urothelium (arrowhead), and a slight dilatation of the renal pelvis (distance arrow).*

Renal swelling in children with a UTI was rarely described before 1980s, when Johansson et al. and Dinkel et al. reported renal enlargement observed on US in children with a UTI (57, 58). They found a mean renal volume increase by 150-175% to normal values for the group with clinically diagnosed upper UTI. Johansson et al. also performed repeated US up to seven weeks after the infection and described regression of swelling in 3-4 weeks, after which no additional significant decrease was seen (57). These findings were not followed up until recently, when reports were published on the relationship between renal swelling and inflammatory parameters, renal damage, and VUR (19, 24, 31, 36). These reports suggest renal swelling on early US, probably reflecting intense renal inflammation, to be a significant predictor for renal damage. However, these findings need to be assessed further in prospective studies including validation of the US method.

## 2.3.2  Magnetic resonance imaging

The first clinical useful image of a patients' internal structures using MRI was produced in August 1980 at the University of Aberdeen. Currently, MRI is a widespread imaging method in clinical practice based on the ability to produce high contrast images of soft tissue without the need of ionizing radiation. In addition to conventional anatomical images, MRI can be applied to obtain microstructural and functional information on the organ systems. MRI is an established method to study the morphology of renal tissue but can also be used to study the renal function. MRI urography is a dynamic contrast enhanced method that is used in clinical practice in several centers to evaluate the kidneys and urinary tract, especially for complex congenital anomalies in children. This imaging method allows depiction of deep anatomical structures together with functional evaluation (59, 60) but the application is limited by the use of gadolinium based intravenous contrast agents, long duration of the examination that might require the use of sedation as well as time consuming data analyses. Several alternative MRI methods for functional renal imaging, such as DWI, arterial spin labelling, blood oxygen level-dependent MRI and elastography have been developed. However, their potential in clinical practice is still being researched. The drawback of using MRI is the need for sedation in younger children that cannot cooperate and lie still during the relatively long examination. Despite this, MRI is often the preferred method over for example CT in examining soft tissue, as it provides superior diagnostic information without exposing the infant to ionizing radiation. Infants up to six months can often be examined after feeding without using medical sedation, using an optimized MRI protocol. As infants are more sensitive to ionizing radiation and have an immature renal function, these new methods are of particular interest for this patient group.

## Diffusion weighted imaging

In the 1990s the development of the MRI technique enabled acquisition of images based on the diffusion rate of water. DWI is a noninvasive MRI method that demonstrates pathology based on water motion states at the cellular level (61). DWI reflects the microstructure of the tissue, as diffusion of water is hindered by tissue structures in the intra- and extracellular space, such as cell membranes and macromolecules in biological tissue (61, 62). Changes in tissue structure, for example increased cell count, cell swelling or cell necrosis, alters the diffusivity and can be measured by DWI (Figure 6) (63). DWI is currently an important diagnostic tool in clinical practice for the whole body and is becoming an increasingly used tool in abdominal imaging. Since DWI does not require any contrast agents and the acquisition time is relatively short, it is frequently added to the standard MRI protocol. Moreover, the use of parallel imaging techniques, stronger gradient systems and multi-channel coils have improved the usability. The high blood flow and water transport function of the kidney makes it especially suitable for DWI imaging. Application of the method in renal imaging has gained increasing interest because it provides information on both the function and morphology of kidneys without the injection of contrast medium (64 , 65). Despite its potential in renal imaging, DWI is still under research, since the clinical use is limited by its motion sensitivity, lack of standardized acquisition parameters and complexity of result interpretation.
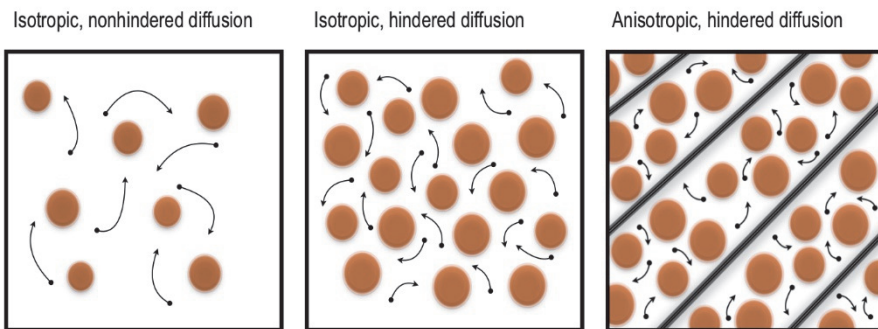


*Figure 6. Illustration of nonhindered, isotropic hindered and anisotropic hindered diffusion in biological tissue. In pathological tissue, as in pyelonephritis, the diffusion is hindered compared to normal tissue. In anisotropic hindered diffusion, the diffusion is hindered in some directions due to tissue structures, such as tubular walls. Figure design Nora Odqvist.*

**Technique**

The DWI image is a modification of a T2 sequence, reflecting the water content of the tissue, with an addition of a diffusion sensitizing gradient. The resulting image reflects the diffusivity as well as the water content of the tissue, where areas of hindered diffusion have high signal in relation to areas of free diffusion that have low signal. The magnitude of the diffusion sensitizing gradient i.e. the diffusion weighting, is expressed by the diffusion factor b. The higher the b value the stronger the diffusion-weighting of the image. At lower b value, the resulting signal is influenced not only by diffusivity, but also to a higher degree by the water content of the tissue.

Apparent diffusion coefficient (ADC) is a parameter calculated from the DWI images used to quantify the diffusivity in the tissue. The ADC value reflects the mean diffusion rate per voxel and is usually expressed in $(x10^{-3})$ $mm^2/s$. The ADC values are visualized voxel-by-voxel, on an ADC map used to confirm and characterize pathological tissue. The ADC value is calculated from at least two images, one with and one without (b0) diffusion weighting, using the equation; $ADC = -ln (Sb/ S0)/b$, where S is the signal intensity for the chosen b value (62). The ADC is generally high in kidneys because of high water content and the contribution of flow (tubular and vascular) that influences the ADC (66). The cortical ADC is often reported as higher than in the medulla in healthy kidneys, but there are diverging reports, which are probably due to variability in for example scanning, hydration status, and age, between the studies (67, 68). The ADC has also been shown to be age dependent in children, with increasing ADCs with age, particularly during first year of life (67, 69, 70). The absolute ADCs are affected not only by tissue properties, but also by factors related to different acquisition strategies, such as choice of b values, technique used to manage breathing artefacts, and diffusion direction in single directional scanning (68, 71).

The usability of DWI is limited due to its high sensitivity to respiratory motion, as intra-scan respiratory motion gives rise to artefactual noise in terms of image blurring and signal decay (72). Three main approaches are used to manage breathing artefacts in abdominal DWI scanning: respiratory navigation, breath-holding, and free breathing scanning. Respiratory navigation and breath-holding techniques are most commonly used for DWI (73-76), but these techniques are impractical when imaging infants. Free-breathing scanning with signal averaging has therefore been proposed for motion artefact reduction in DWI examinations, where breath holding or navigation scanning is not an alternative (73, 77), as for example in infants. Free-breathing scanning affords high signal-to-noise-ratio (SNR) and high spatial resolution using multiple

slices and excitations with signal averaging of motion artifacts over an extended scan time duration (77). However, despite the use of respiratory motion compensation that can reduce the visible artefacts, the DWI technique has an inborn sensitivity to motion and the resulting image and measures can be expected to be influenced by motion effects (72).

**Renal DWI**

There is intensive ongoing research activity on the potential usefulness of DWI as an imaging biomarker for renal diseases, such as fibrosis estimation in chronic kidney disease, acute graft dysfunction and characterization of focal lesions (68, 78, 79). In patients with chronic renal failure, a correlation has been found between the degree of fibrosis, impairment of renal function and reduced ADCs (64, 80, 81). DWI has also shown promising results in the assessment of renal allografts function with decreased ADCs in acute rejection, acute tubular necrosis and immunosuppressive toxicity (82-85). However, the ability to differentiate between these underlying conditions has not yet been shown. There are a few publications on the use of DWI in the evaluation of the functional status in obstructed kidneys in adults, but the results are so far contradicting and more research is needed in this area (86-88). DWI has a potential to characterize renal lesions based on cellular density, but the ability to differentiate between benign and malignant renal masses has so far been reported to be moderate (89). However, studies have shown DWI to be a sensitive tool in the detection of pyelonephritic lesions in native and transplanted kidneys in the adult population (90-95).

**Renal DWI in children**

Despite the benefits of using DWI in children, there are only a few reports on the use of DWI in renal diseases in children. A retrospective study has evaluated DWI on obstructed kidneys and found no correlation between ADCs and morphological or functional changes detected by functional MRI urography (69). The effect of VUR on DWI parameters has also been studied with conflicting results. However, no difference in ADCs could be seen between kidneys with or without VUR (70, 96). In oncology there is evidence supporting the use of DWI parameters as potential biomarkers for preoperative assessment and therapy response in nephroblastoma (97-100).

**DWI in acute pyelonephritis**

Acute pyelonephritis is in DWI characterized by a focal area of hindered water diffusion reflecting the inflammatory response in the renal parenchyma (62, 93, 95, 101-103). In the initial phase of pyelonephritis, there is an accumulation

of inflammatory cells, such as neutrophilic granulocytes, mainly in the tubules. This increased cell count in the affected tissue is suggested to result in decreased diffusion compared to normal tissue. Thus, DWI may be used to evaluate parenchymal involvement of a UTI. There has been a growing interest in the potential usefulness of DWI in the detection of acute pyelonephritis in children. So far, studies in the pediatric population have mainly included sedated or older children (104-108). With the use of respiratory triggering in sedated infants (107, 108), or free breathing in older children (106), DWI has shown high sensitivity for detection of acute pyelonephritis. However, the need for sedation limits the usability in younger children if conventional protocols are used. The use of a short, free breathing scan protocol would enable the method to be used in non-sedated infants, but the feasibility of this method has not been studied in this patient group. There is also no data available on the diagnostic criteria or cut-off ADC values to be used in the diagnosis of acute pyelonephritis. Moreover, the diagnostic performance regarding the potential to predict outcomes, such as acute or permanent renal damage, needs to be tested before implementing the method in routine clinical management.
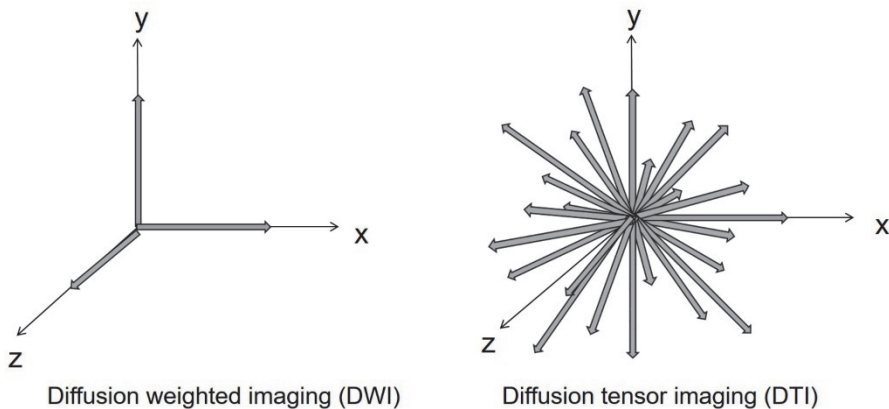
## Diffusion tensor imaging



*Figure 7. Illustration of the diffusion directions measured with conventional DWI with three directions and DTI with 24 directions.*

The number of diffusion sensitizing directions used in DWI scanning varies among different reported image acquisition techniques. Conventional DWI in three orthogonal directions is routinely used in clinical examinations of the

abdomen, but does not account for any directionality of the diffusion that may affect the diffusion in biological tissue. DTI is a technique measuring diffusion in multiple directions and although a minimum of six directions is required, more directions are often used (Figure 7). DTI has the ability to add information on tissue microstructure by analyzing the orientation and degree of anisotropic diffusion (109-111). This method might be especially suitable in directional tissue, as in the kidneys, with radially oriented tubules, ducts and blood vessels (Figure 6) (112). In several studies, DTI has shown to be more sensitive compared with DWI in assessment of renal pathology and altered renal function (75, 85, 109, 113, 114).

**Technique**

Several parametric maps can be calculated from the raw diffusion tensor image. In addition to mean diffusivity (or Trace) map and ADC map, DTI allows for the calculation of a fractional anisotropy (FA) map, describing the amount of anisotropy per-voxel basis (111). The FA-values in the FA map range from 0 to 1, with 0 standing for maximum isotropy and 1 for unidirectional diffusivity. FA maps thus show the directional preference of diffusion in each voxel. Further analysis can give information on the direction of the diffusion, fiber orientation maps, as well as 3D reconstructions of fiber bundles based on the longest vector orientation by defining seed points and propagation of rays (fiber tracking) (67, 109, 110, 115). The first to report on renal application of DTI in healthy volunteers was Ries et al. (75), demonstrating the anisotropic nature of the kidney by using FA maps. Renal FA values in healthy subjects is reported to be higher in the medulla than in the cortex (109). This is considered to be due to the tissue microstructure with radiating tubules, ducts and vessels in the medulla.

Although renal DTI has been proven to be feasible with reliable and reproducible results (116-119), the results are affected by several parameters, such as the number of diffusion directions, choice of b values, strength of the magnetic field, and technique for handling respiratory motion artefacts. DTI needs to be performed in at least six different diffusion directions. Increasing the number of directions will increase image quality, but also alter the FA parameters (120). DTI is in similarity to DWI very sensitive to physiological motion, especially respiratory motion which is mainly directed cranio-caudally. Respiratory motion that occurs during scanning are managed as conventional DWI imaging, using free breathing, single or multiple breath-hold, or respiratory triggering techniques.

## Renal DTI

The feasibility of DTI has been studied in various renal diseases, including chronic renal disease, transplanted kidneys, renal masses, and pyelonephritis. Recent studies assessing the potential of DTI in the evaluation of chronic renal disease all show decreased FA, reflecting the severity of the disease (113, 121, 122). The majority of these studies also demonstrated a correlation between DTI parameters and renal function (121, 122). Transplanted kidneys are well suited for DTI scanning, since their position in the iliac fossa decreases breathing motion artefacts and thus allows free breathing scanning. DTI has been used in the assessment of transplanted kidneys mainly for noninvasive functional evaluation but the ability to differentiate between different allograft pathologies remains to be investigated (85, 114, 123). There are a few reports on the potential of DTI to provide complementary information to DWI in assessment of renal cell carcinoma, but the role in the assessment of other renal lesions is less well established (119, 124, 125).

## Renal DTI in children

Renal DTI studies have shown the potential of the method to evaluate renal pathology in older children (67, 126-129). Reports have suggested that DTI-based parameters, including ADC and FA, are potential biomarkers in the evaluation of kidneys with ureteropelvic junction obstruction (126, 127). By using DTI in children with autosomal recessive polycystic kidney disease for characterization of the renal parenchyma, Serai et al., found changes in diffusion anisotropy and kidney structure, showing the usefulness of the method to stage and monitor kidney disease (128). In a study by Jaimes et al. a small cohort with diverse urological conditions was assessed with DTI, demonstrating disarranged tracks on tractography, but no significant change in ADC or FA values in abnormal moieties (67). In a case report of a patient with histology-confirmed xantogranulomatous pyelonephritis, DTI changes consisting of altered FA and inconsistencies in the tubule pattern on tractography were demonstrated (130).

## DTI in acute pyelonephritis

The value of using DTI in the diagnosis of acute pyelonephritis is sparsely studied. In a study comparing free breathing DTI and conventional respiratory triggered DWI, DTI demonstrated significantly better image quality and showed agreement with DWI in all patients regarding pyelonephritic lesions (104). The authors concluded that a free-breathing DTI scan protocol could be used as a superior alternative to conventional DWI. With the ability of DTI to

be more sensitive to changes in renal microstructure, the method may provide additional information compared to DWI that may improve the characterization of renal lesions. However, the potential of DTI to characterize and distinguish between renal lesions, such as acute pyelonephritic lesions, permanent renal damages or dysplastic changes has not been shown.

**Image analyses**

Conventional anatomical MRI images, such as T2 weighted images, are analyzed qualitatively by visual inspection for the assessment of pathological conditions relative to normal findings. Functional imaging techniques, such as DWI and DTI enables quantification of data derived from variables that can be measured in physical units. This quantitative information has the advantage of more accurate comparison between tissue regions and among subjects. The multiparametric approach can combine qualitative as well as quantitative information from different imaging methods, thereby allowing comprehensive characterization and understanding of the biologic processes. This method has several potential clinical roles and is under development for use for example in characterization of renal masses in adults (131). Furthermore, pattern recognition can be performed either manually or, in case of larger materials, by machine learning to improve the renal characterization.

## 2.3.3 DMSA scintigraphy

DMSA scintigraphy is often regarded as the reference method with a high sensitivity for the detection of acute pyelonephritis and permanent renal damage (8). The method also enables evaluation of relative renal function. In DMSA scintigraphy, 99mTc-dimercaptosuccinic acid is injected intravenously and is then accumulated in the tubular cells with slow excretion. The accumulation of the isotope is then visualized by using a gamma camera (collimator). Images are obtained after a few hours with subsequent evaluation of uptake reductions in the renal parenchyma and estimation of the relative renal function. The acquisition time for the images is approximately ten minutes. Focal or multifocal uptake reductions are often attributed to acute pyelonephritis or permanent renal damage, while generalized changes can be seen in congenital malformations or dysplasia. Since many of the acute defects heal within 2-6 months, a late DMSA scan can be used to confirm permanent renal damage (8, 132).

DMSA scintigraphy has drawbacks, such as the need for intravenous access, exposing the infant to ionizing radiation, limited availability and possible need for sedation. The method also has limitations as a golden standard due to low

spatial resolution, detection of congenital malformations and limited ability to differentiate between acute and permanent lesions or hypo-/dysplasia (133). Alternative methods, such as gadolinium-enhanced MRI and contrast-enhanced CT, have all been suggested to detect renal lesions, but have the drawbacks of requiring the use of contrast agents or ionizing radiation.

## 2.3.4 Voiding cystourethrography

The current primary diagnostic procedure for evaluation of VUR is VCUG. In VCUG, the bladder is filled with contrast via a bladder catheter and radiographs are obtained while the child voids. The procedure lasts approximately 30 min including preparations. VCUG is a well-established method with an international grading system and is also regraded as the method of choice when imaging urethra in infants. The drawbacks of the method are the invasive nature, use of ionizing radiation and the need for sedation in some children. Contrast-enhanced voiding urosonography with intravesical contrast is being increasingly used as an alternative, especially in Europe. Other alternatives to VCUG, including direct or indirect scintigraphy, or MRI voiding cystography, are used less frequently. The US or MRI based methods have the advantages of not using ionizing radiation and the ability of simultaneous imaging of the renal parenchyma.

## 2.3.5 Imaging algorithm

The time-point and extent of imaging for a first febrile UTI has been debated for several years, but no consensus has been reached (40, 134). There is a lack of thorough evidence on the role of risk factors for renal damage and long term sequelae. The difficulties in balancing between high sensitivity and specificity for detection of risk factors against drawbacks, such as increased radiation burden and high cost, have led to the development of several partially different imaging recommendations. Many earlier guidelines have focused on the detection of VUR, but with the knowledge that VUR is neither necessary nor sufficient for renal scarring, the focus has changed to the detection of renal involvement (16). This top-down approach includes early US and DMSA scans. There are currently several different guidelines; the National Institute of Clinical Excellence (NICE 2007)(135), the American Academy of Pediatrics (AAP 2011) (42), European Association of Urology/European Society for Pediatric Urology (EAU/ ESPU 2016) (136), the Italian Society of Pediatric Nephrology (ISPN 2011) (137), the Canadian pediatric society (138), and the

top-down approach used in Gothenburg (26, 27) (Table 1). In the studies included in this thesis, we used the top-down approach with an acute US and DMSA scan. VCUG was performed only if there was renal involvement on the early DMSA scan, or dilatation of the urinary tract on the US. A late DMSA scan was performed in patients with an abnormal first DMSA scan.

*Table 1. Imaging recommendation for a first UTI in infants up to one year of age in seven guidelines.*

| | US | VCUG | Acute DMSA | Late DMSA |
|---|---|---|---|---|
| NICE 2007 <6m | All children | Atypical/recurrent UTI | - | Atypical /recurrent UTI |
| NICE 2007 ≥6m | Atypical /recurrent UTI | Atypical/recurrent UTI and risk factor | - | Atypical /recurrent UTI |
| AAP 2011 | All children | Abnormal US | - | - |
| ISPN 2011 | All children | Abnormal US or risk factor | - | Abnormal US or VCU |
| CPS 2014 | All children | Abnormal US or recurrent UTI | - | Uncertain UTI diagnosis |
| EAU/ESPU 2016 | All children | Either VCUG or DMSA | Either VCUG or DMSA | - |
| Gothenburg | All children | Abnormal DMSA or US | CRP >70 or risk factors | Abnormal first DMSA |

NICE – National Institute of Clinical Excellence; AAP - American Academy of Pediatrics; ISPN - Italian Society of Pediatric Nephrology; EAU - European Association of Urology; ESPU - European Society for Pediatric Urology; CPS – Canadian pediatric society

Atypical UTI is for example seriously ill infant, septicemia, elevated creatinine levels, no response to treatment. Risk factor is for example dilatation on US, family history of VUR, *non E.coli* infection

# 3  SPECIFIC AIMS OF THE STUDIES

The specific aims of the studies in the thesis were:

I. To evaluate renal swelling in infants with a first UTI by studying acute increase and subsequent decrease of renal length and volume on US, and the correlation with CRP and body temperature.

II. To evaluate renal swelling on US as a predictor of acute and permanent renal damage in infants with their first UTI.

III. To prospectively assess the feasibility and performance of DWI for detection of pyelonephritis in non-sedated free breathing infants.

IV. To study the potential of multiparametric DTI to characterize renal lesions in infants with their first UTI.

# 4 PATIENTS AND METHODS

## 4.1 Overview of aims and methods in the studies

Below follows an overview of the aims and methods in the four studies (Figure 8). In study I -II renal swelling was studied by measuring renal size with US. In study III-IV the potential of DWI and DTI in imaging of renal lesions was evaluated.



*Figure 8. Overview of aims, patients, methods and analyses from study I-IV.*

## 4.2 Patients
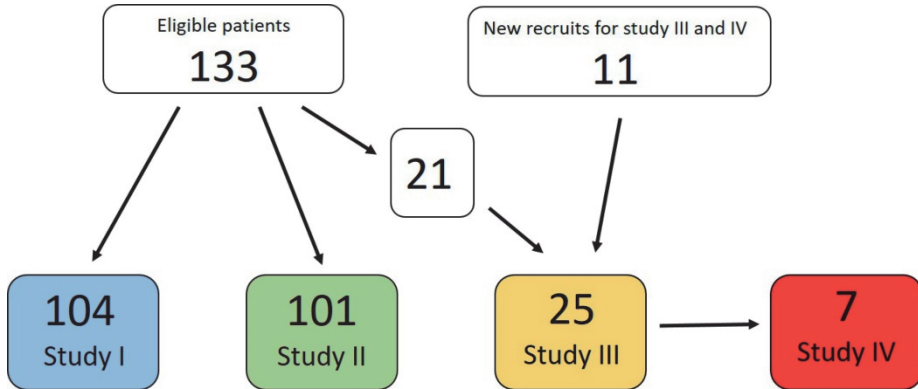


*Figure 9. Flowchart of patient eligible for inclusion in study I-IV. Study dropouts are not displayed.*

The subjects in the studies in this thesis were all included over a period from March 2011 to January 2013 at The Queen Silvia Children's Hospital, Gothenburg, Sweden, which is a primary health center for infants with a UTI. Patients eligible for the studies were infants with their first community acquired symptomatic UTI; <1 year of age for the US studies and <6 months of age for the MRI studies. The majority of the patients were consecutively included as they were diagnosed when visiting the emergency room. Some of the patients in the MRI studies were included at the time-point of the DMSA examination. The diagnosis of UTI required bacteriuria, defined as ≥100 000 colony forming units per mL in clean catch urine samples, or any growth in suprapubic bladder aspirates. Patients with negative urine culture or known urogenital malformations were excluded. The inclusion in study I-II was performed during March 2011- June 2012, and the inclusion in study III-IV from May 2011 to January 2013. There were 133 patients eligible for the studies with 11 new recruits for study III and IV (Figure 9).

The patient characteristics in the cohort of included children, described per study, are shown in Table 2. All studies were approved by the Swedish Regional Ethical Review Board at the University of Gothenburg (Study I, II; Nr: 372-10 and Study III, IV; Nr: 214-11).

*Table 2. Patient characteristics in the individual studies.*

| Study no | I | II | III | IV |
|---|---|---|---|---|
| Number of patients | 104 | 101 | 25 | 7 |
| Number of boys | 58 (56%) | 55 (55%) | 15 (60%) | 4 (57%) |
| Age (months) mean/median | 3.9 | 3.9 | 1.7 | 1.2 |
| Maximum temperature (°C) mean/median | 39.2 ±1 | 39.2 ±1 | 38.4 (37.0-40.3) | 38.6 (37.2-40.3) |
| CRP (mg/L) mean/median | 91.0 ±67 | 91.7 ±68 | 52 (8-180) | 90 (8-180) |

Age, max temperature and CRP are expressed as mean ±SD for study I and II, and as median (min-max) for study III and IV.

# 4.3   Imaging protocols

## 4.3.1   Study I and II

For both the US studies the infants underwent an US during the acute phase of the infection and a follow-up US examination after one month. CRP and body temperature were measured repeatedly during the acute phase and the highest value were used. For study II the imaging protocol included an acute DMSA scan, and a late (one year) DMSA scan in infants that had abnormal acute DMSA scans. As part of our local clinical routine, a VCUG was included in patients with abnormal acute DMSA scans, dilatation at acute US, or in cases with a UTI recurrence.

## 4.3.2   Study III and IV

For studies III and IV MRI examinations were performed during the early phase of the infection The MRI protocol included both DWI and DTI sequences to enable patients to be included in both studies. The imaging protocol in study III also included data from the acute DMSA scan that was performed as part of the local clinical routine.

# 4.4 Imaging methods

## 4.4.1 Ultrasound

The acute US was performed within a few days (1 day±0.9 (mean ±SD)), after being admitted to the hospital, as part of our clinical routine. The follow-up US was performed after one month (32 days±5.8) as a scheduled exam. The examiners who performed the follow-up examinations were blinded to the results of the acute US. The infant was investigated by a radiologist or a specially trained radiographer. No specific patient preparations were used for the examination. The US was performed without sedation, but oral sugar solutions was sometimes used to calm restless infants.

All the US examinations were performed with an iU22 ultrasound system (Philips Healthcare, Best, the Netherlands). The US examinations were performed according to the local routine in our department for evaluation of the urinary tract, including the kidneys and the bladder, with the infant in a supine position. The study protocol included measures of renal size (Figure 10) and dilatation of the urinary tract, in addition to evaluation of malformations, such as duplications. For measurement of renal size, the renal length (L) was measured with the probe positioned to achieve maximum renal length. The kidney depth was measured in the kidney hilar region in both the longitudinal (D1) and transverse planes (D2), and the kidney width (W) was measured in the transverse plane (139). The kidney volume was calculated with the ellipsoid equation: kidney volume (mL) = L x W x (D1 + D2)/2 x 0.52. The duration of the US examination was normally 15-30 min. The height of the infant was measured after the examination if this had not been measured and registered in the hospital information system within three days before the examination.

For validation of the US method, intra- and interobserver variability were determined. Interobserver variability was assessed by two radiologists, who performed independent length and volume measurements in nine infants, immediately after each other. Intraobserver variability was determined by allowing the examiners to perform a second independent length and volume measurement as a renewed examination right after the first examinations, without the patient or the observer leaving the room between the examinations. For all US examinations, examiners were blinded to previous results
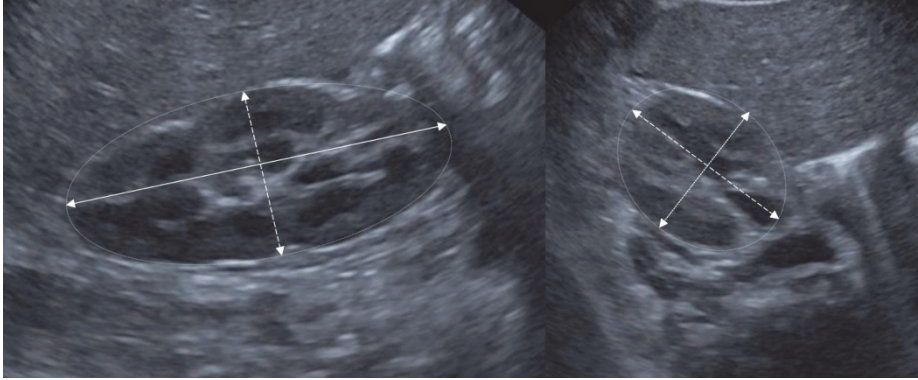
*Figure 10. Illustration of length (full line), width (dotted line) and depth (dashed line) measurements on the longitudinal and transverse planes for evaluation of renal size with the ellipsoid as a reference.*

## 4.4.2 Magnetic resonance imaging

The MRI examination was performed in median 10 days (range 1-17) from admission to the hospital. The included patients were scheduled for an MRI examination as close to the DMSA examination as possible. Of the 25 infants, 10 underwent the MRI examination the same day as the scintigraphy, but due to parent preference, the time interval varied from -23 to 6 days, with a median of 0 days. The instructions to the parents were to time the feeding and sleep of the infant to the time-point of the scheduled examination. The infants were fed at the radiology department prior to the MRI examinations and were moved to the MRI camera and prepared for the examination after being brought to rest. The scanning was performed with the infants resting in the camera with the parents being present during the examination. Sedation or anesthetics were not used (Figure 11). The examinations were performed on a 1.5 T MRI system (GE Signa HDx Twinspeed, GE Medical systems, Waukesha, WI, USA). The scan protocol included a fat saturated T2 weighted scan, a DTI scan (b=0,700 s/mm², 24 diffusion directions) and a DWI scan (b=0,100,400,700). In cases where the infants were restless and there was a risk of not being able to finish the whole protocol, the DTI scan was prioritized over the DWI scan. If possible, any scans with motion artifacts were repeated. The total examination time including the preparations was 30 min, with an effective acquisition time under 15 min. The acquisition times for the DWI and DTI scans were 2.34 min and 58 seconds, respectively. The workstation on the scanner (Advantage

Workstation, GE Healthcare, USA) was used to calculate images used for further analyses. For DWI; b700 and ADC and for DTI; b0, b700, ADC, FA and tractographies were produced.



*Figure 11. Image illustrating MRI examination of an infant in our department.*

### 4.4.3   DMSA scintigraphy

DMSA scintigraphies were performed in accordance with the guidelines of the Pediatric Committee of the European Association of Nuclear Medicine (25). In short, the scanning was performed 2-4 hours after the intravenous injection of DMSA. Planar images were obtained from one posterior and two oblique projections, with a total acquisition time of approximately ten minutes. Hermes software package (Hermes Medical Solutions, Nuclear Diagnostics AB, Stockholm, Sweden) was used of the evaluation of uptake reductions and split renal function. All images were reviewed by the same senior nuclear medicine specialist, blinded to the other results. A kidney without uptake defects and relative renal function ≥45% was classified as normal. An uptake defect or split function <45% on the acute DMSA scan was classified as acute renal damage, and a corresponding finding at the late scan was classified as permanent renal damage.

### 4.4.4   Voiding cystourethrography

VCUG was performed according to standard procedures at the Pediatric Radiology Department. The images were read by a senior pediatric radiologist, and VUR was graded I-V according to the International Reflux Study in Children (26)

## 4.5   Image analyses

### 4.5.1   Study I and II

All findings on the US examination, DMSA scans and the VCUG were registered in study protocols. Renal length and volume measurements from the US were transferred into standard deviation scores (SDS) using the nomograms developed by Vujic et al. (41). This was performed to be able to evaluate the renal size independent of the increase over time related to patient growth. Renal swelling was defined as renal length and volume >2 SDS, related to body height.

### 4.5.2   Study III

The analyses of the images were performed using the ViewDex software (version 2, Gothenburg, Sweden), where the image series were displayed in a blinded randomized order (140, 141). The analyses of the MRI images were performed by three senior pediatric radiologists and the DMSA images were reviewed by a senior nuclear medicine specialist all blinded to the other results. For evaluation of pyelonephritic lesions on the DWI images, any presence of focal signal increase in the parenchyma was registered, and for DMSA scans any focal uptake reduction was noted. The agreement between the findings of pyelonephritic lesions on the DWI and DMSA images was used to evaluate the diagnostic performance of DWI, using the DMSA scans as the reference. The DMSA, DWI and T2 images were also evaluated for indications of hydronephrosis and duplicated systems, with the use of the T2 image as a reference. Since DMSA scintigraphy, the currently used reference method, has known limitations, the findings on DWI were analyzed further using an approach with the aim to reach a consensus diagnosis (142). Since DWI is a novel method for the evaluation of UTI, the consensus diagnosis was determined by an expert panel including the same pediatric radiologists and nuclear medicine specialist that had performed the initial analyses. The expert panel reviewed and discussed the full image data set of each case and consensus were reached for all patients.

## 4.5.3  Study IV

For the evaluation of the descriptive findings from the visual readings of the DTI images, a diagnostic PACS station and monitor (Coronis 5MP; Braco, Belgium) was used. The images were evaluated by two pediatric radiologists discussing the findings and reaching a consensus. The evaluation included the presence of signal changes in the renal lesions on the b0, b700 and ADC images, as well as distorted or reduced tracks on the tractography. The DMSA images were evaluated as described for study II. The quantitative image analyses were performed with Image J (*1.45s*, National Institute of health, USA) on the b0, b700, ADC and FA maps. The lesions were evaluated with regions of interest (ROI) placed in the center of the lesions, as well as in normal parenchyma in the contralateral kidney. In cases with bilateral findings, normal tissue on the ipsilateral side was used (Figure 12).



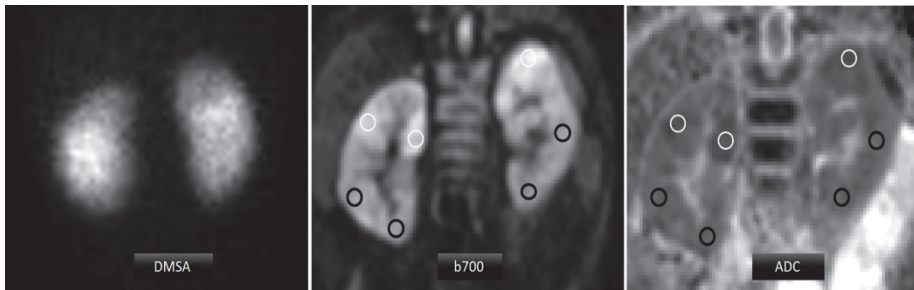*Figure 12. Images showing the placement of the ROI in lesions (white circles), as well as in normal parenchyma (black circles), in a patient with bilateral apical pyelonephritis using the DMSA image as a reference.*

The multiparametric analyses were then performed using a combination of the descriptive and quantitative parameters with the intension of finding different patterns of characteristics for the lesions.

# 4.6 Statistics

Statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) and SPSS software (version 22, SPSS, Chicago, IL). Nonparametric tests were used due to the small sample size of the studies and the presence of not normally distributed variables. A p-value < 0.05 was considered statistically significant.

## 4.6.1 Study I

The Wilcoxon signed rank test was used to compare continuous variables over time. Correlations between measures were assessed using Spearman's rank correlation coefficient. Intraobserver and interobserver variability was expressed as means, coefficients of variance (CV) and intra-class correlation coefficients.

## 4.6.2 Study II

Renal damage was predicted using logistic regression for the patient-level, and using generalized estimating equations with binomial distribution, adjusted for within individual correlations, for kidney-level predictors. The area under the receiver operating characteristic (ROC) curve was obtained for all associations. Sensitivity, specificity and likelihood ratios were used to evaluate the diagnostic performance of renal size in predicting renal damage on DMSA scans.

## 4.6.3 Study III

Agreement between the DWI and DMSA scintigraphy findings was determined using McNnemar's test and Kappa (K) statistics. For K, agreement was defined as: excellent agreement, K > 0.75; fair to good agreement, K = 0.40−0.75; poor agreement, K < 0.40 (143). Kappa statistics was also used for determination of interobserver agreement.

## 4.6.4 Study IV

The Wilcoxon signed rank test was used to compare the DTI variables.

# 5 RESULTS AND DISCUSSION

In this section the major results of the four studies are presented in conjunction with the discussion.

## 5.1 Study I and II

In study I the measurement method was validated and renal size was related to CRP and body temperature. In study II the renal size was related to renal damage on acute and late DMSA. The main results of study I and II are presented in Figure 13.

**Main results of study I**

- There was a significant decrease in renal length and volume between the first and second ultrasound for both the smaller and larger kidney.
- The length and volume of the larger kidney correlated with CRP, but only the renal length correlated with fever.

**Main results of study II**

- Renal damage at the acute and late DMSA scan were significantly correlated with gender, temperature , CRP  and VUR grade 0–II versus III-V .
- Increase in renal length and volume on the first ultrasound predicted acute renal damage.
- Increase in renal length at the first ultrasound and change in renal length and volume predicted permanent renal damage.

*Figure 13. Main results of Study I and II.*

## 5.1.1 Structural abnormalities

At the first US 36% of the 104 patients in study I had structural abnormalities, such as duplication, dilatation of the renal pelvis or other parts of the urinary tract, thickening of the pelvis wall, echogenicity changes or power doppler changes. Duplications were found in three kidneys. Dilatation of the renal pelvis with an AP diameter > 10mm was seen in two kidneys, of which one showed a duplication. Thus, only a few of the patients in the study had structural findings that might have influenced the renal size. There was a decrease in the number of findings of structural abnormalities at the second US, except for findings of duplications. This is in line with earlier studies reporting on transient US findings that can be seen during the acute phase of the infection (55, 56).

## 5.1.2 Renal size

Table 3 gives an overview of the renal size measures from the two studies. Children $\geq$ 6 months had significantly greater renal length (SDS) at both the first and second US examination compared with younger children (p<0.0001, p=0.0012). No differences were found for length measurements in boys compared with girls.

*Table 3. Renal size measures from the first and second US examination.*

| Study no | | I | | II |
|---|---|---|---|---|
| | | Larger kidney | Smaller kidney | All kidneys |
| US 1 | Renal length | 1.90 (±1.54) | 0.86 (±1.01) | 1.39 (±1.43) |
| | Renal volume | 1.67 (±1.13) | 0.84 (±0.90) | 1.30 (±1.08) |
| US 2 | Renal length | 1.01 (±1.33) | 0.46 (±1.02) | 0.74 (±1.22) |
| | Renal volume | 0.65 (±0.93) | 0.38 (±0.94) | 0.55 (±0.89) |
| Reduction in renal length | | 53% p <0.0001 | 53% p<0.0001 | 53% |
| Reduction in renal volume | | 39% p <0.0001 | 45% p <0.0001 | 42% |

Renal length and volume in SDS (±SD)

At a follow-up US after one month, a significant reduction in renal size was shown, indicating swelling of the kidneys during the acute phase of the

infection. Figure 14 shows an example of an infant with reduction in renal size between the first and second US.
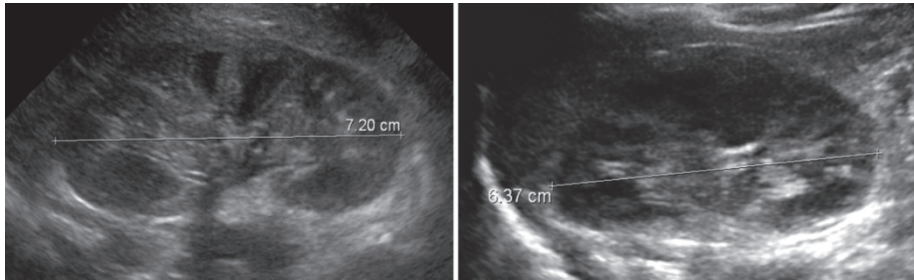


*Figure 14. Six-month old boy with a UTI examined with US during the acute phase showing a renal length of 7.2 cm (4.2 SDS). At follow-up after one month the renal length had decreased to 6.4 cm (1.5 SDS).*

The time-point of the follow-up US was chosen to allow for regression of renal swelling (57, 58), while reducing the risk of any confounding UTI recurrences. The renal size measurements were related to body height using the nomogram described by Vujic et al. in 2007 (41). This nomogram is applicable to the studied population as it is based on a study of a large number of heathy infants from Serbia, including infants up to 12 months of age. The use of body height instead of age, is another advantage of this nomogram, as body height has been shown to be the best predictor of renal length (41, 139, 144). However, the results from study I revealed that the renal size was greater compared to the normal material even at the second US. This might be explained by differences in the methods, which can vary with factors related to both measurement methods and equipment. However, it cannot be excluded that there are differences in renal size between the populations even after relating renal size to body height.

## 5.1.3 Measurement method

US is a highly investigator dependent method, which called for validation of the measurement method used in the studies. In the validation study, we found that volume measurements showed a greater variability than length, which is not surprising as volume estimation is technically more demanding than estimation of kidney length. The volume calculation with the two-dimensional (2D) method also depends upon an approximation of an ellipsoid from the more complex renal morphology (145). As expected, the interobserver variability (CV 3% for length and 17% for volume) was greater than the

intraobserver variability (CV 2% for length and 8% for volume). Since this validation was performed by two experienced pediatric radiologists, the results reflect the potential of the method rather than the application of the method in clinical practice where users with mixed experience perform the measurements. The use of SDS for further analysis made the results independent on renal growth over time. The measurement error is also related to renal size and therefore CV was used for determination of variability. The CV for interobserver variability for renal length was 3%. This means that the measurement error in an infant with renal length of 50 mm would be approximately ±1.5 mm. According to the nomogram, a renal length of +1 SDS would correspond to +5.5 mm compared to the mean, and the mean renal growth in one month would be +5.0 mm. Thus, a renal length >2 SDS, or change in renal length of >1 SDS were regarded as relevant.

Two other aspects could have influenced our renal size results. In children with congenital changes, such as hypo-/dysplasia, the renal size is often smaller than expected and therefore acute swelling might be underestimated. There are also some reports on the influence of the hydration status, with normalized renal size after hydration following a period of restricted fluid intake in older children or adults (146, 147). The influence of hydration is probably less relevant in our clinical setting as the infants were allowed unrestricted oral fluid intake.

By analysing the larger and smaller kidneys separately, we found a significant reduction in renal size, not only for the larger but also for the smaller kidney. This result, in conjunction with the finding of bilateral reduction of renal size > 1 SDS in 16% of the patients is interesting. Bilateral renal swelling has been reported previously (57, 58), but it is unknown whether this swelling is caused by bilateral renal involvement of the infection or merely reflects the general inflammatory process. There are few reports on the incidence of bilateral renal engagement in UTI (148). In the present study, abnormal DMSA scintigraphies were found bilaterally in 11% of the infants indicating bilateral involvement of the infection.

## 5.1.4  Correlation with inflammatory parameters

Renal length, and to a somewhat lesser degree renal volume, correlated with inflammatory parameters, and this was more clearly seen for CRP than for temperature (Table 4). This suggests that the magnitude of renal swelling on US reflects the inflammatory process and the extent of renal involvement of the infection.

*Table 4. Correlation of renal size at first US and change in kidney size to highest CRP and temperature.*

|  | Kidney length at start | Kidney volume at start | Kidney change in length | Kidney change in volume |
|---|---|---|---|---|
| CRP | r=0.45, p<0.0001 | r=0.43, p<0.0001 | r=0.47, p<0.0001 | r=0.31, p=0.0023 |
| Temperature | r=0.33, p<0.001 | p=0.09 | r=0.25, p=0.016 | p=0.87 |

## 5.1.5  Association with renal damage

The association of renal swelling with renal damage has been suggested in a few preceding studies (19, 31, 32, 36). In 2008 Bouissou et al. published a prospective study comparing intravenous treatment alternatives in 383 children with their first UTI (36). They used early US to measure renal length to exclude patients with hypo-/dysplasia, but also found 15 children with enlarged kidneys (>1.5cm above normal for age). This renal enlargement was associated with an increased risk for renal scars as a sign of renal damage. In a study of the value of early US in UTI, Preda et al. examined 290 infants up to 1 year of age with acute pyelonephritis and found that 28% of the patients had a renal length greater than 2 SDS normalized to body height (19). They observed that renal length was correlated with inflammatory parameters, such as temperature and CRP. Kidney length measurements were also related to the presence of acute DMSA scan abnormalities.

The association of renal size with renal damage has been studied by Cheng et al. and Hung et al. in two large scale studies (24, 31). Table 5 shows a comparison of the results from these two studies with the present study. These three studies are in line showing renal length to be a predictor of renal damage at the late DMSA scintigraphy. However, there are differences regarding the

strength of the ability to predict renal damage. This is probably related to the selection of patients, with older children and with more severe disease in the comparison studies. However, despite the lower incidence of renal damage the magnitude of the renal swelling was greater in the present study. Again, this could be related to the selection of patients, but also to differences in measurement methods.

*Table 5. Comparison of results of the present study of renal swelling indicating renal damage with results from Hung et al. and Cheng et al.*

|  | Simrén et al. | Cheng et al. | Hung et al. |
|---|---|---|---|
| Study design | Prospective, first UTI | Retrospective, first UTI | Prospective, first UTI, >38°C, tertiary center |
| Normalized to | body length | age | age |
| Mean CRP (mg/ml) | 92 | 60 | - |
| Number of patients | 101 | 545 | 310 |
| Age included (years) | <1 | <2 | <5 |
| Bag urine sampling | No | Yes | No |
| Time-point DMSA | Acute +1 year | Late > 6 months | Acute + late > 6 months |
| Patients with damage at late DMSA | 24% | 40% | 27% |
| Patients/kidneys with renal length >2SD | 38% / 27% | 15% / 12% | 5% / - |
| Renal damage in patients /kidneys > 2SD | 26% / 21% | 90% / 80% | 87% / - |
| Odds ratio | 1.4 | 18 | 22 |

In study I and II the US examinations included length and volume measurements. This was performed as volume measurements may capture renal enlargement better than renal length. As described in the measurement section above, the variability of the volume measurement was larger than for

renal length. In contrast to renal volume, renal length correlated with both CRP and temperature. In the comparison of renal length to volume in study II, we found that renal volume predicted acute (p=0.0005) but not late renal damage (p=0.069). Thus, the use of renal length measurements at the early US seem to be preferable over volume due to smaller variability, better correlation overall to inflammatory parameters and the ability to predict acute and late renal damage.

The value of using change in renal size in the evaluation of infants with a UTI has not been studied previously. We found a weak correlation between change in renal length to CRP and temperature (Table 4). In addition, change in renal length could not predict renal damage (p=0.054).

In accordance with earlier studies (12, 16, 24, 25, 31), an association between acute and late findings on DMSA scans for sex (female), temperature, CRP and dilating VUR (grade III-V) was found. The increased risk for findings on DMSA scans for girls is not surprising, as the girls had higher temperature (p=0.0031) and CRP (p=0.017) compared to the boys. The strongest association was found for VUR III-V with an odds ratio of 5.84 (1.29-26.47) for acute damage and 13.51 (2.58-70.84) for late damage. In contrast to other studies (24, 31), we did not find any difference in renal size between VUR 0-II versus VUR III-V or VUR O versus VUR I-V. This is probably due to the small number of patients in our study.

## 5.2 Study III and IV

In study III the agreement of DWI with DMSA scintigraphy and consensus diagnosis was tested in 25 patients. In study IV a subgroup of the patients with lesions on DMSA scintigraphy was analyzed further using multiparametric DTI. Figure 15 summarizes the main results of study III and IV.

### Main results of study III

- The DWI method demonstrated a fair to good agreement with DMSA.
- DWI identified all patients with a consensus diagnosis of pyelonephritis and development of permanent renal damage at a follow-up DMSA.
- No false positive findings were detected with DWI compared to consensus diagnosis.

### Main results of study IV

- There was a difference in the ADC, FA and the b700 values between the lesions and the normal tissue for all nine kidneys.
- Six kidneys had focal lesions with increased b700, decreased ADC and FA, that could indicate typical findings of acute pyelonephritis.
- In three kidneys the multiparametric characteristics of the lesions were diverse and differential diagnoses were considered.

*Figure 15. Main results of Study III and IV.*

## 5.2.1 Morphological findings

In study III five kidneys in four patients had duplicated systems, but none of the patients had hydronephrosis. No hydronephrosis or duplications were found in the seven patients in study IV.

## 5.2.2 Agreement diffusion weighted imaging, DMSA scintigraphy and consensus

The findings of pyelonephritis using the three methods, DMSA, DWI and consensus diagnosis in our study are shown in Table 6 in comparison with data from three reference studies (106-108).

*Table 6. Detection of pyelonephritis using DWI in the present study and reference studies.*

| | Simrén et al. | | Bosakova et al. | Lee et al. | Vivier et al. |
|---|---|---|---|---|---|
| Study design | Prospective, first UTI | | Prospective, first UTI | Retrospective, first UTI, >38°C | Retrospective/ uncertain pyelonephritis |
| Number of patients | 25 | | 31 | 22 | 39 |
| Age included | 19 days-5.5 months | | 3-18 years | 1-11 months | 0.5-16 years |
| CRP (min-max) | 52 (8-180) | | - | - | 144 (10-456) |
| Breathing management | Free breathing | | Free breathing | Sedation/ respiratory triggering | Sedation/ respiratory triggering |
| b value (s/mm²) | 0,100,400,700 | | 0, 50, 800 | 0, 25, 50, 75, 100, 200, 500, 800 | 0, 1000 |
| Reference method | DMSA | Consensus diagnosis | Clinical pyelonephritis | DMSA | Contrast enhanced MRI |
| Patients/kidneys with positive findings using the reference | 10 (40%) /12 (24%) | 6 (24%) /9 (18%) | - | - /15 (35%) | 28 (72%) /32 (41%) |
| Patients/kidneys with positive findings on DWI | 5 (20%) /8 (16%) | | 31(100%) /37 (60%) | - /14 (33%) | - /35 (45%) |
| Agreement | K=0.50 (p<0.0001) with DMSA | | K=0.60 (p<0.0001) with DMSA | K=0.94 (p<0.05) | K=0.92 (0.83–1.00) |

Pyelonephritis was diagnosed in 8 (16%) of the 50 kidneys on DWI compared with 12 (24%) on the DMSA scans and 9 (18%) using consensus diagnosis (Figure 16). There were eight kidneys in seven infants with discrepancies between the DWI and DMSA scan findings. Consensus diagnoses for these seven patients were pyelonephritis in two cases and no pyelonephritis in five patients. Two of the patients with discrepancies (positive DMSA scan and negative DWI regarding pyelonephritis) had findings of duplication or hypo-/dysplasia and the consensus diagnoses was negative. In one patient with positive consensus diagnosis, DWI did not show any findings that corresponded to the uptake reduction on the DMSA scan. However, the follow-up DMSA scan in this patient was normal. DWI identified all patients with consensus diagnosis of pyelonephritis and development of permanent renal damage at the follow-up DMSA. No false positive findings were detected with DWI compared to consensus diagnosis.
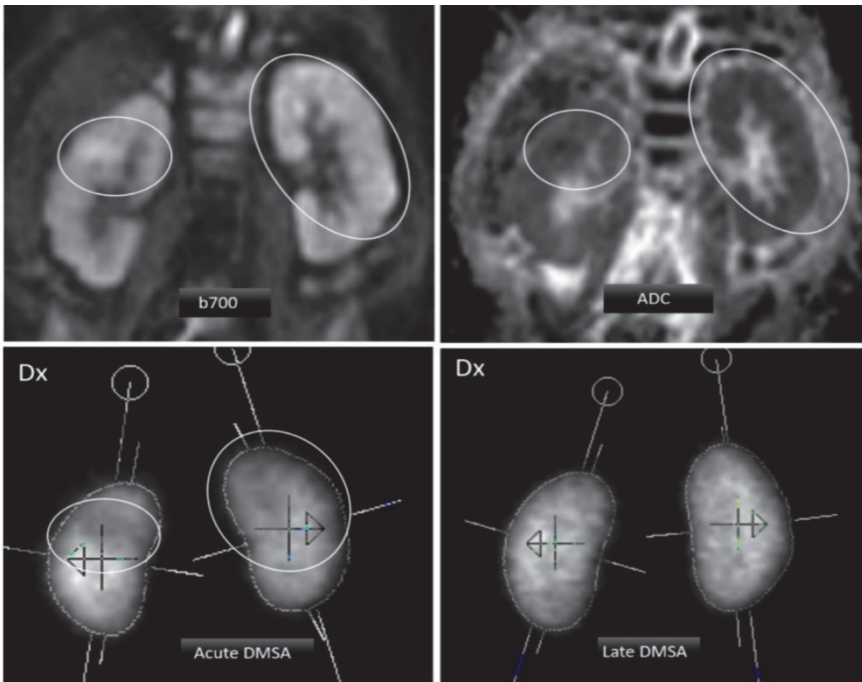


*Figure 16. Images of a one-month old infant with a UTI showing concordant findings of bilateral pyelonephritis on the DWI and DMSA scans. Uptake reduction were found apically in the right kidney and more generalized in the left kidney on the acute DMSA scan with a corresponding increased signal on the b700 and decrease in ADC. The late DMSA scan was normal.*

We found that the DWI method demonstrated a fair to good agreement, K=0.5 (p<0.0001), with DMSA scintigraphy, which is lower compared to previous studies. However, there are several differences between the studies that may have major impact on the results; severity of the disease, age of the children, scanning protocol and reference method used in the different studies. By including children with a UTI based on presence of symptoms or temperature, or by only including children from tertiary centers, children with more severe disease are selected. This leads not only to an increase in children with renal involvement, but also makes the lesions easier to detect on the images, as a more extensive involvement of the renal parenchyma can be expected. The age of the included children is also important to consider as imaging of younger infants using DWI is demanding, due to small kidney size, higher breathing frequency and more voluntary motion. The involuntary movements of organs in the abdomen with breathing can lead to artifacts, low image quality and inaccurate measurements (75, 149). The use of respiratory triggering to reduce these artifacts require the children to be sedated or to be old enough to cooperate. None of the reference studies used free breathing in non-sedated infants. The different b values used in the four studies might also have influenced the results. As higher b values increase the diffusion sensitivity, the choice of b values influence the image used for detection of lesions and determination of the ADC map. The optimal b value for renal DWI scanning is still debated, but is usually estimated to 300-700 s/mm² (1/ADC) (120). The choice of b value is a balance between SNR and minimizing flow and T2 effects. The ADC value is influenced by both the number of, and absolute b values used in DWI scanning. Accurate estimation of the ADC value requires multiple b values, as it results in a better fit compared to a monoexponential model (150, 151). In clinical practice ADCs are most frequently calculated based on the monoexponential model and the perfusion effect is minimized by using higher b values (79).

In the present study we had to exclude six patients that underwent the MRI examination due to motion artefacts. This was probably the result of limited time available for the examinations and suboptimal preparation of the infants, who often underwent DMSA scintigraphy on the same day. The method of MRI scanning after feeding in infants is already in use in clinical practice in our department for other indications with high success rate. With preparations optimized solely for the MRI examinations, the success rate would probably have been higher in our study. Despite the intension to limit the time interval between the DWI and DMSA examinations, there were two patients with more than seven days between the examinations (one with 23 days and one with 8 days). There is limited knowledge regarding the development of pyelonephritic changes over time on DWI or DMSA scintigraphy, but the time interval was

not greater in the discordant compared to the concordant group in this study. The two patients with a time span of more than seven days between the examinations showed normal findings on both methods.

## Reference methods

DMSA scintigraphy is often regarded as the reference method with a high sensitivity for the detection of acute pyelonephritis, but has limitations as a gold standard because of its limited spatial resolutions and difficulties to differentiate between pyelonephritis and congenital malformations (8, 152, 153). Pinhole DMSA scintigraphy has been shown to have a high sensitivity and specificity (95%) for the detection of pyelonephritis, verified with histopathology, in experimentally induced UTI in piglets (152). Since verification of findings with histopathology is not an alternative for infants with a UTI, alternative reference methods, such as contrast enhanced MRI or CT are used, but these methods have the drawbacks of using contrast or ionizing radiation. Consensus diagnosis has been described as an alternative reference standard in diagnostic studies where single and error free reference standards do not exist (142). We decided to use consensus diagnosis as an additional reference method to DWI, as morphological information on the MRI images together with the findings from the DMSA and DWI images could be useful for further evaluation of the findings. The results from the comparison of DWI to consensus diagnosis can be viewed as valuable complementary information in the interpretation of the demonstrated agreement between the DWI and DMSA methods. With the use of the consensus method, several cases with discordant findings between DWI and DMSA scans were identified, where the consensus method had to be used for further diagnosis, highlighting a need for further characterization of renal lesions in infants with a UTI.

## 5.2.3   Multiparametric diffusion tensor imaging

In this study, renal lesions detected by DMSA scintigraphy in seven infants with a UTI, were further characterized using multiparametric DTI. DTI can provide several parameters that can be used for further characterization of the tissue. In addition to the ADC value that quantifies the degree of diffusion, the FA value, a quantitative measure of the fraction of anisotropy in the tissue, can be calculated. The information on the dominant direction of diffusion can be used to reconstruct tractographies that can visualize changes in the architecture of the renal tissue. By combining these parameters with the qualitative characteristics obtained from the visual inspection, patterns can be recognized that can be used in the evaluation of the lesions. Thus, multiparametric DTI has the potential to provide additional information to enable further characterization of renal lesions. Such information may improve the ability to identify and differentiate acute pyelonephritic lesions from permanent renal damage or dysplastic changes in infants with urinary tract infections.

For the nine kidneys with lesions there were differences in b700 signal, ADC and FA between the lesions and normal tissue (p=0.011, p=0.008, and p=0.008). No significant difference could be found in the b0 signal (p=0.38). Based on the multiparametric analyses the findings of the nine kidneys could be divided into two groups; one groups with uniform findings indicative for acute pyelonephritis, and another group with more diverse characteristics where alternative diagnoses were considered. Six kidneys in four infants had focal lesions with increased b700 signal, decreased ADC and FA, which could indicate typical findings of acute pyelonephritis (Figure 17). In three kidneys the multiparametric characteristics of the lesions varied and differential diagnoses were considered (hypoplastic/dysplastic changes, or sequelae after earlier infection). Figure 17 illustrates an example of a patient with discrete but typical findings of pyelonephritis. The corresponding quantitative characteristics with differences in values between lesions and normal tissue were for b0, b700, ADC and FA, 21%, 32%, -12% and -44% for this patient.
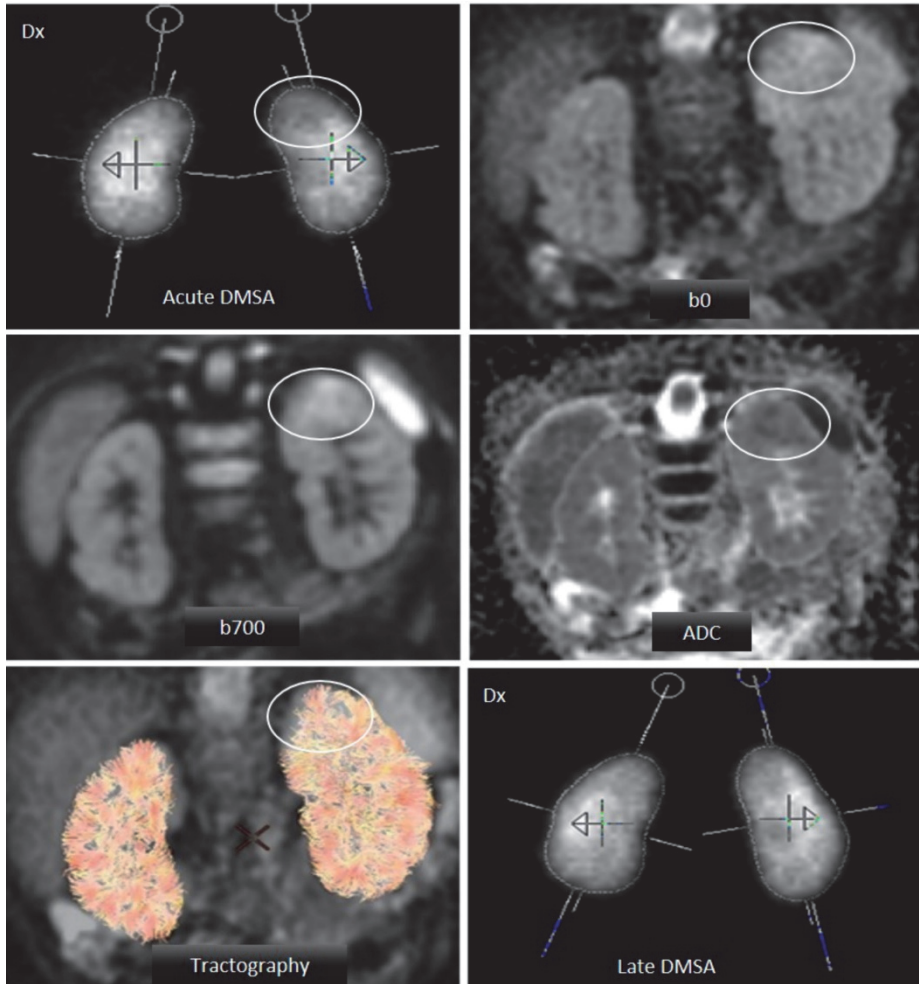
*Figure 17. Images of an infant showing findings of pyelonephritis with an apical uptake reduction in the left kidney on the acute DMSA scintigraphy with a corresponding discrete increased signal that could be visualized on the b0, more distinct increase on the b700, decreased in ADC and distorted tracks on the tractography. The late DMSA scan was normal.*

There is limited knowledge regarding the typical characteristics of pyelonephritis using DTI (104). Based on studies using DWI, focal areas of hindered water diffusion can be expected, reflecting the inflammatory response in the renal parenchyma (62, 93, 95, 101-103). In addition to merely reflecting

the degree of diffusivity, DTI also gives information on the directionality of the diffusion with the potential to provide an insight to the microstructure of the parenchyma. The pathophysiological mechanisms responsible for the abnormalities found in imaging of pyelonephritis is unexplored. However, the inflammatory response to pyelonephritis is suggested to cause infiltration of inflammatory cells, reduced blood flow due to edema or aggregated intravascular granulocytes, and focal ischemia in the parenchyma (154, 155). Parkhouse and Majd et al. conducted experimental studies using histopathological criteria for pyelonephritis in a pig model with induced VUR (152, 155, 156). They noted that a common factor explaining the imaging findings appeared to be focal ischemia secondary to the inflammatory response, causing edema and accumulation of intravascular and intratubular granulocytes. They also suggested that altered tubular cell membrane transport mechanism and cell death could account for the imaging findings. These finding are in agreement with studies in acute human pyelonephritis were areas of distal tubules were affected, containing large number of neutrophils or destroyed by the inflammatory process, whereas glomeruli and proximal tubules were less damaged (157). Since the inflammatory response seems to mainly affect the tubules one can expect that DTI that measures directional diffusivity would be more sensitive compared to conventional DWI in the diagnosis of pyelonephritis. This could explain our findings with larger differences in FA between lesions and normal parenchyma compared to b700 and ADCs. Thus, together with other quantitative and qualitative parameters derived from DTI, FA can provide information that can allow for improved detection and characterization of renal lesions compared to DWI. FA is also considered a more stable parameter compared to ADC, as changes in low b value effects ADC values considerably (120, 158).

There are only a few reports in the pediatric population that we can relate our findings to. The potential of DTI has been described in the evaluation of allograft dysfunction, ureteropelvic junction obstruction, and in the assessment of various focal renal lesions (67, 126-130). In a study by Lair et al., 31 children (age 6 months to 16 years) with suspected acute pyelonephritis were examined with the objective to compare image quality in free breathing DTI with respiratory triggered conventional DWI (104). They found that DTI demonstrated significantly better image quality and agreement with DWI in all patients, and concluded that DTI could replace DWI in the diagnosis of acute pyelonephritis.

The study by Lair et al. highlights one of the potential advantages of using DTI in the diagnosis of pyelonephritis, i.e. the use of the free breathing method. Free breathing scanning with signal averaging has been proposed for motion

artefact reduction in DWI examinations, in cases where breath holding or navigation scanning is not an alternative, which is the case in infants (73, 77). Free breathing scanning also enables multidirectional scanning with the large number of signal averages being spread out over a large number of diffusion sensitizing directions (104). By using this imaging strategy, motion effects in abdominal DW images may be reduced, as the diffusion sensitizing gradients are not oriented predominantly in the direction of the respiratory motion. However, some contradictory reports have shown improved image quality and reproducibility by using respiratory triggering compared to free breathing in adults (120).

# 5.3   General discussion and future perspectives

This thesis has shown the potential of US, DWI and DTI in the initial evaluation of the urinary tract in infants with their first UTI. In the US studies, an association was found between renal swelling and inflammatory parameters, as well as renal damage, but the diagnostic performance in predicting renal damage was weak. Therefore, our results do not support replacement of DMSA scintigraphy in the present follow-up protocol. However, renal length assessment is easy to perform and is commonly performed as part of the routine US examination in the evaluation of malformations. Thus, information on renal length may add value in the decision-making process regarding further follow-up of the patient. Therefore, determination of renal length to assess renal swelling can be a recommended as a complement in the evaluation of infants with their first UTI.

The potential of the DWI method in the detection of pyelonephritis was demonstrated by showing agreement between DWI and DMSA scintigraphy. Furthermore, the finding that a short (58 seconds) DTI scan may add valuable information on the characteristics of a renal lesions that can be used to confirm or reject the diagnosis of acute pyelonephritis, is also of great interest. It is still unclear how to use DWI or DTI in clinical practice, but if our results would be confirmed in prospective studies in larger cohorts, the methods can be viewed as attractive alternatives, potentially replacing both US and scintigraphy, in the initial evaluation of infants with a UTI. It would be of value to study the development of the findings on DWI and DTI over time in the follow-up of infants with a UTI. The potential of the methods to predict outcomes, such as permanent renal damage also needs to be explored. In addition, future studies

should also focus on investigating healthy children for reference and finding cut-off values for ADC and FA.

Thus, despite the demonstrated potential of US, DWI and DTI, their future role in the evaluation of infants with a UTI remains to be defined. The imaging protocols currently used varies greatly among centers. This is not only due to uncertainties in balancing the detection of risk factors against drawbacks, such as radiation burden, but also due to the availability of imaging methods. Even if DMSA scintigraphy is well-tolerated, there are concerns about accessibility, radiation exposure, and lengthy protocols in the pediatric population. The availability of MRI cameras is rapidly increasing with the expanding use of the method in a wide range of applications. With the use of DWI and DTI, MRI has a potential not only to replace the present methods, but also to add valuable morphological and functional information. The development of DWI and DTI in renal imaging in children could also be of value for other patient groups, for example in the evaluation of renal transplants, in oncology or in other renal diseases with impaired renal function, where CT or MRI with intravenous contrast media should be avoided. US has the advantage of being most readily available and the full potential of the method has probably not yet been utilized.

Based on the findings in the present studies there has been an increased awareness in our clinical setting of the potential of US and DWI/DTI in the evaluation of UTI in children. Although our imaging guidelines have not yet changed, the knowledge on the usability of renal length measurement as an indicator of renal swelling has led to an increasing number of US reports where this information is included. The close collaboration between the uro-nephrology and radiology departments is an important factor for the implementation of new methods in clinical practice. We have seen a shift towards performing MRI examinations at an earlier stage in infants, to be able to use the advantage of performing the examination without sedation. The potential of MRI with DWI has been recognized and the method is being increasingly used in complicated UTI. However, the use of the method as an alternative to DMSA scintigraphy, for example in cases with difficulties in getting intravenous access, is still to be discussed.

Imaging is becoming an increasing part of diagnostics in general and there is a rapid ongoing development in the field. The superior soft tissue contrast in MRI is ideal for visualization of the renal parenchyma, and the method is currently the reference method for evaluation of morphological changes, such as congenital malformations. However, the technical development of MRI is extensive and in addition to improvement of the present methods, there are several new methods that might be used for renal imaging in children in the future. The need for sedation that currently limits the usability of DWI and DTI

in older infants might be overcome with the development of new MRI techniques with faster sequences and improved motion compensation. Recent studies suggest combining DTI with the intravoxel incoherent motion (IVIM) technique from which both pure molecular diffusion parameters and perfusion related diffusion parameters can be calculated (107, 159-162). This technique has the potential to distinguish structural changes from flow effects on renal diffusion anisotropy.

Technical progress in US has increased the image quality considerably, and a number of new tools have become available, for example CEUS and elastography. CEUS involves the administration of intravenous contrast agents containing microbubbles that reflect the microcirculation and perfusion of the renal parenchyma. Intravenous CEUS has potential in the evaluation of focal renal hypovascular lesions, such as complex renal cysts and trauma (163). Thus, the use of intravenous CEUS for renal application in children may become an important imaging tool as soon as more literature and experience is available, and the product is licensed for use in pediatric patients in Europe. US contrast can also be used in contrast-enhance voiding urosonography to detect VUR (164, 165). However, the use of this method for the evaluation of the male urethra is controversial (165, 166). Elastography that can be obtained using both MRI and US, noninvasively measures tissue stiffness by visualizing the propagation of shear waves in the tissue. This method is under investigation for use in the liver in children (167) and could potentially be used in the detection of focal changes in the renal parenchyma. However, the high blood flow and heterogeneous renal tissue limit the use of this method in kidneys. US elastography is also limited by the dependence of the depth of the organs, thus native kidneys are more difficult to evaluate, but the method has been used in the assessment of allografts that are located superficially (168). There are a few reports on the utility of MRI elastography in the evaluation of renal fibrosis (169).

Thus, new attractive methods have been developed that call for further research to be able to not only replace the currently used methods, but also to enable improved evaluation of acute pyelonephritis and prediction of renal damage.

# 6 CONCLUSION

This thesis demonstrates the potential of US, DWI and DTI as valuable tools in the initial evaluation of infants with their first UTIs.

The US studies showed significant renal swelling in infants with UTIs that correlated with inflammatory parameters and was associated with an increased risk of renal damage at the acute and late DMSA scan. This suggests that early US can be a valuable non-invasive method for identifying children with inflammation of the kidney and risk of future renal damage. Although the diagnostic performance compared to DMSA scan was weak, renal swelling can help in identifying patients at risk, which may lead to changes in the management of children with UTIs in clinical practice.

The DWI study showed an agreement between DWI and DMSA scintigraphy in the detection of pyelonephritis with the potential of the method confirmed by the use of a consensus diagnosis. With the use of multiparametric DTI analyses, lesion patterns could be demonstrated with potential to distinguish acute pyelonephritis from other renal lesions, such as permanent renal damages or dysplastic changes. Thus, multiparametric DTI has the potential to characterize renal lesions in young infants with a UTI. As a non-invasive, non-radiating, short scanning technique, multiparametric DTI is an attractive method that may provide valuable information for the evaluation of these patients in clinical practice. Hence, free-breathing DWI and DTI are promising methods for primary evaluation of this patient group.

The results show that US, DWI and DTI are valuable non-invasive, non-radiating tools in the initial evaluation of infants with their first UTI. Renal length US measurements adds value to the early US examination by helping to identify patients at risk for renal damage even though it cannot replace DMSA scintigraphy. DWI and DTI have the potential to be advantageous alternatives to DMSA scintigraphy. However, studies of larger cohorts are needed to verify the results.

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