LONGITUDINAL STUDY OF INFANTS WITH HIGH-GRADE VESICOURETERAL REFLUX

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To my family

Abstract

Background Infants with congenital high-grade vesicoureteral reflux (VUR) have been regarded as a special group amongst children with reflux, with their own characteristics concerning renal damage, gender, resolution rate and causative mechanism. A dysfunctional bladder has been suggested to be associated with the condition. In the past surgical intervention was considered necessary in infants with high-grade reflux to prevent further renal damage. In the last decades there has been a trend towards more conservative treatment and delayed surgical intervention influenced by reports of high rates of spontaneous resolution of VUR in this group of patients. Therefore increased knowledge of the natural course of high-grade VUR in infants and factors affecting the outcome is needed to meet the new trends for management.

Research questions The overall aim was to identify infants with high-grade VUR at risk of persistent reflux and deterioration in renal status and select those from patients with a better prognosis. Evaluation of bladder function and its significance for the VUR prognosis was included in this aim.

Material and Methods 134 infants with dilated VUR (grade III-V) were consecutively included in this prospective observational study. The patients were followed longitudinally according to a study protocol including repeated examinations for determination of grade of VUR, evaluation of bladder function (videocystometry) and evaluation of renal status (DMSA and MAG3 scintigraphy and Cr-EDTA clearance). The first investigations were made after diagnosis of VUR and then yearly during a 3-year study period. Surgical intervention was intentionally late and not performed until the end of the study.

Result A high frequency of renal abnormality was found at entry (85%), with characteristics of the congenital generalised damage in more than two thirds of the study patients. Despite the high frequency of renal damage, total renal function (GFR) was only subnormal in one third (30%). Deterioration in renal status during follow up was seen in 19 patients (18%), but only one had a significant decrease in total renal function. Predictive factors for deterioration were recurrent febrile urinary tract infection, bilateral abnormality and reduced total glomerular filtration rate.

Breakthrough febrile urinary tract infections during follow up were seen in 47%, despite antibacterial prophylaxis, and were more frequent during the infant year, especially in boys.

Bladder dysfunction was found in 42% and was mainly characterised by high bladder capacity and high postvoid residual, a dysfunction pattern described as dilated bladder dysfunction. This dysfunction could only be recognised at the second examination at 20 months, since the results from the first year of life showed an immature pattern with overactivity during filling, high voiding detrusor pressure and low bladder capacity; characteristics not possible to separate from normal function.

A series of factors of importance for spontaneous resolution or downgrading of VUR were identified. Renal abnormality and subnormal renal function were

negative predictors for spontaneous resolution and so was recurrent UTI. Bladder dysfunction significantly correlated to non-resolution, and so did both high bladder capacity and increased residual urine seen as separate variables. Reflux occurring passively during filling and higher grade of VUR at inclusion was also negative predictors for resolution. All these variables were included into a multivariate Cox proportional hazard model with stepwise selection. Three variables were identified as strong independent predictors for non-resolution of VUR in the multivariate analysis; renal abnormality, bladder dysfunction and breakthrough urinary tract infection.

Conclusion In this cohort of patients with congenital dilated VUR the overall spontaneous resolution rate to grade II or less was high (38%). Renal abnormality, bladder dysfunction and breakthrough urinary tract infection were found in many study patients and were also shown to be three strong independent negative predictors for reflux resolution in multivariate analyses.

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Contents

List of publications

This thesis is based on the following articles :

I. **Sjöström S., Sillén U., Bachelard M., Hansson S. and Stokland E.,**
Spontaneous resolution of high grade infantile vesicoureteral reflux.
J Urol, 2004. 172(2): p. 694-8; discussion 699.

II. **Sjöström S., Jodal U., Sixt R., Bachelard M. and Sillén U.,**
Longitudinal Development of Renal Damage and Renal Function in Infants With High Grade Vesicoureteral Reflux.
J Urol, 2009. 181 p. 2277-2283.

III. **Sjöström S., Bachelard M., Sixt R. and Sillén U.,**
Changes in urodynamic patterns in infants with dilating reflux; three year follow up.
J Urol, 2009 . 182(November), in press.

IV. **Sjöström S., Jodal U., Stokland E., Sixt R., Wahll L., and Sillén U.,**
Predictive factors for resolution of high-grade infantile vesicoureteral reflux - Results of uni and multivariate analyses.
Submitted.

Abbreviations and Acronyms

BC	bladder capacity
CI	confidence interval
CKD	chronic kidney disease
51 Cr EDTA	51 chromium edetic acid
DBD	dilated bladder dysfunction
DMSA	dimercapto-succinic acid
FVO	free voiding observation
GFR	glomerular filtration rate
MAG-3	mercaptoacetyltriglycine
OAB	overactive bladder dysfunction
ROC	receiver operating characteristic
UTI	urinary tract infection
VCM	video cystometry
VCU	voiding cystourethrography
VUR	vesicoureteral reflux

Introduction

Vesicoureteral reflux (VUR) is the pathological retrograde back flow of urine from the urinary bladder through the vesico-ureteral junction into the ureter and kidney. VUR is diagnosed using radiological or radioisotopic techniques and is graded I-V by the severity of ureteral dilatation and calyceal changes according to the International grading system[1](figure 1)(table 1).

The milder grades of reflux, grades I and II, appear without dilatation of the upper urinary tract, whereas the more severe grades III-V show various extents of ureteral and calyceal dilatation[1]. The prevalence of VUR in normal children is estimated to be 0.4-1.8%, and it is more frequent in girls (2.2%) than in boys (0.6%)[2]. The prevalence of VUR after urinary tract infection (UTI) is as high as 31% and after prenatal diagnosis of hydronephrosis 20%, which is significantly lower than in children with UTI[2]. The prevalence of reflux is inversely correlated with the age of the study population, and spontaneous resolution of reflux occurs in many patients with growth[2, 3].

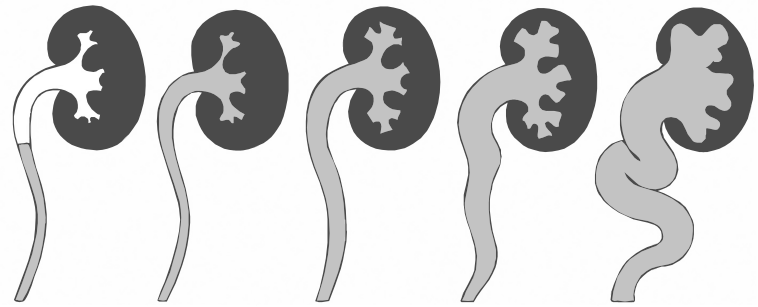


Figure 1. International system of radiographic grading of vesicoureteral reflux according to Lebowitz et al.[1]

Table 1. Definitions of grading of VUR according to the International Radiographic System.

The International System of Radiographic Grading of Vesicoureteral Reflux	
Grade I	Appearance of contrast in the ureter only
Grade II	Appearance of contrast in the ureter and renal pelvis without associated dilatation or blunting of calyces
Grade III	Mild calyceal dilatation without ureteral tortuosity
Grade IV	Moderate calyceal dilatation and blunting without ureteral tortuosity
Grade V	Severe calyceal dilatation with ureteral tortuosity

Vesicoureteral reflux is variable in severity, aetiology and prognosis. Dilating reflux is more likely to be associated with renal abnormality whereas mild grades of VUR in children can probably be seen as delayed maturation of the uretrovesical junction. The dilated vesicoureteral reflux diagnosed during infancy has been considered a

special condition with its own characteristics and prognosis. There is a marked preponderance of males. A high frequency of associated renal abnormalities and bladder dysfunction has also been suggested to accompany the condition.
This thesis focuses on infantile dilated vesicoureteral reflux, diagnosed after findings on prenatal ultrasound or urinary tract infection during the first year of life. The thesis provides a description of longitudinal development of renal and bladder function and identifies prognostic factors for long-term outcome of reflux and renal status in a cohort of children followed in a prospective observational study at the Queen Silvia Children's Hospital in Gothenburg.

History

In the second century A.D. Galen described the anti-reflux mechanism of the vesicoureteral junction as he noted that fluid did not pass up the ureters when he filled a human bladder obtained at autopsy [4]. In the fifteenth century Leonardo da Vinci was the first to illustrate ureteral reflux accompanied by scarred kidneys in his anatomical drawings in brown ink (figure 2) but, according to a notice in The Lancet, there is no evidence that Leonardo da Vinci recognised the association between them and nor did doctors for another four centuries[5]. Pozzi, in 1893, was probably one of the first to observe vesicoureteral reflux in man, and he noted that the phenomenon was abnormal[6]. The efficacy of the normal uretro-vesical junction in the prevention of vesicoureteric reflux and the essential features of the mechanism involved was described by Bell in 1812 and Young in 1897 [7-9].

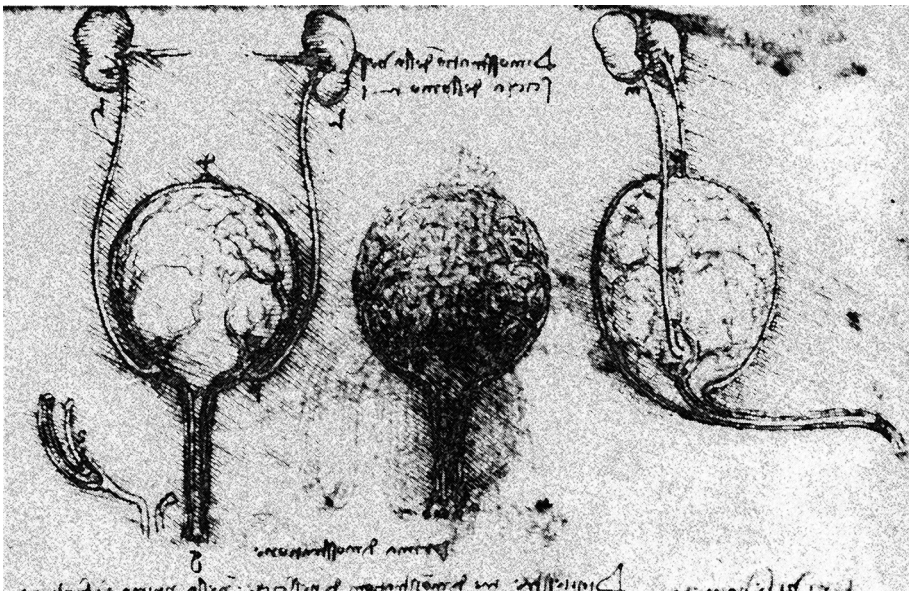


Figure 2. Anatomical drawing in brown ink by Leonardo da Vinci entitled 'Three views of the bladder, with kidneys, ureters and detail of entry of ureter into the bladder'.

The possible significans of vesicoureteral reflux in urinary tract infections was proposed by Sampson in 1903 and again by Bumpus in 1924 [10-11]. Methods for radiological diagnosis of VUR developed and were systematically used by many clinicians in evaluation of children with UTI after Bartrina had introduced cystography in 1935[12]. Still, it was the work of Hodson and Edwards in 1960 showing the association between renal scarring and vesicoureteral reflux and Hutch, Politano and Leadbetter in the 1950s, presenting techniques for surgical correction of reflux, that evoked a new interest in the congenital VUR condition and treatment strategies for children with this diagnosis[13-15]. Bailey introduced the term 'reflux nephropathy' and Smellie et al. made their contribution to confirming the close association of reflux, urinary tract infection and renal scarring in the early 1970s[16, 17]. They stressed the importance of early diagnosis of reflux and of keeping both the bacteriuria and the renal status under observation[17]. Ransley and Risdon made experimental studies in 1975, confirming the studies of Tanagho, showing that reflux could be created in animals by modifying the urethrovesical junction and showing the relation between reflux, renal papilla anatomy, pyelonephritis and renal damage[18, 19].

In 1970 Rolleston et al. showed that gross vesicoureteral reflux (grade IV and V) diagnosed during infancy was found to be accompanied by a high incidence of *initial* and *progressive* renal damage and that if this reflux was allowed to continue, it could lead to depressed renal growth and further loss of renal substance[20]. In infants showing moderate (grade III) and slight (grade I-II) reflux, it did not appear to be associated with renal damage. The same researchers found evidence that the grossly refluxing ureter of infancy was potentially dangerous to the kidney and should be surgically corrected as soon as possible [20]. Stephens found a strong tendency for spontaneous cessation of reflux over the course of several years as early as 1963[21] and this was confirmed by Edwards et al. in the 1970s [22], but the intention to treat severe reflux in infants surgically was still common since they were seen as a special group with poor prognosis unless intervention was done.

In the 1970s and early 1980s there were widely divergent opinions as to the optimal management and treatment for infants and children with dilating primary VUR.

The International Reflux Study in Children (IRSC) was set up in 1980 to compare the outcome of medical or surgical treatment of children after the infant year, managed according to a strict protocol, who had grade III or IV non-obstructive VUR and a history of at least one symptomatic urinary tract infection (UTI)[23]. Using renal status and UTI frequency as effect variables the study showed that there was no difference between outcomes of medical versus surgical treatment.

A change of paradigm gradually evolved, with delayed surgical intervention in infants with dilated VUR, since renal damage associated with the condition became known as a congenital abnormality rather than an aquired lesion. This trend of delayed surgical intervention was also supported by the findings of high rates of spontaneous resolution in prenatally detected severe infantile VUR[24]. Surgical intervention of the refluxing vesicoureteral junction in infants had also proven to be technically complicated. If prophylactic antibiotics could protect from pyelonephritis in the first year of life, a surgical procedure should be easier to perform later and be more likely to succeed.

Embryology and pathophysiology of the urinary tract

Embryology

After fertilization, the human body with its organ systems develops during the first 10 weeks of gestation. The remaining 28 weeks of the pregnancy are spent in maturation, growth and development of function of the body, enabling independent life after separation from the placental support system [25]. The diploid zygote divides to form a blastocyst, which turns into the two layer embryonic disc in the second week of gestation, surrounded by the amniotic cavity on the ectoderm surface and the yolk sac on the endoderm surface. During the third week a third layer of mesoderm forms in between the other cell layers, differentiated from a midline area, called the primitive streak. It is largely from the intermediate mesoderm that the urinary and genital organs will develop[25].

Renal development

From the fourth week of gestation, three nephric structures develop in succession from the intermediate mesoderm. The first of the three structures, called the pronephros, regresses rapidly without forming any nephrons. The second structure, called the mesonephros, develops from tubular structures in the mid-portion of the intermediate mesoderm and establishes a connection to the cloaca; the Mesonephric or Wolffian duct. In the mesonephric kidney primitive nephrons are formed which function between the sixth and tenth weeks, producing small amounts of urine. During the tenth gestational week, the lower part of the mesonephros degenerates, leaving the upper nephrons to develop into the genital duct system. The mesonephric duct remains and takes part in the formation of the permanent urinary system (figure 3)[25].

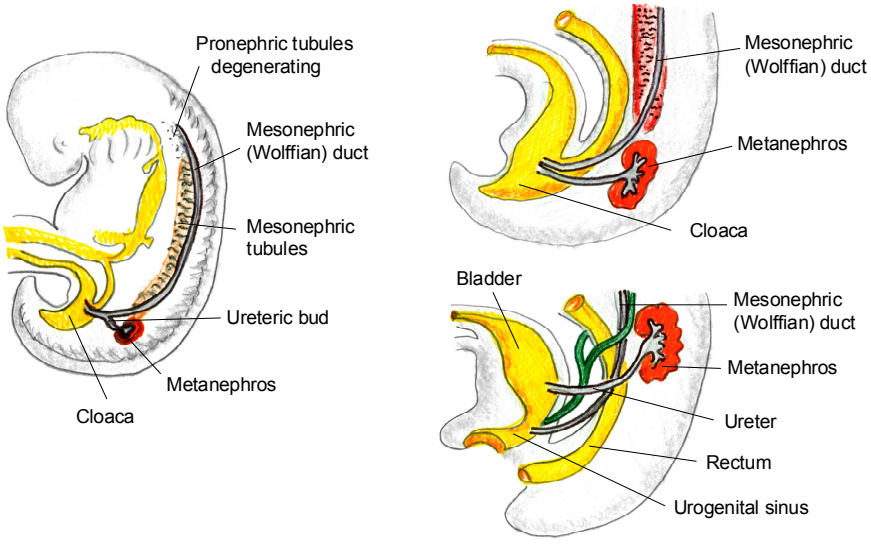


Figure 3. Development of urinary organ precursors.

As early as during the fifth gestational week the structures of the permanent kidney begin to develop. A diverticulum called the ureteric bud forms in the lower portion of the mesonephric duct close to the junction with the cloaca (figure 3). The ureteric bud branches to form the renal pelvis, the major and minor calyces, and a portion of the collecting tubules, which merge with the third nephric structure condensed from the mesoderm: the metanephros (which forms the glomeruli and the upper part of the nephrons). Thus the renal development is induced in the fifth week, and the development of nephrons continues until the thirty-sixth week of gestation[25]. No new nephrons are formed after birth, but growth and maturation are important factors in increasing renal function during the first two years of life.

Bladder, trigone, and lower ureteric development

Around the fourth to fifth week, the mesonephric ducts extend caudally and reaches the portion of the cloaca that forms the urogenital sinus (figure 3). Fusion allows the mesonephric duct to drain into the cloaca and the segment from the mesonephric duct caudal to the ureteric bud is absorbed into the urogenital sinus. The right and the left sides, merge into the midline, to form the trigone. By the sixth week the cranial portion of the urogenital sinus dilates to form the primitive bladder. At the seventh to eighth gestational week development of detrusor and bladder wall muscles starts[26].

Normal ureterovesical junction and vesicoureteral reflux pathophysiology

Normal ureterovesical junction

The uretrovesical junction is structurally and functionally adapted to allow the intermittent passage of ureteral urine to the bladder and to prevent reflux backwards of bladder urine to the upper urinary tract, by a flap-valve mechanism. It separates the upper urinary tract (with low capacity and low pressure) from urine storage and pressure changes normally confined to the bladder. The long spiral muscle fibers of the ureter terminates as the ureter passes through the bladder wall in the posterior lateral aspect of the bladder. In the intravesical ureter only longitudinal muscle fibers continue, covered by bladder mucosa, supported by underlying detrusor muscle (figure 4)[27, 28]. The competence of the valve is influenced by the length, diameter and course of the intramural part of the ureter, which increases in length from 0.3 cm at birth to 1.3 cm in adults[28-30].

Pathophysiology

Vesicoureteral reflux is the abnormal retrograde flow of bladder urine into the upper urinary tract through an incompetent ureterovesical junction. Reflux of urine with bacterial contamination is a risk factor for pyelonephritis, which might lead to reflux nephropathy in children[13, 16, 17, 20]. Primary reflux in itself, without bacterial contamination and low in pressure, has not been documented as deleterious, although renal abnormality can bee seen in severe primary reflux without a history of urinary tract infection, probably owing to congenital maldevelopment [31, 32].

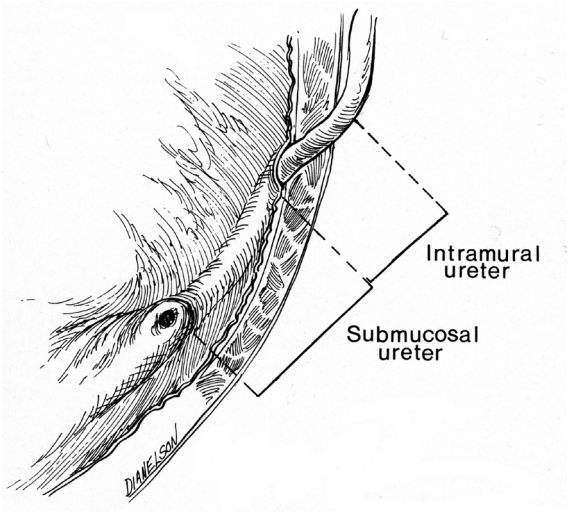


Figure 4. Anatomy of normal uretrovesical junction[4].

Reflux is classified as primary or secondary. Primary vesicoureteral reflux is the result of a congenital deficiency in the formation of, or delayed maturation of the ureterovesical junction. This is often seen as a lateralised ectopic ureteral orifice, with a deficient submucosal ureteral tunnel, with the appearance of a ”golf hole orifice” (figure 5).

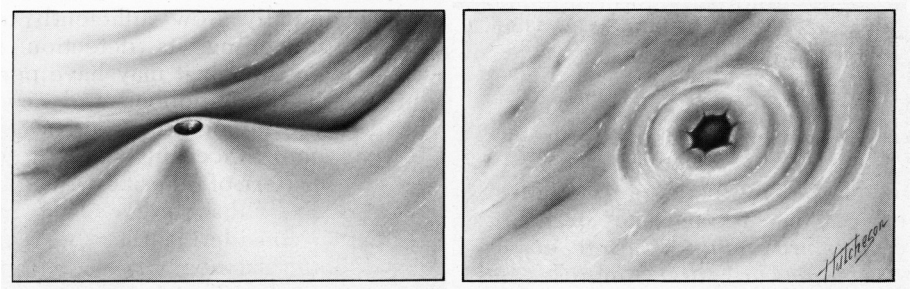


Figure 5. Anatomy of vesicoureteral reflux with lateralised ectopic ureteral orfice, with a deficient submucosal uretral tunnel, seen as a golf hole orfice[4].

Secondary reflux is caused in most cases by changes in the bladder wall due to neurological (in myelomeningocele or spinal cord injuries) or obstructive (seen in posterior urethral valves or ectopic ureteroceles) disorders. Low-grade secondary reflux can also be seen during urinary tract infection when cystitis can probably predispose a marginally competent uretrovesical junction to demonstrate reflux[33].

The severe primary vesicoureteral reflux diagnosed early in infancy, with dilated upper urinary tract associated with dysplasia or hypoplasia of the kidneys, is seen as a congenital malformation and different theories have been presented to explain the reflux anomaly complex. Experimental studies have shown renal dysplasia if

obstruction of the fetal kidney occurs during the first half of gestation[34], resembling the condition seen in patients with posterior uretral valve, often associated with severe vesicoureteral reflux and renal dysplasia. This could suggest a temporary fetal obstruction of the urinary outlet as the causative mechanism of the disorder even in primary reflux[34, 35]. The most widely advocated theory, however, is misplacement or maldevelopment of the ureteric bud early in fetal life, resulting in a dysplastic or hypoplastic kidney and abnormal insertion of the ureter within the bladder wall, leading to an incompetent vesico-ureteric junction[36-38].
Familial clustering of vesicoureteral reflux implies that genetic factors play an important role in the pathogenesis of reflux[39]. There are many implicated candidate genes, some of which regulate the position of ureteral budding, thus strengthening the theory of maldevelopment of the ureteric bud as the cause of the vesicoureteral reflux anomaly complex [40, 41]. VUR is genetically heterogeneous and at least 14 genes and ten additional potentially interesting loci have been identified associated with VUR [41].

Renal function in infancy and childhood

Glomerular filtration is low in the newborn and even lower in the premature infant but it increases rapidly during the first months of life[42]. Nephrogenesis is complete at birth, but glomerular and tubular function continue to mature during the first two years of life, through both cellular proliferation and enlargement[42]. Glomerular filtration, normalized for body surface area, increases between 0 and 2 years of age and remains unchanged thereafter. Low filtration at birth is caused by low perfusion, low blood pressure and limited filtration surface.
The plasma creatinine level at birth is the same as that of the mother but decreases during the first weeks after birth down to 20-30µmol/l. As the child grows and increases in muscle mass the plasmacreatinine level again rises. Schwartz formula can be used to relate the growing child's plasma creatinine level to normal for age and to corresponding GFR. This formula is based on the ratio of body length, plasma creatinine and a coefficient that differs from one age group to another[43, 44]. A simplification of the formula can be used. For children < 2 years of age GFR = 32 x length (cm)/ plasma creatinine (µmmol/l) and for children > 2 years of age GFR = 38 x length (cm)/ plasma creatinine (µmmol/l).

Bladder function in infancy and childhood

In the healthy adult, the lower urinary tract is controlled by a series of cortical, subcortical and spinal central nervous centers, resulting in unconscious permanent continence and conscious, voluntary initiation of voiding[45]. In the newborn the voiding is neither conscious nor voluntary, indicating that the micturition reflex is driven by lower subcortical and spinal levels. However, there is evidence of an existing cortical connection as early as in the neonatal period, since children of this age have been shown to wake up just before voiding in almost 90%[46]. The newborn healthy child should be considered continent from an anatomical, but not from a social point of view. Although the cortical connection already is established it lacks functional importance. Number of voidings is correlated to intake and bladder capacity as in

older children, and voiding is repeated once every hour if the child is fed regularly during 24 hours[47]. The voiding pattern at this age has immature characteristics with undeveloped coordination between detrusor contraction and sphincter relaxation, resulting in interrupted voiding and incomplete emptying in many infants[47-49].

Urodynamic studies of healthy young infants have shown high voiding detrusor pressure and low bladder capacity, especially in males[50]. The dyscoordination is easily recognised, with fluctuation in voiding detrusor pressure simultaneously with intermittent increase in EMG activity of the pelvic floor. Overactive contractions, on the other hand, are only seen in 10% of healthy infants[48, 50, 51]. During the first months of life a voiding contraction can be seen at the start of filling, with leakage of urine (premature contraction) in 20%, which should probably not be interpreted as overactivity[50]. After the first year of life voiding pressure normalises and is similar to what is seen in older children[51, 52].
During the second year of life there is an increasing awareness of the desire to void and the functional bladder capacity increases. The detrusor sphincter coordination develops and there is normally little if any residual urine left in the bladder after voiding. From 2-4 years the normal child develops conscious, voluntary control of the lower urinary tract[45, 47]. The bladder capacity increases with age, but the increase is probably not a linear correlation, at least not during the first years of life[53, 54]. Still, linear correlations universally serve as clinical instrument for estimation of expected bladder capacity[55]. Many different formulas have been suggested[45, 56, 57]. There is evidence of achieving higher bladder capacity (BC) in catheter-based investigations as compared with free voiding studies, at least after the first year of life[58]. (figure 6).

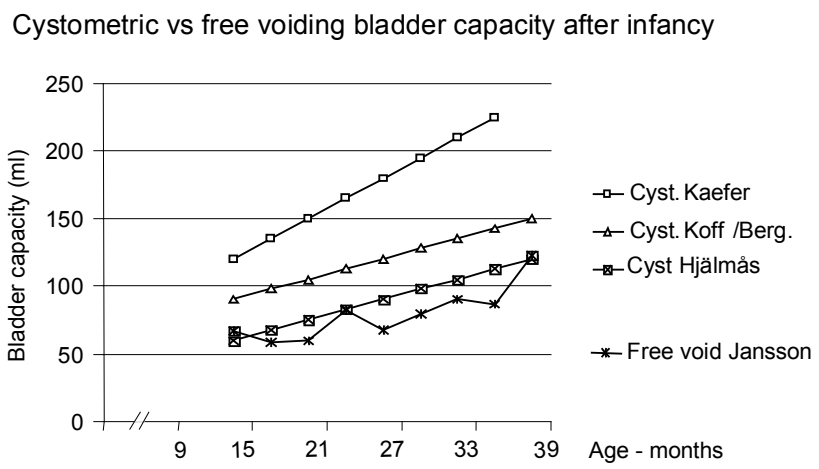


Figure 6. References for estimated bladder capacity in infants and small children with estimated normal bladder capacity in ml plotted against age in months according to three urodynamic studies and one free voiding study. Note that cystometric capacity is higher than free voiding capacity [58].

But even in formulas in which the results are derived from catheter-based studies very different estimated bladder capacities at given ages have been found[45, 56, 57](figure 6).

Vesicoureteral reflux, urinary tract infections and renal damage

The association between vesicoureteral reflux, urinary tract infections and renal damage in children is well established[13, 16, 17, 20]. The distinction between the congenital renal damage, or abnormality, and the acquired renal scarring, both associated with higher grades of reflux, has developed gradually over recent decades, after contributions from both clinical and experimental studies[17, 18, 24, 36, 59, 60]. Suggested mechanisms of renal damage in VUR are: bacteriuria-reflux into upper urinary tract-renal scarring[13, 16, 17, 61], intrarenal reflux of sterile urine with high pressure and renal scarring[62, 63], a genetic basis of susceptibility to acute pyelonephritis[64]. There are two types of renal papillae in the human kidney, the simple (convex) papillae and the compound (concave) papillae. The simple papillae are mainly located at nonpolar regions and possess oblique, slitlike, ductal orfices that close upon increased intrarenal pressure and thus prevent intrarenal reflux. The compound papillae possess gaping orfices that are perpendicular to the papillary surface and that remain open upon increased intrarenal pressure. They allow free intrarenal reflux and if the urine is infected, the presence of bacterial endotoxins activates the host's immune response with release of superoxide and other mediators. This cascade of inflammation results in local tissue ischemia and fibrosis and eventually leads to scar formation at the infected polar region [61]. Aquired renal lesions are most often localised at the polar regions of the kidney but initial scar formation can distort the local anatomy of the papillae and convent simple papillare into compound papillae. This might lead to further intrarenal reflux and additional renal scarring.

In experimental studies with obstruction of the urethra in piglets and mini-pigs, creating a high intravesical pressure transmitted to the renal pelvis, formation of renal lesions were observed even in the absence of bacteria[62, 63].

Lately, genetic explanations of susceptibility to acute pyelonephritis have evolved showing that some individuals have a genetic predisposition to renal injury[64]. Single gene defects in mice have been shown to confer susceptibility and develop and exaggerated acute inflammatory response, which leads to renal scarring[65].

Treatment

The principal goal in both medical and surgical management of reflux is to prevent recurrent febrile urinary tract infections, development of pyelonephritis which might lead to progressive renal parenchymal damage, and future renal impairment and hypertension[66-69].

Medical management of reflux consists of low-dose continous antibiotic prophylaxis. It is initiated in infants with suspected dilated vesicoureteral reflux, after findings of fetal hydronephrosis on prenatal ultrasound, or after pyelonephritis in infancy. After the diagnosis of reflux grades III-V is confirmed on voiding cystourethrog-

raphy (VCU) the prophylaxis is continued until spontaneous resolution or surgical treatment of reflux. Reflux of lower grades (grades I and II) does not require medical or surgical treatment[70]. The major disadvantages of medical therapy is increased antibiotic resistance, which has become an increasing worldwide problem in the last decades [71]. In recent years antibiotic prophylactics has been questioned, since there is limited evidence based data supporting the use of long time prophylactics[72].

Surgical treatment of reflux is indicated when medical treatment is unsuccessful, seen as recurent febrile pyelonephritis and progress of renal damage, despite antibiotic prophylaxis. The goal of surgery is to create a competent vesicoureteral junction. This is achieved either by open surgery with neoimplantation of the ureter through the bladder wall according to Cohen's procedure (intravesical technique)(figure 7a),which is the most popular technique today, or reimplantation of the ureter according to Lich-Gregor's procedure (extravesical technique)(figure 7b).

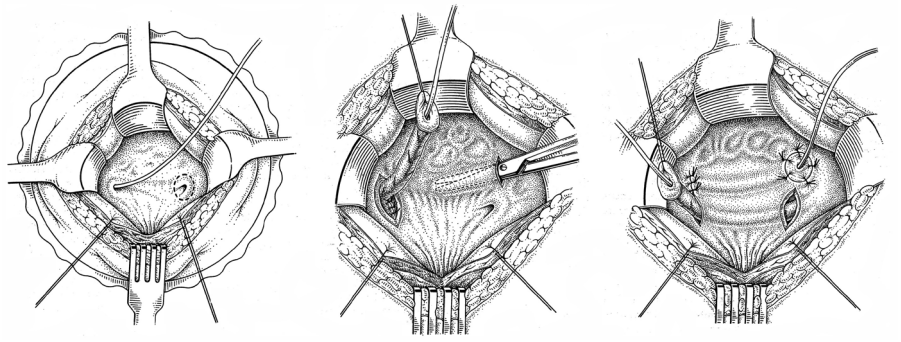


Figure 7a. Neoimplantation of the ureter through the bladder wall according to Cohen´s procedure (intravesical technique).

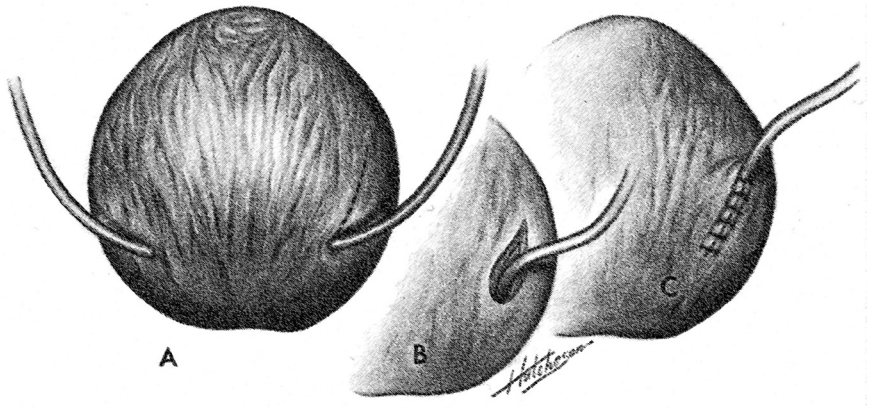


Figure 7b. Reimplantation of the ureter according to Lich-Gregor´s procedure (extravesical technique)[4].

Surgical success rates varies depending on technique and grade of reflux but is reported to be 95% (range 81-99) overall [71]. The main complication to surgery is obstruction of the orfice (2% range 0-9%), and reoperation is needed in 2% (range 0.3-9)[71].

Endoscopic treatment with injection of dextranomer/hyaluronic acid copolymer (Deflux^{R}) has gained popularity in recent decades. The procedure is performed by inserting a cystoscope through the urethra, and injecting a small amount of the copolymer into the bladder wall near the refluxing ureter orfice[73](figure 8).

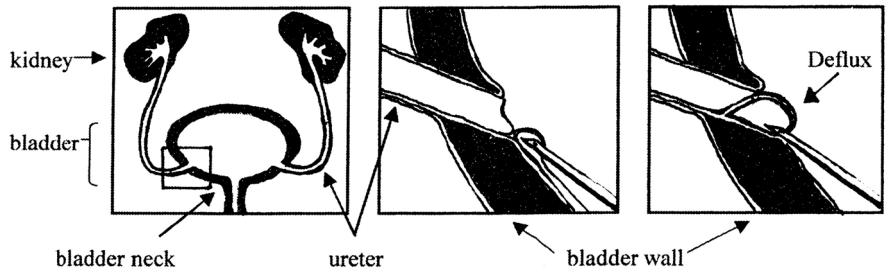


Figure 8. Endoscopic treatment of vesicoureteral reflux with injection of dextranomer/hyaluronic acid copolymer (Deflux^{R}) in the bladder wall near the refluxing ureteral orfice[33].

The overall success rate for endoscopic treatment after a single injection is 77%, and varying results are reported, with higher success rates for lower grades of reflux (range 41-87% in studies on reflux grades II-IV)[71, 74, 75]. Complications to endoscopic treatment are rare and are reported as persistent postoperative ureteral obstruction in 0.4% of cases. The technique does not preclude open surgical correction if not successful[71]. The presence of voiding dysfunction is reported to be a limiting factor in the success of endoscopic treatment in some studies while in other studies no difference have been found[74, 75]. No long-term side effects of the injected substance have been identified.

Aims of the study

At the beginning of this study there was a particular interest in the selected group of children found to have severe VUR diagnosed in infancy. Increased knowledge of bladder function or dysfunction was seen as a key to better understanding and management of children with dilated VUR. Sillén et al. identified pronounced depressor hypercontractility in infants with gross bilateral reflux[76], and Yeung et al. reported findings of increased bladder wall thickness in infants with gross bilateral reflux[24]. These findings initiated the present study in infants with dilated VUR at our hospital with longitudinal investigation of bladder and renal function and observation of the natural course of VUR.

Research questions

- What is the spontaneous resolution rate in dilated infantile VUR?
- Which factors affect the spontaneous resolution in this selected group of VUR patients?
- Can we select patients with a high chance of resolution from patients with a low probability of resolution and make individual clinical decisions about follow up and intervention in line with these findings?

- What are the bladder function characteristics in VUR patients of this age group?
- How does bladder function develop over time in infants with VUR?
- How can we identify patients with bladder dysfunction?

- What are the frequencies and types of renal abnormality in infants with dilated VUR?
- Is renal function impaired?
- How many infants deteriorate in renal status during the first years of life?
- Can we prevent deterioration or identify patients at increased risk?

Study design and inclusion criteria

To respond to these research questions, a study was set up at the initiative of Sillén et al. at the beginning of 1992. It was prospective and mainly observational in design. A cohort of children with severe VUR diagnosed during the first year of life, were conservatively treated with prophylactic antibiotics, with monitoring of bladder and renal function and observation of the natural course of reflux. Registration of breakthrough urinary tract infections was performed throughout the study and infections were treated promptly and without delay. Surgical intervention was intentionally late and indications for surgery were persistent high-grade reflux at the end of the observational time and/or repeated breakthrough infections.

Inclusion and exclusion criteria

Patients born in 1992 to 1999 with dilated primary VUR (grades III-V) on at least one side, diagnosed during the first year of life, and referred to or primarily treated at the Queen Silvia Children's Hospital in Gothenburg were eligible for the study. The hospital is a secondary referral centre for high-grade VUR (grades IV and V) from a region with a population of 1.8 million people but manages all patients with dilating VUR (grade III-V) from the local area which explains the relatively low number of patients with VUR grade III included in the study population. Patients were eligible both when diagnosed after findings of hydronephrosis on prenatal ultrasound as well as when diagnosed after pyelonephritis. Infants with secondary reflux due to neurological (myelomeningocele or spinal cord injuries) or obstructive (posterior urethral valves or ectopic ureteroceles) disorders were excluded.

Investigation programme

After the first diagnostic voiding cystourethrography (VCU) the patient was investigated with videocystometry (VCM), which includes simultaneous VCU and cystometry, free voiding observation (FVO), renal scintigram (DMSA or MAG-3) and Cr-EDTA-clearance. Serum creatinine and urinary culture were taken at follow up appointments.

These investigations were repeated *one year after diagnosis, two years after diagnosis* and *three to four years after diagnosis.*

Parents received information about early potty training to improve bladder function and enhance bladder emptying.

After spontaneous resolution of reflux or downgrading to grade II or less, spontaneously or after surgical intervention, no further VCMs or VCUs were performed. Monitoring of renal damage and function was continued even after VUR resolution.

When the treating physician was worried, sometimes investigations were done more frequently than recommended in the study program. The results from all investigations in each study patient were collected by the study centre for analyses. Table 2 shows median number of investigations per study patient and median age at first in-

vestigation. The number of patients at follow up decreased with increasing age of the child depending on a series of factors, the most important being increasing number of children with cessation of reflux, thus with a lower risk of recurrent pyelonephritis, resulting in less parental interest in continuing the study program.

Table 2. Summary of investigation program, median age at first investigation, median number of investigations and median follow-up time in 115 infants with dilated VUR from infancy.

	Median number of investigations per child (range)	Age at first investigation Median months (range)	Follow-up time Median months (range)
VCM, (VCU) & Free voiding studies	3 (2-5)	2.7 (0.03-12)	36 (2-69)
Scintigrams (DMSA&MAG-3)	4 (1-10)	4.7 (0.2-54)	62 (4-135)
Plasmaclearance (51Cr-EDTA-clearance, 98 patients)	3 (1-11)	7.7 (0.5-72)	53 (1-145)

Ethical approval

The study received approval from the Committee of Ethics at the University of Gothenburg, and parents gave their consent for the children to participate in the study program. Information was given by the treating physician and after parental approval the patient was included in the study.

Material

This study comprises a total of one hundred and thirty-five patients born in 1992-1999 and referred to the Queen Silvia Children's Hospital for dilating VUR (grades III-V) on at least one side. Distribution of sex, pre- or postnatal diagnosis and grade of VUR at inclusion are shown in table 3.

Table 3. Material, paper I-IV.

Paper	I, II, IV	III
Patients, year of birth	1992-1997	1992-97 / 1998-99
Number of patients	115	114 (93 / 21)
Sex, N (%) : boys	80 (70%)	89 (78%)
girls	35 (30%)	25 (22%)
Presentation: prenatal	30 (26%)	30 (26%)
pyelonephritis	82 (71%)	84 (74%)
Grade of VUR: Grade III	18 (16%)	22 (19%)
Grade IV	52 (45%)	52 (46%)
Grade V	45 (39%)	40 (35%)

Papers I, II and IV comprise one hundred and fifteen patients born 1992-1997 (table 3).

Paper III comprises patients born 1992-1999 (table 3). The extended inclusion period for the longitudinal study of bladder function was needed since some patients or parents did not comply with repeated VCM but preferred VCU during follow up. The cystometry results were necessary for estimation of bladder function variables but were not needed for evaluation of VUR resolution or bladder capacity.

One weakness of the study is that we do not have any record of the number of patients that were eligible but not included because of the individual choice of the child's parents or treating physician. We have notes about 4 patients included but who did not participate in any study investigations and who were thus immediately lost to follow-up and therefore excluded. Patients later lost to follow-up were analysed as long as they followed the study program.
This is not an epidemiological study and the patient material is highly selective. Although the majority of infants with VUR grades IV and V in the region were referred to the study, referral of infants with grade III only occurred from the local area, explaining the relatively low number.

Methods

Videocystometry (VCM)

VCM is a simultaneous VCU and cystotometric investigation that enables imaging of occurrence of reflux and monitoring of bladder function variables, performed with computerized equipment (figure 10).

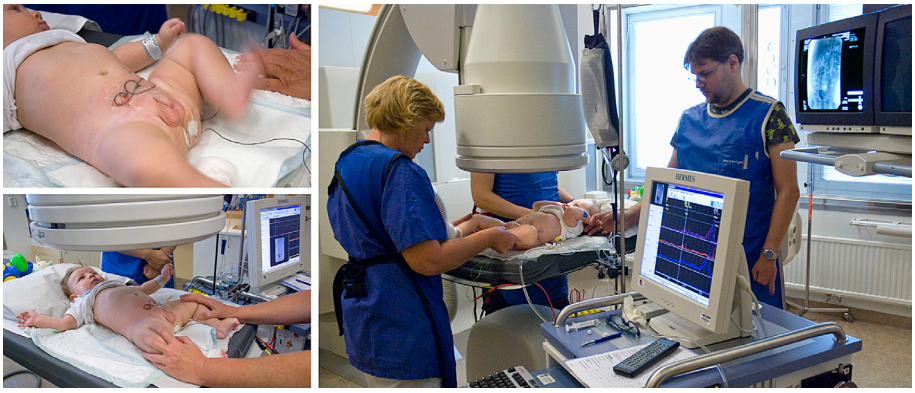


Figure 10. Investigation situation in performing videocystometry (VCM) with simultaneous voiding cystourethrography (VCU) and cystometry in an infant child. Spot fouroscopy is taken as the contrast medium fills the bladder and cystometry curves are obtained. (Photo: Anna-Karin Larsson)

Intravesical, abdominal and subtracted detrusor pressures were recorded simultaneously and perineal electromyography was performed using skin electrodes. Bladder filling and pressure recordings were obtained via a 6 Fr double lumen transurethral

catheter during follow up, exept in boys younger than 1 year, were two supra pubic 5 Fr tubes were used. The latter technique was also used in children older than 1 year in whom difficulties introducing a urethral catheter could be expected. Contrast medium (100 ml iodine per ml isopaque) was used for bladder infusion at a slow rate of 3-5 ml per minute depending on patient age and expected bladder capacity. Spot fluoroscopy was performed at regular intervals during filling and at noticeable increase in detrusor pressure. X-rays were exposed at occurrence of reflux and during voiding. The bladder was filled twice in all patients except those with extremely high capacity and time consuming filling during follow up. After the last filling the catheter was removed to obtain a view of the urethra at voiding. Infants with reflux only at the second filling were also included into the study.
The radiation burden of VCM according to this method is calculated as approximately 0.68-1.0 mSv/examination (The higher number accounts for investigation in boys, which also included a lateral exposure).

Grading of VUR was done from the VCU part of the investigation according to the International System of Radiographic Grading of Vesicoureteric Reflux[1]. All voiding cystourethrographies, including copies of the initial radiographic investigations performed elsewhere, were reviewed by one of two specialist in pediatric radiology (E.S./ M.B.) and pediatric surgery-urology (U.S./ S.S.), respectively.

Cystometric variables
Cystometric or maximum bladder capacity was evaluated from the VCM. Bladder capacity was estimated as the sum of voided volume and residual urine withdrawn from the bladder directly after voiding. *Expected bladder capacity* for age in ml was calculated from the formula 30+ 2.5x age in months[45].
Refluxing volume was estimated from urine withdrawn from the bladder 5 minutes after voiding and repeatedly again after 5 minutes if the upper tracts still contained considerable amounts of contrast seen on spot fluoroscopy. Refluxing volume was not included in either BC or residual urine. (figure 11)
O*veractive contractions* were defined as an increase in pressure during the filling phase exceeding 15 cmH_2O above the baseline. The strength of the detrusor overactivity was estimated using the maximum pressure of the detrusor contractions.
Premature voiding contraction was seen as a single detrusor contraction early during filling with a maximum pressure equal to or higher than the maximum voiding pressure with leakage of small amounts of urine. This was not considered as detrusor overactivity.

Free Voiding Observations

The voiding pattern and difficulties in emptying the bladder were evaluated on the basis of the non-invasive four-hour voiding observation (FVO) designed by Holmdahl et al.[49]. In infants and non toilet trained children the patient was observed for 4 hours by the parents under the surveillance of a trained urotherapist. Initial bladder volume was assessed by ultrasonography and a dry weighed diaper was applied. Every 5 minutes the diaper was checked by the parents using a “gossip strip” placed

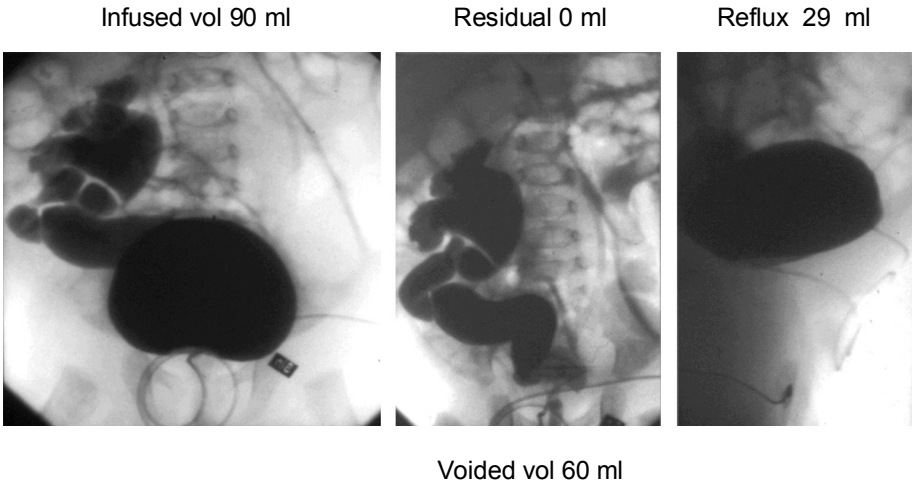


Figure 11. Spot flouroscoy image from VCM in an infant boy, 2 months of age with unilateral VUR grade V, showing the relationship between bladder capacity, residual urine and refluxing volumes.
A. Filling of the bladder (infused volume of 90 ml). B. Image taken directly after end of voiding (voided volume 60 ml). C. Bladder refilled 5 minutes after end of voiding (refluxing volume 29 ml).

in the diaper demonstrating when voiding occurred. Urine volume was evaluated by weighing the diaper, and residual urine was determined using ultrasound with a 7.5 MHz linear scan probe. The diaper was not opened until 30 seconds after the gossip strip was noted as wet, to avoid disturbance of voiding.
In toilet trained children the free voiding observation was carried out with repeated measurements of flow and residual during a four-hour observation period under the surveillance of an urotherapist.
Results from the FVO were recorded in a protocol, and residual urine was calculated as the mean of repeated measurements after voidings during 4 hours.

Definition of bladder dysfunction
Bladder dysfunction was defined as residual urine > 25% of bladder capacity (taken from FVO) combined with bladder capacity > 200% of expected (taken from VCM) and/or more than 5 overactive contractions with maximum detrusor pressure of more than 30cmH_2O on more than one occasion (taken from VCM).
Based on these criteria bladder dysfunction was subgrouped as:
Dilated bladder dysfunction (DBD) defined as high-capacity bladder and incomplete emptying, with or without overactivity according to the definitions given above.
Overactive bladder (OAB) defined as significant numbers of overactive contractions either with normal emptying and bladder capacity or with one of these variables elevated.
Uncertain bladder dysfunction defined as either high-capacity or increased residual urine but without pronounced overactivity.

Categorization of bladder function was done in VCM and FVO performed between 1-2 years of age, since bladder dysfunction was difficult to evaluate at the investigation during the first year of life. Children with none of the above mentioned criteria were considered to have normal bladder function.

Methodological considerations: VCM and FVO

Some artefacts may influence the results of the cystometric examinations, induced by anxiety in the child, irritation of the catheter, and the filling process. The artifacts to be expected are overactive contractions during filling and incomplete or postponed voiding[77]. To reduce the risk of artefacts filling rate should be constant and at maximum 10% of expected bladder capacity/minute, temperature of the contrast medium should be 25-36 ºC and fillings should be repeated two or three times, at least in older children[77]. Since anxiety about the investigation situation is less pronounced during the first year of life, fewer artefacts could be expected to be present in this age group. Therefore the use of cystometric results from the first filling in the present study during infancy was considered acceptable.
There are many formulas suggesting different expected normal bladder capacity (figure 6)[45, 56, 57]. The differences are probably mainly related to different type of investigations: catheter based or free voiding observations. The formula used in this study was defined by Hjälmås and calculated from cystometric investigations in children from 2 months to 4 years[45, 52]. It correlates well with the data found in a longitudinal study of free voiding observations in healthy children from birth to three years of age[53]. Therefore, we used this formula as a reference for expected maximal or cystometric capacity as also recommended by the Standardisation Committee of the International Children's Continence Society (ICCS)[55].

Using bladder volume from the VCM gives information about maximal bladder capacity (BC) rather than functional BC, and cystometric BC is considered more constant than functional BC, which has greater intra-individual variation[54].
We believe that residual urine is best reflected in the FVO. Taking the information about residual urine from VCM would mean the risk of more pathological values caused by disturbance of a transurethral or suprapubic catheter.

Renal Scintigraphy; ^{99m} Tc-DMSA & ^{99m} Tc –MAG-3

The presence of renal abnormalities was evaluated by means of static or dynamic renal scintigraphy. The investigations were performed according to European standards[78, 79].
^{99m}Tc- DMSA scintigraphy was the first method of choice, but we used ^{99m}Tc-MAG3 scintigraphy in the presence of severely dilated renal pelvis and calices when obstruction or poor drainage could be expected. In addition to the scintigram image both static and dynamic renal scintigraphy allow the side distribution of renal function to be determined, expressed as split function in percent of the total renal activity uptake.

^{99m}Tc- DMSA is the most appropriate tracer available for static cortical imaging, with a high sensitivity for detection of acute and chronic cortical abnormalities in the kidney[78]. The tracer is taken up by the tubular cells directly from the tubular vessels. Indications for using ^{99m} Tc-DMSA scintigraphy are detection of focal renal parenchymal lesions in acute pyelonephritis, as well as detection of renal sequele 6 months after acute infection. This method can also be used for diagnosis of associated anomalies: small kidney, dysplastic tissue and detection of ectopic kidney[78]. In the case of marked hydronephrosis, tracer may accumulate in the renal cavities, causing difficulties in the interpretation of cortical images and give falsely high differential function[78].

Investigation procedure

The radionuclide was injected intravenously through a Venflon needle and images were acquired 2-3 hours after tracer injection. The gamma camera (collimator) collected counts with the child in a supine position. Three images were aquired: posterior and left and right oblique posterior. The differential renal function was calculated, based on background subtracted kidney uptake. The reference value for split function is between 45 and 55%[78].

The initial evaluation and classification of renal abnormalities was done directly on the computer screen but hard copies in both grey scale and colour were taken and used for review according to the study protocol.

The radiation burden of ^{99m} Tc-DMSA scintigraphy is approximately 1 mSv/examination regardless of the age of the child, provided that the dose is adapted according to the body surface area[78].

^{99m}Tc-MAG3 is a dynamic tracer with high excretion rate used for standard and diuretic renography in children. The investigation allows for estimation of two aspects of renal function: estimation of split function, i.e. the extraction of a tracer from the blood and distribution between the right and the left kidney, and the excretion, or disappearance, of the tracer from the kidney. The disappearance can be estimated by inspecting the renogram curve: an early peak followed by a rapidly descending phase is typical of normal excretion[79]. Any major delay in excretion is characterized by a continuously ascending curve[79].

^{99m}Tc-MAG3 scintigraphy can also be used for imaging of renal parenchymal damage but it is less sensitive than the static ^{99m} Tc-DMSA. Common indications for this investigation are all uropathies which require evaluation of individual renal function and/or drainage function at diagnosis and during follow up of surgical or conservative treatment[79]. When dilatation of the collecting system exists, the standard renogram is usually supplemented with a diuretic renogram.

Investigation procedure

The child was fitted with a Venflon catheter and (adequately) hydrated perorally by means of 15 ml/kg body weight of a suitable liquid during the hour before the study. The radionuclide was injected intravenously through the Venflon needle. Image acquisition began immediately before the injection. The gamma camera was

positioned with the collimator facing up with the child lying in a supine position. The minimum data set was 0-20 minutes. If a diuretic was administered (diuretic renogram) after the injection of the tracer, an additional 15-20 minutes of acquisition was obtained. The relative function of each kidney was computed after subtraction of background activity, and expressed in percent of the total function; normal values are between 45 and 55% uptake[79].
The radiation burden of ^{99m}Tc-MAG3 scintigraphy is 0.2-0.4 mSv/examination[79].

All renal scintigrams were reviewed by a team of specialists (R.S., U.J. and S.S.) according to a study protocol. Notations were made at each investigation regarding split function and renal abnormalities. The complete series of investigations in each patient were compared to identify signs of deterioration during follow up. Renal abnormality was classified as focal or generalised, unilateral or bilateral. A small kidney with reduced tracer uptake or diffuse parenchymal anomaly was classified as generalised abnormality (figure 12).
None of these had split function of > 45% (if unilateral damage). Focal damage or abnormality was defined as areas with reduced uptake or indentation of the renal outline.

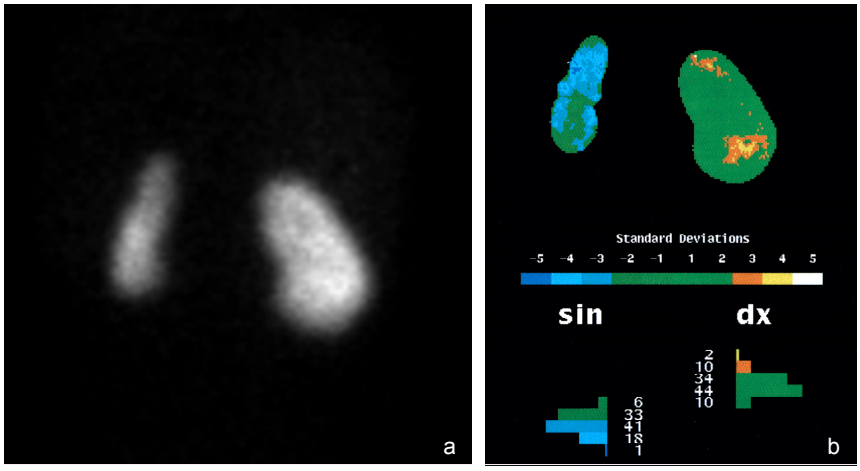


Figure 12. 99mTc-DMSA image of a generalised renal abnormality with reduced tracer uptake in a small left kidney with split function of 28% of total uptake.

Methodological considerations: renal scintigraphy

There are possible objections against using two separate techniques in illustrating renal parenchymal abnormalities:
Although DMSA is considered most sensitive for detection of renal scars and abnormalities MAG-3 scintigraphy can provide a reliable semi quantitative and qualitative detection of renal inflammatory lesions in acute pyelonephritis and during recovery, according to a comparative study with MAG-3 and DSMA scan performed in chil-

dren after acute pyelonephritis [80]. Furthermore MAG-3 and DMSA can both be used for estimation of differential kidney function and their result can be compared during follow up according to studies on patients investigated with both static and dynamic scintigraphies within 5 days to 3 months [81, 82].

^{51}Cr-EDTA-clearance and other GFR estimates

Glomerular filtration is the initial and generally rate limiting step in the renal excretory process, and measurement of glomerular filtration rate (GFR) provides an overall estimate of renal function. The classic technique for measuring GFR requires the infusion of a substance that is filtered freely and is not reabsorbed, secreted, or metabolized by the kidneys. The direct assessment of GFR with inulin clearance constitutes the reference method for measurement of GFR, but this method requires constant infusion and plasma and urine samples, and is not used by routine in clinical practice. The agent used in this study was ^{51}Chromium-ethylenediaminetetraacetic acid (^{51}Cr-EDTA), and evaluation of absolute GFR was based on the plasma disappearance curve after a single bolus injection of the tracer. The clearance of the substance is obtained by dividing the injected dose by the area under the curve [83]. The reference value used for normal GFR was 110 mmol/l/1.73m^2 after two years of age according to Brochner-Mortensen[84] and GFR<80% (<2SD) of expected was considered subnormal.

Investigation procedure

The patient should be adequately hydrated. The tracer was given through a Venflon needle and blood samples were drawn at 5, 15, 60, 90 and 120 minutes after the injection from the back port of the Venflon, according to Brochner-Mortensens method[84]. The samples were centrifuged and activity measured in each sample in a well counter.

The radiation burden for ^{51}Cr-EDTA is approximately 0.011 mSv/examination regardless of the age of the child, provided that the dose is adapted according to body weight [83].

Methodological considerations: GFR measurements

Since steady state of GFR is not achieved until after two years of age we operationally used the equation of Winberg for estimation of expected clearance in investigations performed before two years of age[85] (figure 13). GFR measurements performed during the first months of life showed variability and seemed less reliable, which was our reason for using the last available GFR in each patient to describe renal function. When looking at GFR over time, in repeated investigations in each patient, we only found significant loss of GFR (>12%) in one patient during follow up.

In some patients where there was no direct GFR measurement available, we used indirect estimation of glomerular filtration rate according to the formula of Schwartz[86]. This is not as accurate as direct measurement of GFR but provides a reasonable estimate of renal function[86], and combined with the renal scintigram it gives a good description of renal status in the individual patient.

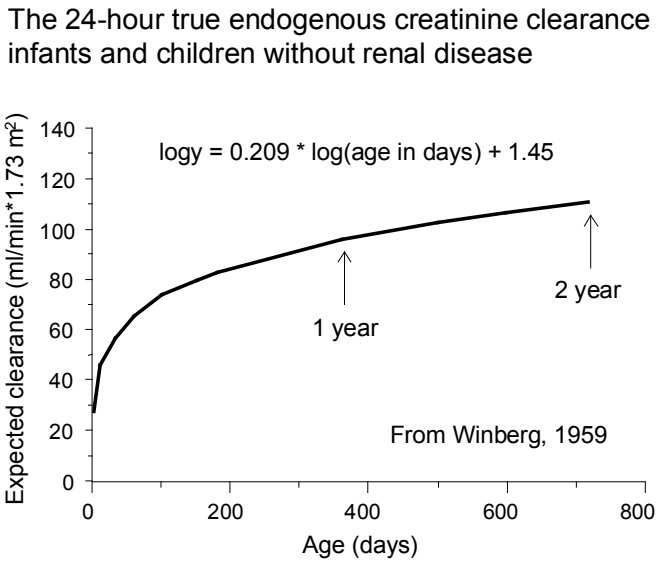


Figure 13. Estimation of expected clearance (ml/minute/1.73 m2) in age 0-2 years according to Winberg's logarithm[85].

Definition of deterioration of renal status during follow-up

Deterioration of renal status in an individual was determined as new lesions on the renal scans or loss of ≥7% in split function or loss of ≥12% of GFR during follow-up.

Methodological consideration and references for definition of deterioration of renal status

The rationale for using loss of ≥7% of split function in renal scintigrams as one sign of deterioration in renal status was based on the following considerations:

When evaluating the reproducibility of renal scintigrams in adults and children one standard deviation (SD) of differences between measurements of relative function was 2-3%, depending on method used[87]. Two SD should thus be 4-6% change in relative function[87]. As some of our investigations performed at very young age were difficult to interpret, it seemed reasonable to use the higher reference (≥7%) to define significant loss.

One could speculate as to whether loss in split function reflects actual deterioration or is a sign of compensatory growth of the contralateral kidney. In a study by Piepsz et al the majority of study patients with a deterioration of >5% in split function remained stable or improved in single kidney glomerular filtration rate[88]. This indicates that loss of split function of the affected kidney actually represents a functional compensation of the contralateral kidney. In pediatric patients there is evidence that functional compensation occurs in a kidney when the function of the contralateral kidney is absent or decreased below 30-35 ml/min/1.73m^2 [89].

To detect a significant change in renal function, two determinations have to differ by more than 12% at GFR values higher than 30 ml/min according to Brochner-Mortensen et al., which was our reference when setting the limit for deterioration in renal function[90].

Infection control

All children received antibacterial prophylaxis, consisting of 0.5 mg/kg trimethoprim or 5 mg/kg cefadroxil given as a single daily dose after diagnosis. The prophylactic agent often had to be changed to nitrofurantoin or ciprofloxacin, depending on bacterial species and resistence in children with recurrent breakthrough urinary tract infections (UTI). Urine cultures were obtained regularly at follow-up visits and during febrile episodes. Recurrent UTI was defined as growth of at least 100 000 cfu/ml in urine obtained by bag or midstream sample with fever of 38.5 or more. A single dose of prophylactic antibiotics was given at VCM; in infants 2 mg/kg tobramycin were given intravenously and in older children an antibiotic different from the ordinary prophylaxis was given orally. In boys with recurrent infections circumcision or dorsal insision was considered in an attempt to minimize bacterial contamination of the urethra and ascending infections. In girls labial synechiae that could contribute to recurrent infections were treated. In patients with recurrent infections and bladder dysfunction clean intermittent catheterization was considered during infancy to minimise residual urine and bacterial growth. In patients at an appropriate age for achieving bladder control toilet training was instituted as the first step. Finally, in patients with repeated recurrent infections despite the above mentioned strategies, anti-reflux surgery was considered.

Methodological considerations: infection control

Although this was a prospective study, there was a certain element of retrospective sampling of data, especially concerning registration of breakthrough UTIs. The acute infections were often treated at hospitals and medical centres elsewhere and reported with a delay to the study centre. Details about bacterial species and resistance were in many cases missing from our files, and were difficult to find afterwards. The UTI data are therefore incomplete and bacterial species are only available in 85% of the registered infections. Collecting data retrospectively also made it almost impossible to evaluate whether or not the infection occurred after non-compliance in taking the prophylaxis or actually was a breakthrough despite the antibiotics.

Results and comments

Demographic data at inclusion split by grade of VUR at inclusion are shown in table 4 (Study population for Papers I, II and IV).

Renal abnormality

Renal abnormality or damage was seen in 98 of the 115 children (85%) at inclusion and in 103 (90%) at the last followup[91]. Generalised abnormality was seen in 72 individuals (63%) and isolated focal damage in 26 (23%) at inclusion. Bilateral abnormality was seen in 23 (20%) at inclusion. There were no differences in frequency and type of renal abnormality in boys and girls or in patients prenatally diagnosed compared with those who presented with UTI. The frequency and type of renal abnormality did not differ between patients with VUR of different grades (III-IV) at inclusion (table 4)[91].

Renal function

Total GFR was subnormal (<80% of expected) in 34 of 112 (30%) at follow-up[91]. There was a significant difference in GFR between cases diagnosed prenatally and those diagnosed after pyelonephritis (p=0.019), which is in line with the finding of significantly higher VUR grades in individuals identified prenatally in this study (table 4)[91]. There was no difference between boys and girls in terms of mean GFR or number of patients with subnormal GFR. After 2 years of age 27 of 84 patients (32%) had chronic kidney disease (CKD) stage 2 (GFR 60 - 89 ml per minute per 1.73m^2) and 3 had reached CKD stage 3 (GFR 30-59 ml per minute per 1.73m^2)[92]. No patient in our study was in the more severe CKD stages 4 or 5.
Subnormal individual kidney function of less than 40% of expected total GFR was seen in 79 (71%) of the patients at the last followup[91].

Renal status over time

The renal scintigrams and GFR measurements of the majority of patients remained unchanged over time. Of the 108 patients with repeated investigations 84 (78%) had unchanged renal status and 5 (5%) had improved (recovering from focal lesions seen in previous scintigrams)[91]. Deterioration was seen in 19 (18%), defined as a decrease in total GFR of 12% or more (1 patient), additional renal scars on scintigraphy (6), a decrease in split function of at least 7% (10) or a combination of these findings (2)[91]. Characteristics of patients with deteriorated vs unchanged renal status are summarised in table 5. Mean age for detection of deterioration was 38 months (range 1.7-105). In survival analyses, breakthrough UTI was shown to be a predictive factor for deterioration (figure 14a). Other predictive factors for deterioration were bilateral renal damage (fig 14b) and subnormal renal function (figure 14c). Deterioration was seen more frequently in patients with prenatal diagnosis than in patients diagnosed after UTI. Only 5 of the 19 patients with deteriorated renal status were without signs of parenchymal abnormality at the first scintigram[91].

Table 4. Demographic data, renal abnormalities and renal function results split by grade of VUR at inclusion.

Variable	Grade III (n=18)	Grade IV (n=52)	Grade V (n=45)	p-value
Uni/Bilateral (reflux)				
Unilateral	5 (28%)	20 (38%)	9 (20%)	
Bilateral	13 (72%)	32 (62%)	36 (80%)	0.310
Sex				
Girl	9 (50%)	20 (38%)	6 (13%)	
Boy	9 (50%)	32 (62%)	39 (87%)	0.001
Presenting symptom				
Prenatal	2 (11%)	12 (23%)	16 (38%)	
UTI	16 (89%)	40 (77%)	26 (62%)	0.023
Age in months at first VCU	3.9 (2.5) 3.6 (0.5-11)	3.8 (3.2) 2.8 (0.1-12)	2.9 (3.4) 1.2 (0.03-11)	0.020
Breakthrough UTI	5 (28%)	23 (44%)	26 (58%)	0.033
Renal abnormality				
None	5 (28%)	6 (12%)	6 (13%)	
Focal	4 (22%)	13 (25%)	9 (20%)	
Generalised	9 (50%)	33 (63%)	30 (67%)	0.212
Uni/Bilat renal abnormality				
None	5 (28%)	6 (12%)	6 (13%)	
Unilateral	10 (56%)	39 (75%)	26 (58%)	
Bilateral	3 (17%)	7 (13%)	13 (29%)	0.092
GFR (% of expected)	90 (16) 91 (51-115)	90 (16) 89 (56-138)	83 (22) 83 (41-140)	0.019
Subnormal GFR (<80%)				
<80	3 (19%)	14 (27%)	17 (39%)	
≥80	13 (81%)	38 (73%)	27 (61%)	0.136

For categorical variables n (%) is presented.
For continuous variables Mean (SD) / Median (Min ¯ Max) is presented.
For comparison between groups Mantel-Haenszel Chi Square Exact test was used for ordered categorical variables and Spearmans rang correlation test was used for continuous variables.

Table 5. Characteristics of patients with deteriorated versus unchanged or improved renal status during follow up.

	The deteriorated group (n:19)	The unchanged group (n:89)	p value
Presentation of VUR	(n:18)	(n:87)	0.047[3]
Prenatal ultrasound	9 (47%)	20 (22%)	
Pyelonephritis	9 (47%)	67 (75%)	
Grade of VUR at inclusion			0.058[2]
Grade III	1 (5%)	15 (17%)	
Grade IV	7 (37%)	42 (47%)	
Grade V	11 (58%)	32 (36%)	
Uni/Bilateral VUR at inclusion	5/14	28/61	0.886[3]
Renal damage (at the latest follow-up)			0.580[2]
Focal	7 (37%)	21 (23%)	
Generalized	10 (53%)	52 (58%)	
Focal and generalized	2 (11%)	6 (7%)	
None	0	10 (11%)	
Uni/Bilateral renal damage	10/9	61/18	0.068[3]
Renal function (at the latest follow-up)			
Median GFR in % of expected for age	77 (Range 41-99) (n:18)	86 (Range 41-140)	0.0015[4]
Patients with reduced GFR (<80% of expected for age)	11 (61%)	21 (24%)	0.0054[3]
Breakthrough infections			
Patients with breakthrough infections	13 (68%) (n:19)	39 (44%)	0.089[3]
Number of infections in those patients	Median 2 (Range 1-10) Mean 3.6 (Std Dev 3.3)	Median 2 (Range 1-7) Mean 2.5 (Std Dev 1.3)	0.718[4]
Surgery			
Number of surgically treated patients	12 (63%)	23 (26%)	
Median age at time of surgery (months)	47 (Range 24-91)	41 (Range 14-78)	
Chi-Square1, Mantel-Haenzel Chi-Sqaure[2], Fisher's Exact Test[3],Wilcoxon Two-Sample Test[4].			

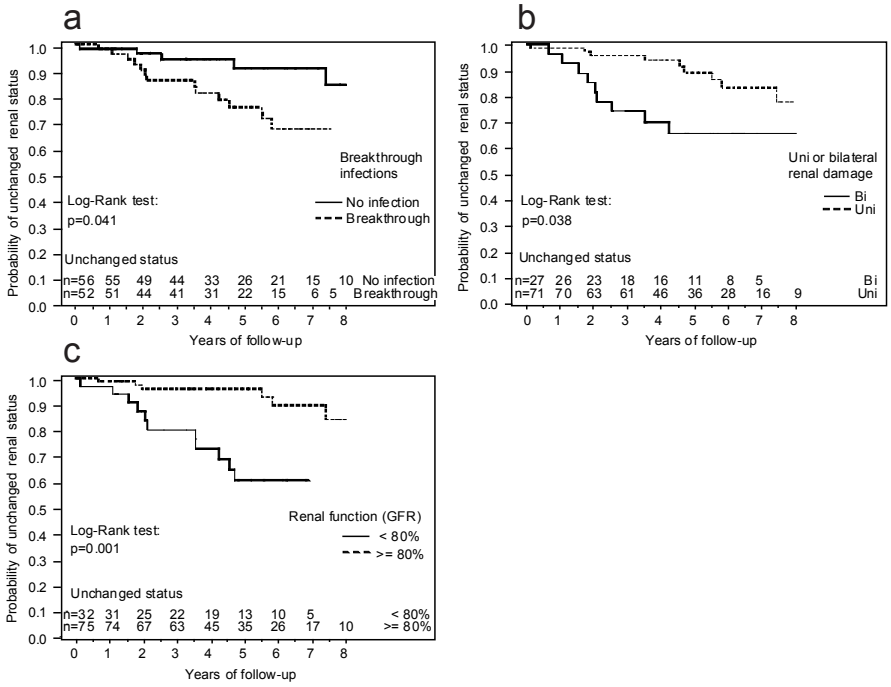


Figure 14a. Probability of unchanged (or improved) renal status in 108 children with dilating VUR with or without breakthrough UTI's. (p=0.041)
Figure 14b. Probability of unchanged (or improved) renal status in 98 children with unilateral (Uni) versus bilateral (Bi) renal damage at follow-up. (p=0.038)
Figure 14c. Probability of unchanged (or improved) renal status in 107 children with normal or subnormal (<80% of expected) renal function. (p=0.001)

Survival curves was produced by Kaplan –Meier estimates and formally tested with log rank test. n = number of patients.

Changes in bladder function over time (Paper III)

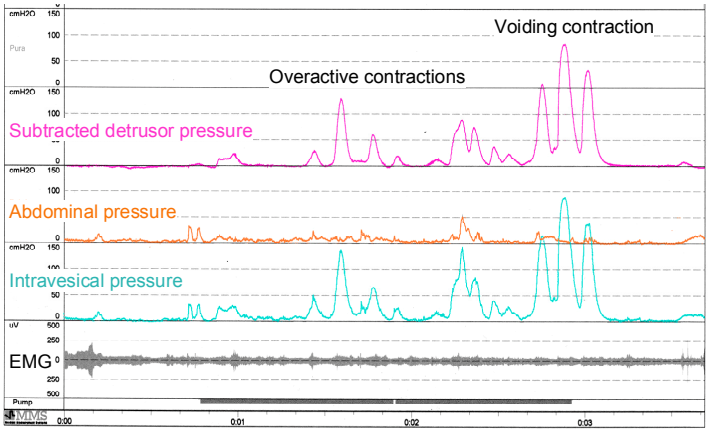
In Paper 3 we investigated changes in urodynamic variables during the first three years of life. We evaluated results from repeated VCM performed at mean age (±SD) 6 ±4, 20 ±6 and 40 ±10 months[93] (table 6).

At age 6 months the boys had significantly lower bladder capacity (p=0.0002) and higher voiding pressure (p<.0001) than the girls. Median bladder capacity was 104% (range 14-482) of expected in boys as compared with 176% (55-335) in girls. Voiding detrusor pressure was median 108 (range 37-275) cmH_2O in boys and 62 (range 27-178) cmH_2O in girls. Overactive contractions at filling were seen in 56% of the patients, and dyscoordination at voiding in 88%, with no differences between boys and girls. (figure 15a)

Table 6. Urodynamic results on repeat VCM.

	Examination One 6 months n=112	Examination Two 20 months n=97	Examination Three 40 months n=59
Number			
Male	n = 87	n = 73	n = 44
Female	n = 25	n = 24	n = 15
Bladder capacity (ml). Median (range)			
Male	41 (6-227)	160 (32-380)	286 (85-416)
Female	87 (25-200)	198 (23-325)	253 (70-428)
Male vs Female, p-value	0.0001	0.309	0.180
Bladder capacity (% of expected)			
Male	104 (14-482)	203 (39-467)	207 (79-372)
Female	176 (55-335)	235 (27-338)	172 (84-287)
Male vs Female, p-value	0.0002	0.936	0.177
Residual urine (ml)			
Male	9 (0-172)	59 (0-380)	61 (0-285)
Female	19 (0-150)	40 (0-190)	10 (0-290)
Male vs Female, p-value	0.070	0.407	0.220
Residual urine (% of bladder capacity)			
Total	24	33	12
Reflux urine (ml).			
Total	8 (0-43)	11 (0-93)	20 (3-98)
Reflux urine (% of bladder capacity).			
Total	15 (0-154)	11 (0-71)	10 (1-35)
Voiding detrusor pressure (cmH2O)			
Male	108 (37-275)	67 (27-178)	64 (28-200)
Female	62 (19-118)	51 (30-82)	51 (19-93)
Male vs Female, p-value	<.0001	0.003	0.054
Number of patents with overactive contractions n (%)			
Total	60 (56%)	49 (55%)	29 (59%)
Number of overactive contractions Median (range)			
Total	4 (1-20)	6 (1-20)	10 (1-40)
Detrusor pressure at OAC (cmH2O)			
Male	44 (15-170)	38 (9-90)	33 (15-90)
Female	22 (9-61)	30 (14-138)	35 (15-77)
Male vs Female, p-value	0.0005	0.501	0.964
EMG: increased / available n/n (%)			
Total	79 / 90 (88%)	45 / 59 (76%)	18 / 36 (50%)

a



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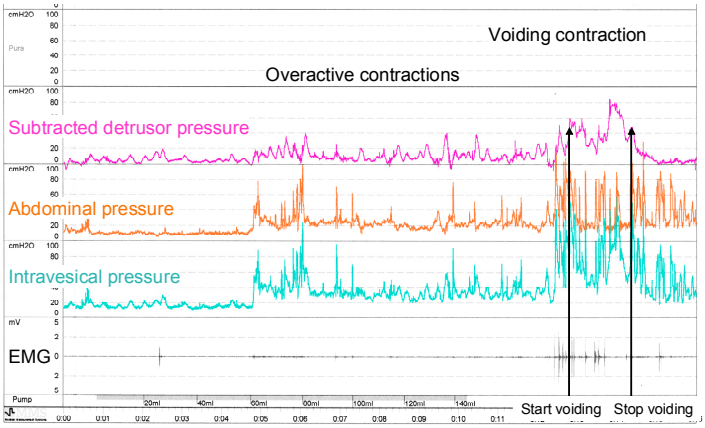


Figure 15a and b. Infant cystometry of a 3 month old boy with overactive contractions, voiding contraction, indicating dyscoordination and with filling of 13ml.
Follow up cystometry at 29 months of age, showing overactive contractions during filling, straining during voiding and incomplete emptying. Bladder capacity; 445 ml, residual volume; 140ml.

At age 20 months the urodynamic pattern had changed. Bladder capacity was increased in both boys and girls with median 203% (39-467) and 235% (27-338) of expected, respectively. Residual urine was equal in boys and girls (table 6). Overactive contractions were seen in 49 (55%) with no difference between boys and girls, but the number of children with dyscoordination at voiding had decreased (76%). (figure 15b)
At age 40 months the urodynamic pattern was similar to that found at 1-2 years (table 6).

The changes in bladder capacity and residual urine over time are shown in figure 16a and 16b, illustrating that male infants changed significantly during the first years of life whereas female infants developed high capacity bladder with incomplete emptying at an earlier age. Voiding detrusor pressure plotted against bladder capacity in each patient is shown in figure 17 showing a correlation between high voiding pressure and low bladder capacity.

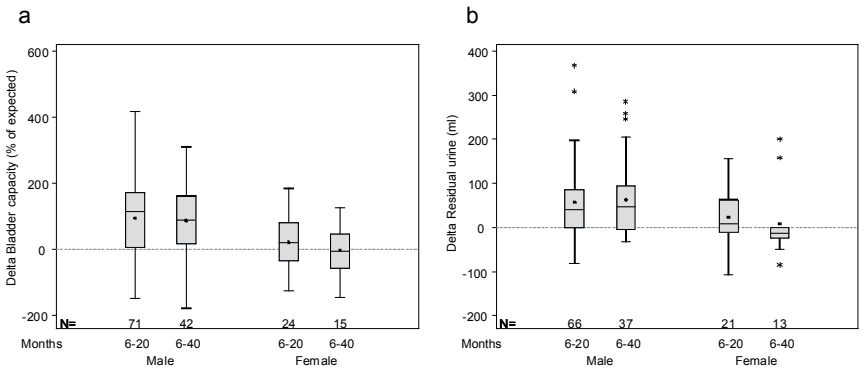


Figure 16a and 16b Change in bladder capacity and residual urine respectively in boys and girls with comparison of values at 6 versus 20 months and 6 versus 40 months.

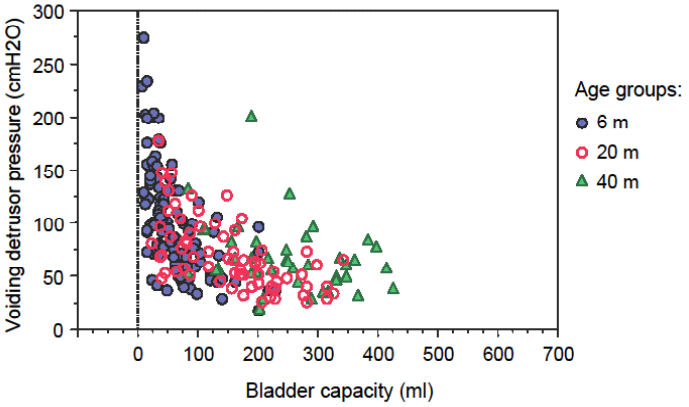


Figure 17. Voiding detrusor pressure (plotted against bladder capacity) at investigations at mean age 6, 20 and 40 months. Dotted line=level at 6 months.

It was not possible to evaluate bladder dysfunction at 6 months of age, owing to an immature voiding pattern with high pressure levels, low capacity and dyscoordination, while during the second year of life high capacity and incomplete emptying could be recognised as bladder dysfunction[93]. We tried to find predictors at presentation for development of bladder dysfunction at 1-2 years of age. We found a strong correlation between breakthrough UTI's and bladder dysfunction (p<.0001),

but UTIs could not be used as an early predictor even if the majority of infections occurred already during the first year[91,93]. Incomplete emptying with residual urine at the 6-month examination could, however, be used as a predictor for bladder dysfunction at 1-2 years of age (p=0.047)[93].

Bladder dysfunction (Paper IV)

Bladder dysfunction was seen in 48 patients (42%) at age 1-2 years, and was more frequent in higher grades of VUR (p=0.001)(table 7). Bladder dysfunction was seen in 14 girls and 34 boys. High capacity and incomplete emptying, dilated bladder dysfunction (DBD), was seen in 36 (32%), overactive bladder (OAB) was seen in 12 (11%) and uncertain bladder dysfunction in 24 (21%). Bladder capacity and residual urine were significantly higher with increasing grade of VUR (p=0.016 and p=0.0005)(table 7).

Fifty-one individuals (45%) had overactive contractions, with no differences according to sex or grade of reflux at inclusion.

Registration from the first VCM in each patient showed that VUR occurred passively, during filling, in 41 (40%), as a response to overactive contractions in 25 (24%), and at micturition in 37 patients (36%).

Table 7. Bladder function variables split by grade of VUR.

Variable	Grade III (n=17)	Grade IV (n=52)	Grade V (n=45)	p-value
Bladder dysfunction				
No BD	9 (53%)	25 (48%)	8 (18%)	
Uncertain	4 (24%)	9 (17%)	11 (24%)	
BD	4 (24%)	18 (35%)	26 (58%)	0.0010
Bladder capacity, % of expected	158 (60) 151 (80-255) n=17	185 (113) 154 (29-467) n=52	216 (89) 229 (46-404) n=45	0.0165
Bladder capacity ≥ 200%	5 (29%)	23 (44%)	27 (60%)	0.0309
Residual urine, % of bladder capacity	18 (14) 20 (1-45) n=16	20 (12) 18 (1-47) n=45	29 (12) 30 (1-49) n=44	0.0005
Residual urine ≥ 25%	3 (19%)	16 (36%)	29 (66%)	0.0003
Bladdervolume at reflux onset, % of bladder capacity	57 (36) 56 (14-100) n=10	66 (31) 67 (11-100) n=35	74 (30) 80 (5-100) n=31	0.1836
Mode of occurence				
Passive	4 (27%)	22 (46%)	15 (38%)	
Over active	3 (20%)	11 (23%)	11 (28%)	
Micturic	8 (53%)	15 (31%)	14 (35%)	0.5155

For categorical variables n (%) is presented.
For continuous variables Mean (SD) / Median (Min¯ Max) / n= is presented.
For comparison between groups Mantel-Haenszel Chi Square Exact test was used for ordered categorical variables and Spearmans rang correlation test was used for continuous variables.

Urinary tract infections

Symptomatic breakthrough urinary tract infection (UTI) during antibacterial prophylaxis was seen in 54 of 115 patients (47%), 34 (43%) boys and 20 (57%) girls[91, 94]. The difference according to sex did not attain statistical significance.

Mean number of infections in the patients with breakthrough was 2.8 (SD 2.0, Median 2, Range 1-10)[91]. The total number of infections peaked during the first year of life and then declined (figure 18a). Mean number of infections in boys was significantly lower than in girls after 3 years of age (p= 0.031, figure 18b). Bacterial species were dominated by *Escherichia coli* (41%), Other gram negative bacteria was found in 24% of urine cultures and gram positive bacteria in 34% [91], (table 8).

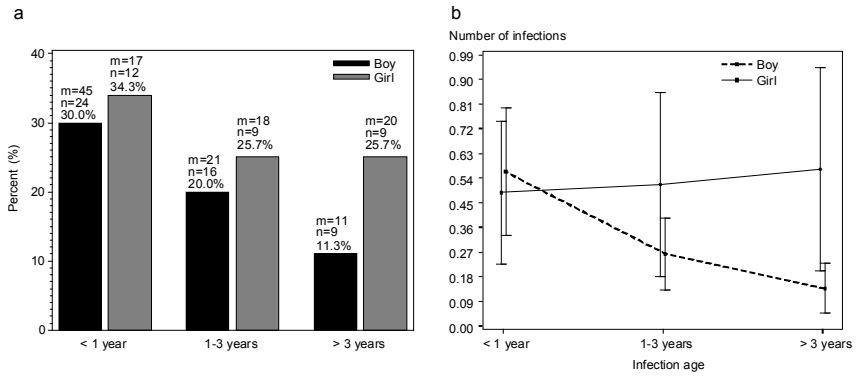


Figure 18a Percent of boys and girls with registered febrile UTI's by age group. m=total number of infections in the group, n=number of patients with infections.
Figure 18b Mean number of infections pr child with 95% CI in 80 boys and 35 girls by age. Number of infections significantly lower in boys (mean ± SD 0.14 ± 0.41) than in girls (0.57 ± 1.12) after 3 years of age (p = 0.031).

Table 8. Pathogens at febrile recurrent UTI (split by age).

	Age <1 year	Age 1-3 years	Age >3 years	Total
	n (%)			n (%)
Escherichia coli	18 (36)	13 (41)	15 (50)	46 (41)
Enterococci	8 (16)	6 (19)	4 (13)	18 (16)
Staphylococci	7 (14)	4 (12)	3 (10)	14 (13)
Pseudomonas aernigiosa	5 (10)	4 (12)	4 (13)	13 (12)
Enterobacter cloacae	4 (8)	1 (3)	0	5 (4)
Klebsiella	4 (8)	1 (3)	1 (3)	6 (5)
Streptococci	0	1 (3)	2 (7)	3 (3)
Others	4 (8)	2 (6)	1 (3)	7 (6)
	50	32	30	112

Circumcision or dorsal incision was performed in 14 boys in an attempt to prevent recurrent infections. Two girls were treated for labial synechia that could possibly have contributed to breakthrough infections. In 2 cases recurrent infections were caused by urethral instrumentation.
Among patients with bladder dysfunction the frequency of breakthrough infections was 69% (29 of 42 cases)[94]. Twenty individuals with bladder dysfunction and voiding problems were treated with clean intermittent catheterisation (CIC) in an attempt to reduce the number of recurrent UTIs. This treatment was introduced before potty training at median age 10 months (range 2-18) but later also included the introduction of a good voiding regimen as soon as the child achieved control of bladder function (after 18 months of age)[95]. The number of infections decreased with CIC treatment, but treatment of bladder dysfunction did not affect the reflux since VUR was persistent after observation time in all but one[95]. Residual urine decreased during the CIC treatment even though VUR persisted. Eighteen of these 20 patients were treated surgically at the end of the study and treatment with CIC was stopped after a few months in all but two patients[95].

Spontaneous resolution or downgrading of VUR

Overall spontaneous resolution or downgrading to grade II or less was seen in 44 patients (38%). Complete resolution occurred in 30 (26%) and downgrading to grade I-II in 14 (12%). Age for detection of spontaneous resolution on VCM was median 27 months (range 2.3-62). Distribution of grade of VUR at follow up is shown in table 9.

Table 9.

Distribution of VUR grades at study end n (%)						
	Paper I, II & IV (n=115)			Paper III (n=114)		
	Males	Females	Total	Males	Females	Total
No VUR	23	7	30 (26)	21	3	24 (21)
Grade I-II	9	5	14 (12)	11	6	17 (15)
Grade III	9	6	15 (13)	8	4	12 (11)
Grade IV	23	15	38 (33)	32	10	42 (37)
Grade V	16	2	18 (16)	17	2	19 (17)
Unilateral VUR	25	11	36	31	8	39
Bilateral VUR	32	17	49	37	14	51

We found several factors affecting spontaneous resolution and downgrading of VUR in Kaplan Meier survival analyses.
-In the higher grades (IV and V) we found higher cessation in *boys* than in *girls* during the infant year, but when grade III was included no sex difference was seen (figure 19a and 19b).
-*Grade of VUR at inclusion* influenced the resolution rate with higher frequency at lower grade of VUR when comparing grades III-V (figure19c)

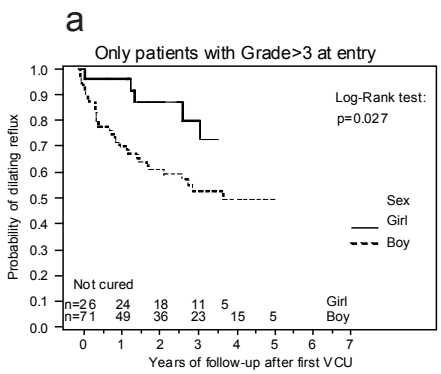
-*Breakthrough UTI* and *bladder dysfunction* were also shown to be strongly negative predictors for VUR resolution (figure 19d, e, f). Bladder dysfunction was defined as a combination of high-capacity bladder and incomplete emptying or significant numbers of overactive contractions. *Bladder capacity ≥200%* than expected was a negative factor seen as a separate variable and so was *residual urine ≥25%* of bladder capacity (figure 19g-h). The presence of overactive contractions did not affect the resolution rate.
-*Renal abnormality* at inclusion was a negative predictor, as was *subnormal renal function < 80%* of expected (figure 19i-j).
-*Mode of occurrence of VUR* at the first VCM was a predictor of VUR resolution (or downgrading) with higher resolution in VUR occurring during micturition as compared with those occurring as a response to overactive contractions or occurring passive during filling (figure 19k).
There was no difference in resolution depending on pre or postnatal diagnosis or uni or bilateral VUR at inclusion[94].

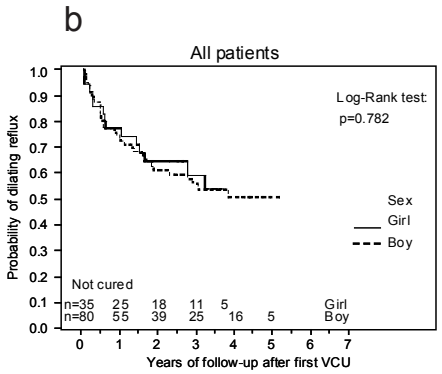
Results of uni- and multivariate analyses of variables affecting VUR resolution

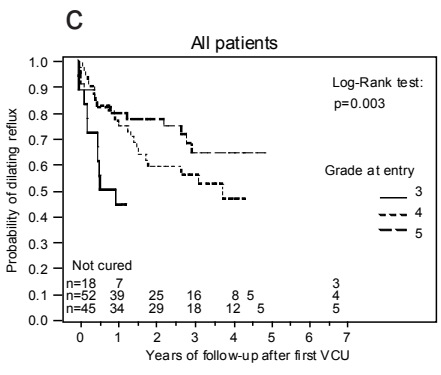
Univariate Cox proportional hazard model was used for all variables showed in table 10. When the variables with significant association with VUR resolution were included in the multivariate Cox proportional hazard model with stepwise forward selection, renal abnormality, bladder dysfunction and breakthrough UTIs proved to be three strong independent negative factors for spontaneous VUR resolution (table 10).

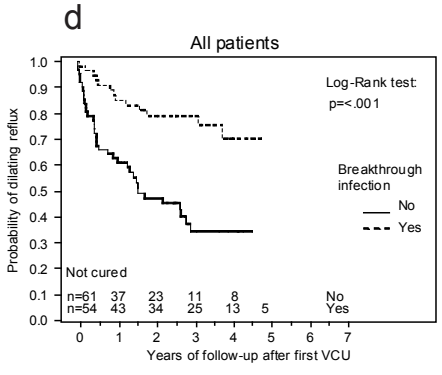
The multivariate model was able to predict VUR resolution by the age of 3 years with area under the curve of 86% (figure 20). Probability of VUR resolution (taken from logistic regression) of different clinically relevant combinations of these variables is shown in figure 21.

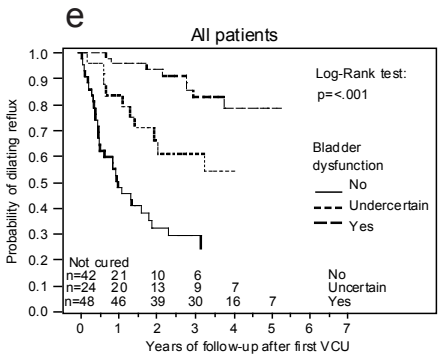
In this model a child without renal abnormality, bladder dysfunction or breakthrough UTI had a 96% probability of VUR resolution to VUR grade II or less before three years of age. On the other hand, a child with bladder dysfunction, breakthrough UTI and generalised renal damage had only an 8% probability of VUR resolution at the same age (figure 22). Of the 115 patients, 32 (28%) had a combination of bladder dysfunction, breakthrough UTI and renal abnormality (focal or generalised) at the end of follow-up and thus a very low probability of VUR resolution (figure 21).

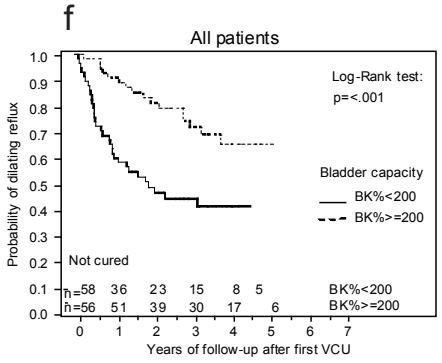

a
Only patients with Grade>3 at entry
Probability of dilating reflux
Log-Rank test:
p=0.027
Sex
Girl
Boy
Not cured
n=26 24 18 11 5
n=71 49 36 23 15 5
Girl
Boy
Years of follow-up after first VCU


b
All patients
Probability of dilating reflux
Log-Rank test:
p=0.782
Sex
Girl
Boy
Not cured
n=35 25 18 11 5
n=80 55 39 25 16 5
Girl
Boy
Years of follow-up after first VCU


c
All patients
Probability of dilating reflux
Log-Rank test:
p=0.003
Grade at entry
3
4
5
Not cured
n=18 7
n=52 39 25 16 8 5
n=45 34 29 18 12 5
3
4
5
Years of follow-up after first VCU


d
All patients
Probability of dilating reflux
Log-Rank test:
p=<.001
Breakthrough infection
No
Yes
Not cured
n=61 37 23 11 8
n=54 43 34 25 13 5
No
Yes
Years of follow-up after first VCU


e
All patients
Probability of dilating reflux
Log-Rank test:
p=<.001
Bladder dysfunction
No
Undercertain
Yes
Not cured
n=42 21 10 6
n=24 20 13 9 7
n=48 46 39 30 16 7
No
Uncertain
Yes
Years of follow-up after first VCU


f
All patients
Probability of dilating reflux
Log-Rank test:
p=<.001
Bladder capacity
BK%<200
BK%>=200
Not cured
n=58 36 23 15 8 5
n=56 51 39 30 17 6
BK%<200
BK%>=200
Years of follow-up after first VCU

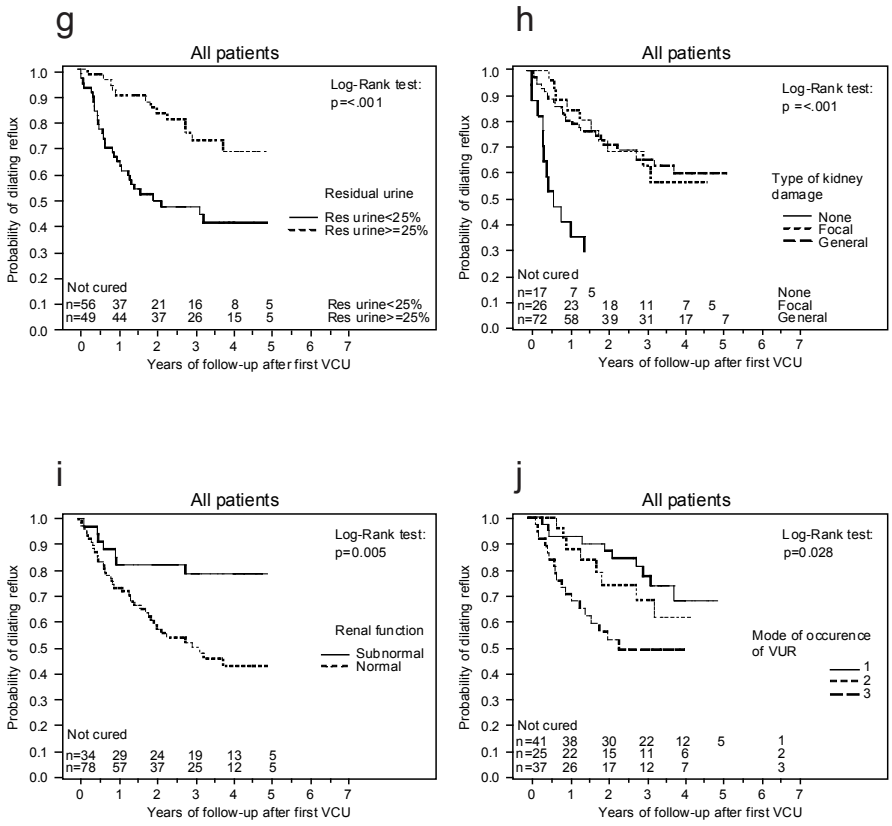


Figure 19 a-j. Survival curves were given by Kaplan-Meier estimates and formally tested with the log-rank-test. VUR grade II or less was used as the end point and patients remained in the survival curves until endpoint was achieved, until the last VCM prior to operation or until only 5 individuals were left. n=number of patients.
a,b. Probability of persistent dilated VUR in boys and girls with high-grade VUR (97 patients with VUR grade IV-V)(p=0.027) and probability of persistent dilated VUR in boys and girls with VUR grade III-V (115 patients, p=0.782).
c. Probability of persistent dilated VUR split by grade of VUR at inclusion (p=0.003).
d. Probability of persistent dilated VUR with or without breakthrough UTI (p<0.001).
e. Probability of persistent dilated VUR with or without bladder dysfunction (p<0.001).
f,g. Probability of persistent dilated VUR with or without high-capacity bladder (>200% of expected)(p<0.001), and with or without increased residual urine (>25% of bladder capacity)(p<0.001).
h,i. Probability of persistent dilated VUR with or without renal abnormality (p<0.001) and with normal or subnormal (<80% of expected) renal function (p=0.005).
j. Probability of persistent dilated VUR according to mode of occurence 1. Passive during filling, 2. As response to overactive contraction, 3. During micturition (p=0.028).

Table 10. Uni and multivariate Cox proportional hazard models for estimation of probability of spontaneous resolution of dilated VUR in 115 infants. (Hazard ratios presented with 95% CI)

		Univariate		Multivariate	
Variable	**Number of missing values**	**Hazard Ratio (95% CI)**	**p**	**Hazard Ratio (95% CI)**	**p**
Sex (Girl=1, Boy=2)	0	1.25 (0.66-2.37)	0.4889		.
Presentation of VUR (Prenatal ultrasound=1, UTI=2)	3	1.13 (0.57-2.23)	0.7273		.
Grade of VUR at inclusion (III-V)*	0	0.55 (0.37-0.84)	0.0050		.
Uni/Bilateral reflux (Unilateral=1, Bilateral=2)	0	0.62 (0.34-1.12)	0.1095		.
Breakthrough UTI*	0	0.31 (0.16-0.59)	0.0003	0.49 (0.25-0.97)	0.0397
Renal abnormality (None=1, Focal=2, Generalised=3)*	0	0.55 (0.38-0.79)	0.0012	0.43 (0.29-0.63)	<.0001
Uni/Bilateral renal damage (None=0,Uni=1,Bi=2)*	0	0.44 (0.26-0.75)	0.0025		.
Bladder dysfunction (No=0, Uncertain=1, Yes=2)*	1	0.34 (0.24-0.50)	<.0001	0.36 (0.24-0.53)	<.0001
Bladder capacity (% of expected) (%/10)*	1	0.94 (0.91-0.97)	0.0003		.
Residual urine (% of bladder capacity) (%/10)*	10	0.64 (0.51-0.82)	0.0004		.
Occurrence of VUR (Passive=1, Overactive=2, Micturic=3)	12	1.65 (1.12-2.44)	0.0117		.
Bladder volume at reflux onset (%/10)	39	1.12 (0.98-1.27)	0.1028		.
Overactive contractions (Yes=1, No=0)	2	0.96 (0.54-1.72)	0.9033		.
Number of overactive contractions	2	0.94 (0.88-1.00)	0.0641		.

* Indicates that the variable has been included into the stepwise multivariate Cox model.
Mode of occurence of VUR (passive, overactive, micturic) was not included into the stepwise logistic model due to many missing values. Missing value of this variable in all patients with very early VUR resolution.(Noted at the first study VCM).

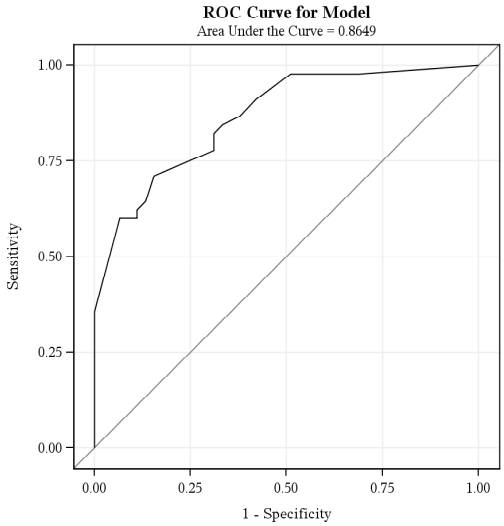


Figure 20. ROC curve for the multivariate model for prediction of VUR resolution at three years of age in children with dilated VUR from infancy.

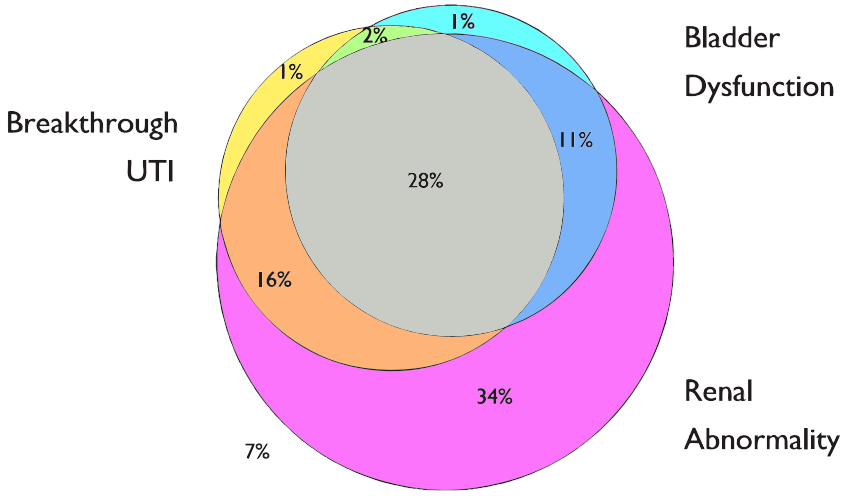


Figure 21. Diagram illustrating covariation of the variables renal abnormality (seen in 90% at the last follow up), breakthrough UTI (47%) and bladder dysfunction (42%) in 115 children with bladder dysfunction from infancy.

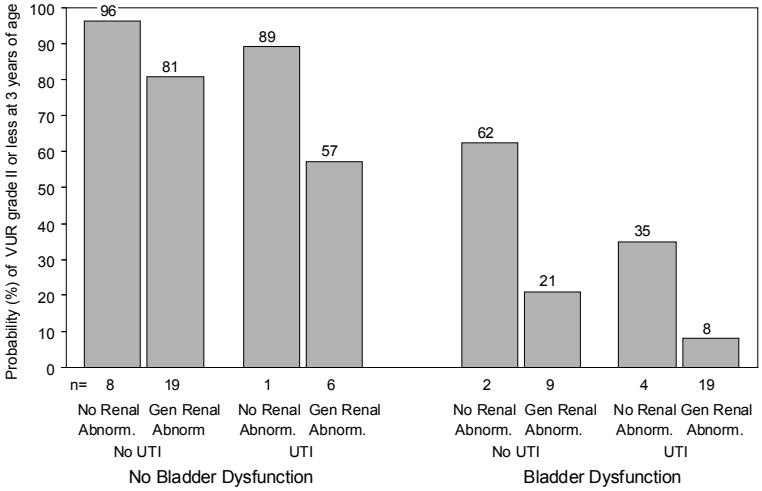


Figure 22. Probability (%) of VUR resolution (or downgrading to I-II) at three years, taken from logistic regression. Illustrates probability level for different linear combinations of the variables renal abnormality (none or generalised), breakthrough UTI and bladder dysfunction.
*n=number of patients with each combination in this study. Formula for linear combination; lc=4.15-1.37*bladder dysfunction-1.13*breakthrough UTI-0.91*renal abnormality.*

Surgical interventions

Of the 115 children 35 were surgically treated for persistent high-grade VUR at the end of the observation period and/or repeated recurrent infections. Median age for open surgery was 42 months (range 14-91) and the majority (29 individuals, 21 boys and 8 girls) were operated on using Cohen's procedure[94]. Three boys with duplex kidneys and poorly functioning lower system with dilating reflux underwent heminephrectomy and 4 boys with dilating VUR to a hypoplastic kidney of less than 10% of total function underwent nephrectomy[94].
Injection of dextranomer microspheres in hyaluronic acid solution (Deflux^{R}) was performed in 7 cases at median age 59 months (range 18-67) for similar indications as antireflux surgery but this was not considered for patients with grade V reflux[94].

General discussion

In this longitudinal study of children with dilating infantile VUR, reflux resolution, renal status, bladder function and UTI recurrences were followed for 3-5 years. The study population was relatively large, homogenous in age and severity of reflux, and has provided a wealth of results. However, some inconsistencies should be commented on.
The inclusion of a small number of infants with VUR grade III (15%) can be questioned since this group is not seen as high grade even though the reflux is dilated to some extent. However, this subgroup has been useful for comparisons, highlighting differences between moderate (grade III) and high grade (IV-V) VUR. Among patients with VUR grade III at inclusion we also identified patients with kidneys with the typical generalised abnormality described in other studies as exclusive to VUR grades IV and V[20, 96]. In VUR grades IV-V the proportion of boys was higher, as was the proportion with prenatal diagnosis, results that were shown in other studies both in series of prenatal diagnosis and of diagnosis after UTI[24, 97].

The inclusion of infants with various ways of presentation, prenatally detected or diagnosed after pyelonephritis, can also be questioned since previous studies have claimed a better prognosis for those prenatally detected[97, 98]. This was not seen in our group in a population where all mothers have one antenatal ultrasound performed during the 16-18th gestational week with no further investigations other than at medical complications. The infants detected in this investigation constituted the worst group with higher VUR grades and prenatally damaged kidneys. In our study there was also a higher frequency of children with deteriorated renal status during follow up in the prenatally detected group. Therefore, in a region with the routine of doing only one early pregnancy ultrasound, prenatal screening did not seem to alter the outcome for infants with dilated VUR. There are also studies that found no difference in the outcome of infants presenting prenatally with infants presenting with UTI in terms of VUR grade, percentage of initial scarring, or clinical course[99]. Once diagnosed, irrespective of reason, infants with reflux had neither great morbidity nor frequent need of surgery since worsening of renal status was uncommon[99].

Generally, a high incidence of patients with renal abnormality, referred to as generalized damage, has been reported to have high-grade VUR[24, 98, 100], as was found in the present study. The small kidney described by Risdon et al. was shown to have dysplastic features[101]. Dysplasia is a histological diagnosis characterized by primitive renal tubules with or without cartilage formation and is seen as poor and heterogenous uptake of DMSA. However, since dysplasia is a histological diagnosis, the small kidney associated with infantile dilated VUR often is referred to as hypoplastic. Developmental hypoplasia refers to a small kidney with homogenous and good tubular uptake of DMSA scintigraphy. In our material there probably is both variants, according to the DMSA results. This is described by Mackie and Stephens in studies of the abnormal ureteric bud[36]. Despite subnormal single kidney GFR in

80%, only 30% of our patients had subnormal total GFR, indicating the compensatory capacity of the contralateral kidney. The number of kidneys with deterioration during follow up is probably too high since loss of split function may in some cases reflect a compensatory growth of the contralateral kidney[88, 89].

The dilated infantile VUR and the hypoplastic kidney can both be related to maldevelopment of the ureteric bud[36]. It is not known whether the high frequency of bladder dysfunction in these patients, in addition to VUR and renal malformation, has the same background, but it is an appealing hypothesis. An alternative explanation for the high capacity bladder with incomplete emptying could be that it is secondary to VUR, and induced by the extra burden of the reflux urine from the upper urinary tract. There are, however, findings that make this explanation less likely. In a follow-up study of 20 children with dilated bladder dysfunction, who presented with dilating VUR during infancy, the majority were still infrequent voiders at the age of 7-9 years although their VUR had been cured since they were 4 years of age[102]. Similar results have been reported from the International Reflux Study, when comparing surgically or medically treated children, the latter having persistent dilating VUR; no difference in bladder capacity were found between the groups[54].

The cystometric findings of high pressure, dyscoordination at voiding and small bladder capacity in infants with dilated VUR, previously reported as a unique pattern for this group of patients, was later demonstrated in infants with no urinary tract disorder as well [48, 50, 76]. These results were described during the course of the study and revealed that this urodynamic pattern, frequently seen in boys, could not be used as a sign of bladder dysfunction in infants. The study design with serial VCM investigations resulted in a detailed description of bladder function over time in relation to VUR. After one year of age there was a change from the immature pattern and a large number of study patients developed a dysfunctional pattern with high-capacity bladder and incomplete emptying. This is the common development in boys with persisting VUR. In contrast, high capacity bladder with incomplete emptying was seen already during the infant year in girls. They also had a lower resolution rate than infant boys. Since bladder dysfunction was a negative predictor of VUR, the difference in bladder function in infant boys and girls could explain the difference in first year resolution rates. After the first year of life there were similar resolution rates for boys and girls.

The frequency of breakthrough infections was high but comparable to other cohorts with a high proportion of uncircumcised infant males [103-105]. Another suggested reason for the high incidence of breakthrough UTIs may be the choice of antibiotic prophylaxis, cefadroxil or trimethoprim. Resistance rates among common uropathogens appear to be increasing in both of these commonly used agents[106-108]. Over 70% of the boys with breakthrough UTIs had infections registered during the first year of life and the frequency decreased significantly after three years of age[91]. Of girls with breakthrough UTIs, 53% had the infection during the first year of life[91]. This corresponds well with other studies showing that breakthrough UTI in

boys after the first year of age is rare[103, 109]. Another risk factor for recurrent UTIs is bladder dysfunction[110, 111] with incomplete emptying of the bladder which was seen in as many as 42% of our patients.

There are widely divergent opinions about indications for VUR treatment. Sweden and most northern European countries have similar policy in not treating or performing further investigations in low-grade VUR[112]. In North America treatment of all grades of VUR surgically, or conservatively with antibiotics is common[71]. Therefore, a drawback of the present study as seen from an American point of wiev is the fact that according to Swedish tradition no further VCMs were performed after cessation or downgrading to VUR grades I-II. Thus we cannot evaluate how many individuals ultimately reached complete resolution. However, we have analysed the factors related to downgrading to VUR grade II or less towards complete resolution, and our results were similar, with the same factors significantly affecting resolution, although with lower power. Altogether the frequency of downgrading and resolution of VUR was high (38%) and comparable to other studies in children with dilated VUR[22,24,115].

In studying a medical phenomenon such as cessation of VUR and factors associated with the resolution rate, univariate methods alone may be insufficient when the phenomenon being studied is actually multivariate. We have explored the study material by multivariate techniques and three factors remained as strong independent negative predictors for VUR resolution, renal abnormality, breakthrough UTI and bladder dysfunction. These findings need to be confirmed in future studies, since the outcome is dependent on the characteristics of the study population. Two of the above mentioned variables were found in a study by da Silva using multivariate techniques, namely renal damage and dysfunctional voiding[113]. That study had a young study population similar to ours with the majority of patients diagnosed before 2 years of age, which may contribute to the resemblance to our results. Knudsen et al. found high bladder volumes at onset, age younger than 2 years and prenatal diagnosis to be three independent variables in a multivariate analysis. None of these variables could be confirmed in our multivariate model where, all patients were younger than 2 years at inclusion, so young age could not be evaluated[114].

As factors for prognostic evaluation, variables from study inclusion should preferably be used, or at least results from early investigations. This was possible for most variables but GFR, for example, was taken from the last investigation owing to the variability in values from investigations performed at very young age; it was therefore excluded from the multivariate analyses. Of the factors identified as independent predictive factors affecting reflux resolution, renal abnormality was available at study inclusion with median age at the first scintigraphy of 4 months. Breakthrough infections occurred during the first year of life in the majority with UTIs. This variable was therefore regarded as early. Bladder dysfunction was the third strong independent factor predicting non-resolution in multivate analyses but this variable could not be evaluated until the second year of life, which was a drawback. However, in

many countries interventions for dilated VUR are rarely considered before one year of life and evaluation of bladder function can be done during the second year of life to help predict the chances of spontaneous resolution at three years according to the proposed model.

It should be remembered that severe VUR with repeated infections is a potentially dangerous disease which can lead to uremia if uncontrolled and untreated[67, 115]. The results of our study are mainly applicable in countries with ready access to pediatric emergency units and rapid treatment of infections.

Concluding remarks and clinical usefulness of the study

There is still no evidence of one single superior treatment strategy when it comes to severe congenital vesicoureteral reflux diagnosed during infancy[116]. This observational study has resulted in a detailed description of the characteristics of the disease, a description of the natural course of dilated VUR during the first years of life, and definitions of risk factors affecting outcome. Development of bladder function during the first years of life has been monitored and kidney status thoroughly investigated repeatedly. Infection control has been sought by administrating prophylactic antibiotics, and infections have been treated and registered. The study has generated results that can be used to formulate hypotheses for future management of this selected group of VUR patients. Our results have provided tools for distinguishing infants with a high chance of spontaneous resolution from those with a high risk of remaining dilated reflux, and we have tried to identify risk factors for deterioration of renal status.

Suggestions for modified investigation program for infantile dilated VUR

The results of this study could suggest a modified strategy for investigations and management of this selected group of VUR patients. On the basis of the results from the multivariate analyses, we can propose a focus on three independent prognostic factors: *kidney status, bladder function and occurrence of UTIs.*

The longitudinal study of *renal abnormalities and function* revealed a very high frequency of renal abnormality already at inclusion in a cohort of infants with dilating VUR. Renal status remained unchanged during the first 5 years in the majority of study patients and only one patient showed significant loss of renal function. Predictive factors for deterioration of renal status were recurrent UTIs, bilateral renal damage and subnormal GFR (< 80% of expected).

Renal abnormality also proved to be an independent factor for prediction of non-resolution of dilating VUR. Information about renal status or abnormalities at an early age seems reasonable both for estimation of the outcome of VUR and for evaluation of the risk deterioration in renal function.

The longitudinal study of *urodynamics* has improved our understanding of the relationship between dilated VUR and bladder dysfunction. We found that bladder dysfunction can only be diagnosed after the first year of life and demonstrated how this condition correlates with grade of VUR, increased risk of recurrent infections and non-resolution of reflux. Estimation of bladder dysfunction variables such as high-capacity and incomplete emptying can be done on the basis of FVO and ordinary VCU. These variables were the only ones that showed impact on the probability of resolution or downgrading of VUR, whereas the presence of overactive contractions and increased infantile detrusor pressure were of less importance. Thus, we cannot claim that cystometry should play a role in investigation of infants and children with

dilated primary VUR in the future.
The third independent factor was *recurrent febrile UTI*. Infection control seems urgent knowing that the patient with breakthrough infections is less likely to have spontaneous resolution or downgrading of VUR and is at greater risk for progress of renal damage.

Thoughts about treatment strategies

An observational study like this one can only be descriptive and present risk factors affecting the outcome and generate new hypotheses but cannot provide evidence for evaluating different treatment strategies. The results of the study need to be confirmed in further studies, but in the meantime the basic principles can be used in the individual patient-consultant situation where the decision about the appropriate treatment should be made based on expert knowledge, medical factors and family preferences.

Infection control, antibiotics to whom and for how long?

This study does not evaluate the use of prophylactic antibiotics as compared with surgery or non-medication. However the results, describing frequency and distribution of infections, may have some clinical implications. Protection first year seems reasonable since there is an increased risk during that year, especially in boys seen both in this and other studies.
If there are repeated infections, management and evaluation of bladder dysfunction should be considered rather than surgical correction as a first action.

Management of bladder dysfunction?

We suggest focus on emptying difficulties, but the use of clean intermittent catherisation should probably only be recommended in very rare cases since this method is invasive and demanding for both the parents and the child. Early potty training, starting at 12-18 months, can probably enhance bladder emptying sufficiently and is preferred by both parents and children.

Surgical intervention or endoscopic injection; in whom and when?

If investigations have shown that the child has generalised renal abnormality combined with bladder dysfunction and recurrent UTI, the likelihood of spontaneous resolution is low, whereas a child with dilating VUR, without any of the above mentioned risk factors, has a better probability of spontaneous resolution of VUR. However, if renal status is considered the primary effect variable in VUR treatment, our findings do not support early surgical intervention, since few patients deteriorated during follow up.

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ERRATA

Paper I: VUR grade at inclusion in results and table 1 should be grade III in 18, grade IV in 52 and grade V in 45. One patient with grade III mistakably noted as grade V, corrected in paper II and IV.

Spontaneous cessation or downgrading to grade II or less was found in 44 cases, not 45 as written in the result section and table 3. One patient was recorded as spontaneously resolved while he had actually undergone surgery. This mistake was corrected in the following papers.

Paper II: Investigation program should say VCU,VCM and FVO since Median age at first investigation is taken from the first VCU. Corrected in paper IV where median age at the first VCU as well as first VCM is presented.

Bacterial agens only available in: should read 85% of registered infections, instead of 80% as written.