Radionuclide techniques for the detection of vesicoureteral reflux and their clinical significance

Christodoulos Likartsis[1] **MD, BSc**
Nikoleta Printza[2] **MD, PhD**
Athanasios Notopoulos[1] **MD, MDA, PhD**

1. Nuclear Medicine Department, Hippokration Hospital, Thessaloniki, Greece
2. 1st Pediatric Department, Aristotle University, Thessaloniki, Greece

Keywords: Radionuclide cystography,
- Vesicoureteral reflux
- Urinary tract infection
- Continuous antibiotic prophylaxis

Corresponding authors:
Christodoulos Likartsis MD, BSc
Nuclear Medicine Department,
Hippokration Hospital,
Thessaloniki, Greece
+302310892093/+306941675606
l2000tolis@yahoo.com

Received:
15 April 2020
Accepted revised:
16 July 2020

Abstract
We discuss and try to evaluate the detection of vesicoureteral reflux (VUR) by radionuclide techniques and especially direct radionuclide cystography (DRC). Direct radionuclide cystography is applied for more than half a century mainly in children. Vesicoureteral reflux has a complex pathology not yet completely understood and is often related to urinary tract infection (UTI) and renal parenchyma scarring that can lead to long-term renal function impairment. Since there is no consensus on the optimal imaging algorithm after the first febrile urinary tract infection, many imaging strategies have been proposed for VUR detection in the last decade, including or not DRC. Views opposing or accepting its use are also presented.

Hell J Nucl Med 2020; 23(2): 180-187 *Epub ahead of print: 27 July 2020* *Published online: 24 August 2020*

Introduction

About 30% to 40% of children diagnosed with urinary tract infection (UTI), have primary vesicoureteral reflux (VUR) [1]. Children with VUR have 2.6 times higher prevalence of renal scarring compared to children without VUR [2]. For most of children with VUR, treatment involves continuous antibiotic prophylaxis (CAP). Micturating cystourethrography (MCU) and direct radionuclide cystography (DRC) are mainly applied for the detection and follow-up of VUR respectively [3] although some authors are not agreeable as for their indications [4, 5]. We shall describe and try to evaluate the radionuclide techniques used for the detection and the follow-up of VUR.

Methods

Historical background

During mid-1950s, the available methods for VUR detection were those using roentgenography or fluoroscopy as well as the so called 'delayed' cystography up to 3 hours after the instillation of contrast medium into the urinary bladder. Other techniques included voiding cystourethrograms, voiding cystoureterograms and the instillation of floating lipiodol into the urinary bladder [6]. However, these methods were time consuming, tiresome and offered a high radiation burden to patients, especially to children. Furthermore, the widely used delayed cystography was not able to detect an intermittent VUR. Moreover the contrast agents used in these techniques were readily absorbed by the urinary bladder and excreted by the kidneys, so there was doubt whether the images obtained were the result of VUR or due to contrast agent excretion [7]. The need for an imaging technique that could safely provide a continuous record was obvious.

In a paper titled: "Vesicoureteric Reflux. Diagnosis with iodine-131 sodium ortho-iodohippurate', published on February 1963 in The Lancet, E. A. Dodge described the first radioisotopic cystography in 300 individuals (250 patients, 50 controls). He used 0,0111 MBq/kg of iodine-131 (^{131}I) sodium ortho-iodohippurate (^{131}I-Hippuran) as a radiotracer injected intravenously, while patients were not catheterized [8]. Since then the method underwent refinements to meet the evolving requirements, making DRC a widely used, safe and reliable technique for VUR detection [9, 10].

Indications and contraindications for direct radionuclide cystography

Main clinical indications of DRC are: a) To diagnose VUR after UTI in children, b) To follow-up of children with diagnosed VUR under CAP, c) To evaluate surgical or endoscopic intervention outcomes, d) To screen parents and siblings of children with known VUR, e) To diagnose VUR in renal transplant recipients, f) To diagnose VUR in bladder dysfunction diseases such as neurogenic bladder, where a serial evaluation is required [11], especially in hydro-ureteronephrosis, after surgery for meningomyelocele [12].

The main contraindication for DRC is an active urinary tract infection. So, every patient who is to undergo a DRC must have a recent (up to a week) negative urine culture.

Patient preparation

For correctly performing DRC, a safe and tranquil environment is crucial. Parents should be well informed by their practitioner and if necessary, by a psychologist, about all steps of the test. In some cases, children must be sedated prior to cystography procedures. Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4]benzodiazepine) has been suggested as an acceptable sedative (midazolam 0.5mg/kg in orange squash or intranasal 0.2mg/kg). Midazolam may cause anterograde short-term amnesia but does not affect the voiding phase neither the grade of existing reflux [1].

The technique

It is generally agreed that a small diameter catheter according to the child's size (F6 or F8 feeding tube) is introduced into the lower urinary tract up into the bladder under aseptic conditions in order to empty the bladder. Catheterization must be performed only by a trained person. For boys, some anesthetic gel (lidocaine 2%) into the urethra preceding catheterization is recommended to reduce the pain [13].

The child or the adult is positioned supine on the gamma camera. To minimize the chances of contamination, if the patient is not well toilet trained, a plastic-lined absorbent paper is placed underneath the patient [14].

There are three techniques mentioned in medical literature for DRC, two of them use a catheter and the third a percutaneous supra-pubic injection of the radiotracer [15, 16]. One of the two first techniques is performed by filling the bladder slowly under hydrostatic pressure with a solution containing the radiotracer in 500mL sterile isotonic saline, warmed to body temperature and placed 40-60cm above the bladder level. Gradual filling of the bladder is obligatory in order to prevent increase of bladder tone and premature voiding. With a steady, non-pulsatile flow the bladder should be filled to its capacity in about 10 minutes. The functional bladder capacity increases with age up to 300-400mL in adulthood. In children the bladder capacity is lower [17].

The second technique, which is widely used, is performed by the instillation into the bladder of the full dose of the radiotracer diluted in 10mL of saline followed by gradual filling of the bladder with normal saline, up to the bladder capacity, calculated using the formula: Bladder volume=(Age in years+1)x30mL, recommended by the European Association of Nuclear Medicine or using the formula: Bladder volume=(Age in years+2)x30mL recommended by the Society of Nuclear medicine (SNM) of the United States [11, 18]. Bladder filling must stop when the child has the urge to void or when flow from the bottle ceases as the differential pressure reaches zero. During the filling phase, sequential images are acquired. After either of the above techniques has been completed the child is asked to void [11].

The voiding phase images can be obtained in either sitting or standing position with the back of the patient facing the camera. The collimator must also be covered with plastic and absorbent pads or other non-impermeable material [19]. In non-toilet trained children, the voiding phase images are obtained in the supine position (Figure 1). In co-operative children, the preferred position is sitting upright on the bedpan with their back facing the camera [20]. According to our experience, micturition chairs and similar devices can be used.

For both techniques: in non-toilet trained children the voided volume should be measured by weighing the dipper prior and after voiding. In toilet trained children it is recommended to measure the voided volume after micturition, especially when the first technique is chosen. Bedpan cover and calculation of the urine absorbed is suggested [20].

The percutaneous technique is performed after the consent of the patient or its guardians. Anesthetic gel is applied to the supra-pubic area 45 minutes prior to the procedure. The child is asked to drink fluids up to the point that the ultrasound device confirms distended urinary bladder. Ultrasound guided injection of the radiotracer (10-20 megabecquerel (MBq) of ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3) in a volume of 1-2mL) into the distended urinary bladder is performed while the patient lies on the gamma camera bed. Imaging begins as soon as the needle for injection is withdrawn. Posterior images of the urinary bladder and renal areas are obtained at 10sec frames for 5 minutes with the patient in the supine position. Micturition-phase images are obtained by the patient seated in front of the camera [16].

Except for the appearance of VUR, which is shown by the presence of activity in the pelvic and/or ureteral regions during the filling of the bladder or voiding, there are many other parameters that can be derived or calculated during DRC. Some of them (bladder pressure and volume at which the reflux happens, urine reflux volume etc) have already shown a variable prognostic significance [21-23]. However, even though many nuclear medicine laboratories calculate these parameters, there has not been a general consensus on the value of quantification of volumes and this issue remains unclarified due to the lack of published large patient series [11].

Dosimetry, sensitivity and specificity of the DRC technique

The effective dose of the DRC technique is about 0.048mSv/20MBq of technetium-99m pertechnetate ($^{99m}TcO_4^-$). For children 1-10 years old the estimated absorbed dose to the critical organ (bladder) using 20MBq of $^{99m}TcO_4^-$ is 0.09-0.14 mGy, to the ovaries 0.005-0.01mGy and to the testes even smal-

ler [11]. It has been estimated that DRC delivers 50 to 200 times less radiation to the gonads than MCU [24].

Many studies in the past have assessed the sensitivity and specificity of DRC for the detection of VUR. There is an important variability in their findings, due to the inhomogeneity of the patients' age, sex and/or the conditions studied. The reported sensitivity ranges between 71% to 100% and the specificity between 67% to 100% [3, 25, 26].

Today MCU is considered the golden standard for VUR detection and is usually preferred as the first imaging study, especially in boys. However, some authors claim that DRC and MCU have comparable [27, 28] or higher [29, 30] sensitivity for VUR detection.

In a group of 35 children with hydronephrosis and a history of febrile UTI episodes, in which MCU was negative, VUR was detected with the aid of DRC in 49.1% of the cases. It was reported that 51.5% of VUR not detected by MCU were of low grade, 42.4% of medium grade and 6.1% of high grade [29]. In another analytical study of 60 children with recurrent UTI (mean age 4.7 years) and negative MCU, DRC had higher diagnostic value (54.5%) in detecting missed VUR cases [30]. Furthermore in another study with 62 infants, DRC and MCU sensitivity was estimated as 91% and 45% respectively, independently of the grade of VUR. Sensitivity of DRC was high in detecting moderate and high grade VUR and low for low grade VUR. On the other hand, MCU sensitivity was high in detecting low grade (first and second grade) VUR [31]. It is obvious that more studies with larger number of patients are warranted.

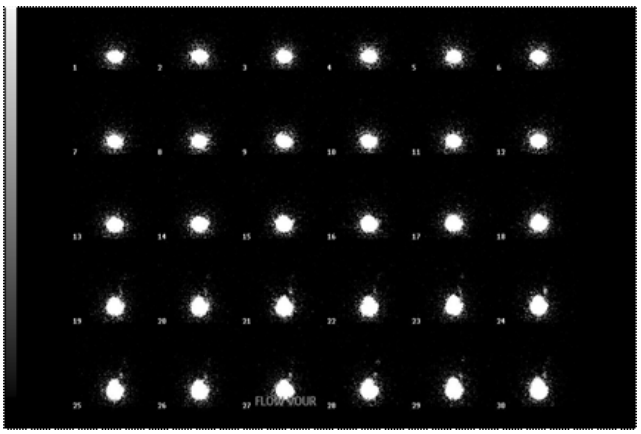

Figure 1. Images of DRC (voiding phase) showing VUR (right) of a 2 years old patient in the supine position. (Source: Nuclear Medicine Department, Hippokration General Hospital, Thessaloniki).

Other nuclear medicine techniques

There are two other nuclear medicine techniques for VUR diagnosis besides the already described DRC the indirect radioisotope cystography (IRC) and the 'cyclic' DRC (CDRC).

Indirect radionuclide cystography (IRC)

The IRC is performed at the end of the usual renogram. A radiotracer of high extraction rate as ^{99m}Tc-MAG3 is used. The child is asked to micturate in front of the gamma-camera and the voiding phase is recorded. The effective dose depends on the radiotracer used (approximately 0.007mSv/MBq for MAG-3, 0.005mSv/MBq for DTPA) [32]. The main advantages of IRC are: a) The non-invasive nature of the method, b) The normal conditions for bladder filling and voiding, c) The additional functional parameters obtained from the renogram. The disadvantages of this method are: a) The necessity of patient's co-operation, since the child must be toilet-trained, b) The absence of the filling phase recording, c) The lower sensitivity compared to DRC, d) The greater difficulty in images interpretation compared to DRC, due to possible remaining renal and/or ureteral activity [14].

Moreover, reflux may not be detectable in cases of overhydration, recent i.v. administration of contrast agents or in case of a previous diuretic renogram causing increased urinary flow rate [33]. Another disadvantage of IRC lies on the fact that the gamma-camera must be readily available at the time the child is ready to micturate [14]. It is obvious that the exclusive use of IRC, as sole technique for the detection of VUR, due to all the aforementioned, may give rise to an increased number of false negative results. On the contrary, few authors claim a positive correlation (86%) between the diagnostic sensitivity of IRC compared to MCU [34], as well as lack of significant difference in accuracy in detecting VUR [35]. Others reported a sensitivity of IRC of 74.1% and specificity of 90.5% [36]. Anyhow, IRC is of limited use today, mainly used for the follow-up of children diagnosed with VUR who are toilet trained [37].

Cyclic direct radionuclide cystography

The CDRC is a variation of the conventional DRC, where after the completion of the filling phase the child voids around the catheter without catheter withdrawal and dynamic images during the voiding phase are acquired. After micturition, a new cycle (filling-voiding) starts and repeated. The procedure ends when the second or the third cycle is completed. The second and the third cycles have dramatically increased the sensitivity of the technique, with an additional VUR diagnosis of 35.7%, when they are both performed [38]. In an older (1990) study, the CDRC performed in 428 patients, with only a second additional cycle, detected 43% more cases of VUR than in DRC [39]. The total effective dose depends on the number of cycles performed. The effective dose of 0.0024 mSv/MBq (^{99m}Tc) for one cycle of CDRC is only 1%-2% of that from MCU [31] and its total radiation burden much lower while this technique has a better sensitivity. However, this technique has not been widely adopted either for VUR diagnosis or for follow-up, since it is time-consuming.

Micturating cystourethrography

Micturating cystourethrography, also known as voiding cystourethrography (VCUG) is one of the most commonly used fluoroscopic studies. The aim of MCU is to assess the bladder, urethra and micturition and to determine the presence or absence of anatomical and functional abnormalities, including VUR. Although considered as a stressful procedure for patients as well as for the medical diagnostic team, it has been previously reported that in 2001 accounted for 40% of all fluoroscopic procedures performed on children in Europe [40].

Prior to this procedure, the child must receive the appropriate dose of an antibiotic, one day prior to the examination and continue for three days after [41]. Psychological preparation of patients and parents is mandatory to avoid sedation of the child. Under aseptic conditions, using an anesthetic gel, a catheter (F5, F8) is introduced into the urinary bladder, with the patient in supine position on the X-ray bed. After catheterization, the urinary bladder should be drained prior to instillation of a warmed to body temperature contrast media (typically a 12%-18% weight/volume solution) by gravity drip. Allergic reactions to the intravesical contrast media are rare. However, in patients with a history of anaphylaxia to the contrast media, prophylactic measures must be taken [42].

A preliminary abdominal image is obtained before the instillation of the contrast media to detect any findings that may affect the performance of the study (presence of contrast media in the gastrointestinal tract, skeletal anomalies, calcifications). Intermittent images are taken throughout the study as follows: a) To confirm the presence of a radio-opaque shadow in the urinary bladder or urethra, b) To show the early filling of the urinary bladder, c) To show full urinary bladder view, d) To show voiding urethra view; in boys, a right or left anterior oblique image is obtained, e) For a full-length view of the abdomen to demonstrate any unnoticed reflux into the kidneys and to record the post-void residue. In infants, cyclical filling of the urinary bladder, voiding and refilling 2-3 times with the catheter in place may be helpful in the detection of VUR [41]. The mean absorbed dose is 0.69mGy [43]. With modern imaging technology and a tailored examination, MCU gonadal radiation dose is 17 to 52μGy [44].

Vesicoureteral reflux and urinary tract infection

Definition

Primary VUR is congenital due to an abnormal development and malfunction of the ureterovesical junction. Secondary VUR is acquired caused by increased intravesical pressure due to an anatomical or neurological bladder obstruction of urine outflow.

A considerable number of neonates and infants with a history of febrile UTI episodes present with findings compatible with VUR when examined by MCU and/or DRC. Infection of the urinary tract is one of the major health concerns during infancy and childhood, especially in female children during the 1st year of life and in uncircumcised male infants younger than 3 months of age. In a meta-analysis conducted in 2008 approximately 7% of febrile infants younger than 24 months of age and 7.8% of children presenting with urinary symptoms were diagnosed with UTI [45].

Vesicoureteral reflux is classified into 5 Grades based on MCU imaging findings [46]. Grades I, II and III are considered of low and medium clinical significance, whereas VUR of Grades IV and V are of high significance (Figure 2).

Since gamma-camera images have poor resolution, accurate scintigraphic description of grades II and III as well as of IV and V is not possible. The grading of VUR in DRC consists merely of 3 grades, where VUR grade I is considered mild, grade II as with moderate VUR (represents the second and the third previous grades) and grade III as with severe VUR (represents the fourth and the fifth previous grades in MCU) (Figure 3). However since renal scarring pathogenesis is still not yet clear and the two main prerequisite factors for scarring are VUR and UTI [47], it is possible that the only valuable distinction between the Grades is to specify if the pressure in the ureter is high enough to cause a retro grade flow up to the pyelocalyceal system. From this point of view the physicians report of a related cystogram should include one of the following findings: a) No VUR presence b) VUR grade 1 and c) VUR grade >1. Considering the previous statements, the high diagnostic sensitivity of DRC for the detection of VUR grade I [29] may be important. Moreover, new technical advances in DRC may improve its sensitivity for the accurate detection of VUR grade I.

The presence of VUR is related to UTI recurrence and renal parenchyma scarring. Furthermore, high grades of hydronephrosis and dilatation of the ureters are related to UTI. For this reason, accurate diagnosis of UTI and detection of VUR as well as early CAP are of great importance [48].

The reduction in UTI recurrence confirmed by the Swedish Reflux Trial [49] is 19% on CAP and 57% on a surveillance performed in girls over the age of 1 year having dilating VUR. Furthermore the RIVUR reflux trial showed reduced risk of UTI recurrence by 50% in children on CAP (trimethoprim/sulfamethoxazole). However, the difference in renal scarring between children on CAP and children taking placebo was not statistically significant [50].

The prevalence of VUR in children is 1%-2% [1]. Vesicoureteral reflux is more common (3:1) in white children compared to children of black race as well as in female patients (2:1) [51].

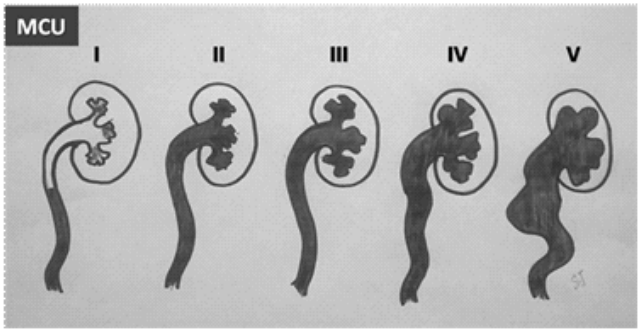

Figure 2. VUR grading in MCU.

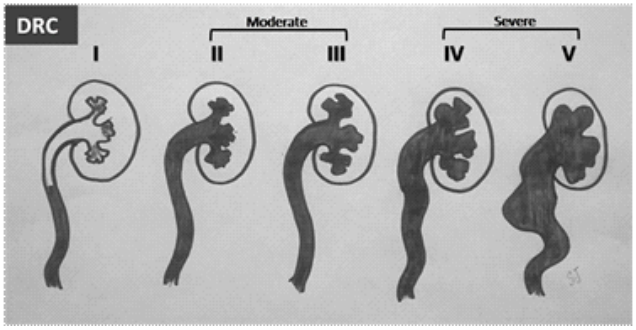

Figure 3. VUR grading in DRC.

Reflux nephropathy-Genetic factors

Among the congenital anomalies of the kidneys and the urinary tract, VUR is of great importance if UTI and renal damage are also present. However, it is still unclear whether the pathogenesis of reflux nephropathy is acquired or congenital. Early detection of patients with VUR is of importance since congenital anomalies of the kidneys and the urinary tract, due to gene mutations associated with kidney development, may manifest later in life [52].

Vesicoureteral reflux nowadays is considered as a complex genetic disorder involving an ectopic embryonic ureteric budding for which many genes have been traced. Previous studies have not yet identified a single major gene or locus responsible for primary VUR, so it is reasonable to say that the different forms of VUR have different genetic determinants. Nevertheless, there are several possible candidate genes for VUR pathogenesis [53].

In a study with 76 children diagnosed after their first UTI, the association between VUR and vascular endothelial growth factor A (VEGF-A)-460C/T functional gene polymorphism was investigated. No significant differences in genotypes or allele frequencies between children with and without VUR were revealed. In children with UTI genotype VEGF-A-460C/T frequencies were different among children with and without lesions of renal parenchyma. Allele C polymorphism of the VEGF-A gene was related to hypodysplastic renal parenchymal lesions, which may have been congenital [54].

Prognosis

It has been observed that children having had before only a single febrile UTI episode are favored by good prognosis [55]. Spontaneous resolution of VUR occurs in 92%, 59% and 25% of children diagnosed with mild, moderate and severe VUR respectively [56]. Certain prognostic factors related to spontaneous resolution of VUR are: a) Age, b) Gender, c) Initial Grade of reflux, d) Bladder bowel dysfunction, e) The presence of renal scarring, f) The volume of the distended urinary bladder at the onset of VUR on cystography, g) Laterality of the refluxing ureter and h) Distal ureteral dilatation. A poor prognostic factor, independent of reflux Grade is an elevated distal ureteral diameter ratio (UDR) which is related to increased prevalence of UTI [48]. Moreover, a child with normal kidneys and recurrent UTI is at low risk (0.3%) to develop chronic kidney disease (CKD) [57].

Other diagnostic methods for VUR

The most reliable diagnosing methods to routinely detect VUR are MCU and DRC. Many authors refer to MCU as the golden standard due to its better depiction of anatomical structures. A dynamic imaging technique performed mainly during the last two decades is contrast-enhanced voiding urosonography (ceVUS) using an intravesical second generation ultrasound contrast agent. The method does not use ionizing radiation and carries a higher reflux detection rate compared to MCU, especially for higher grades of VUR [58, 59].

In a study conducted in 228 children, another technique, described as harmonic voiding urosonography using harmonic imaging modality (VUS HI), showed 77.5% concordance in findings (presence or absence of VUR) with MCU; The development of harmonic imaging ultrasonography has increased contrast and spatial resolution of VUS images [60, 61]. However the difference in the detection rate for VUR was statistically significant between VUS and MCU [62]. Another technique, magnetic resonance voiding cystourethrography has been applied in the last decade in small patient series, with a reported sensitivity of 90%-96% and specificity of 68%-96% [63-65].

Sonographically confirmed dilatation (>3mm) of distal ureter has a total diagnostic accuracy of 74.2% in VUR detection; hence it can be used as a diagnostic tool of reflux in children with urinary tract infection [66].

A number of other diagnostic indices have been suggested for the diagnosis of VUR in several studies, namely serum calcium levels [67], the urine Ca/Cr ratio [68] and serum procalcitonin levels [69]. A recent case-control study with 107 female subjects with VUR grade I- III, showed a higher mean platelet count and a lower mean platelet volume (MPV) in patients with reflux nephropathy compared to those with non-reflux nephropathy. The authors claimed that MPV can be used as an indicator in the diagnosis of VUR [70].

During the last decade, there is a trend to reduce the imaging studies related to VUR diagnosis. A considerable variability in sensitivity and specificity for the diagnosis of VUR along with significant differences in radiation exposure and cost have been considered. Following a primary febrile UTI, the main diagnostic imaging strategy has not yet been established. The aforementioned is partially related to the fact that there are not sufficient data to show the association between renal compromise and VUR and UTI-associated renal parenchyma scarring. Moreover, in the majority of children diagnosed with pyelonephritis and/or VUR the etiology of gradual loss of kidney function may be congenital [55].

Follow-up in children with VUR

It is recommended that in children diagnosed with VUR, depending on clinical and imaging findings, a follow-up should be performed during the next 12-18 months or earlier after conducting DRC and technetium-99m dimercaptosuccinic acid (^{99m}Tc-DMSA) and also biannually with ultrasonography of the urinary tract. However, in patients with febrile recurrent UTI despite CAP, more intensive monitoring and aggressive treatment should be considered [71].

Another point of view for the diagnosis and treatment of VUR

Over the last decades strong evidence has shown that: a) In many children diagnosis and treatment of VUR is of no value, as the disease becomes self-limited [72]. Spontaneous resolution of VUR rates are 92%, 59% and 25% for children diagnosed with mild, moderate and severe VUR respectively [56], b) There is a confirmed correlation between VUR and UTI, as in 30%-40% of infants and neonates with UTI imaging, tests reveal the presence of VUR [1], c) Renal parenchymal scarring can be manifested with UTI in the absence of VUR or with VUR in the absence of UTI [73]. Having the above three statements in mind, the major factors that could be well considered as causes for renal scarring are: a) The presence of VUR, independently of its grade and even with sterile urine [74],

since it results in imbalance of the normal pressure difference between the bladder and the ureter-pyelocaliceal system. It has been reported in children who had VUR grade I that when examined for a second time VUR in up to 20% of them was found to be grade II or higher [75]. b) The presence of UTI, as it can cause damage to renal parenchyma per se, in the absence of VUR. (The rate of renal scarring in children without VUR is about 6%) [76]. c) The retrograde flow of urine up to the pyelocaliceal system as an additional risk factor, for the transmission of pathogens to kidney parenchyma. This occurs when the pressure gradient between the bladder and the ureter-pyelocaliceal system is substantially elevated causing a backflow of urine up to the pyelocaliceal system. In terms of VUR this may happen in VUR grades II-V.

We noticed that the distinction between VUR grades II and III is difficult in cystography techniques. In DRC due to low resolution images, the distinction is almost impossible while in MCU dilatation of the pyelocaliceal system bears a degree of uncertainty and may vary between radiologists. In fact VUR grade II is a 'grey zone' and often misinterpreted. According to the above, it is of great importance to verify the absence of VUR, or of VUR grade I or higher.

The rate of renal scarring in children with VUR grades I-II is about 14% and with grades III-V is about 40% [76]. Vesicoureteral reflux related to renal parenchymal scarring is more frequent with UTI; the odds ratio of renal parenchymal scarring with acute pyelonephritis in the presence of VUR is 2.8 as compared to those without VUR [73].

Despite the aforementioned, many other factors reported in the literature, as mentioned previously, are associated with non-favorable prognosis, which may contribute to renal scarring. There is an obvious need to create a study with multifactorial analysis that could estimate the independent and the relative prognostic value of each factor.

Suggestions for standardizing DRC technique

Suggestions for standardizing the DRC technique may include: a) The need for sedation, the distance of the saline bottle and the manometric device from the patient's bladder, the formula for the estimated capacity of the bladder, the dose of ^{99m}Tc-radiotracer and the patient's position during the voiding phase. Moreover, a standardized acquisition protocol (matrix, zoom etc) is suggested. More semi-quantitative measurements either already tested (e.g. bladder pressure and volume at which the reflux happens, urine reflux volume, etc.) or suggested (e.g. the angle between a refluxing ureter and the body median vertical axis) need to be standardized. b) In female patients DRC could be the first imaging option for VUR detection. In male patients the anatomical depiction of the urethra is of great importance, so MCU may remain the golden standard as a first imaging modality after a UTI episode. c) In some complicated post-surgery conditions, especially when congenital conditions (e.g. megaureter) are involved, the planar DRC images are inadequate to distinguish the anatomical structures and assess the outcome of an operation. Hybrid imaging devices such as SPECT/CT gamma-cameras may provide additional anatomical information using sophisticated software with relatively low radiation exposure of the patient. d) In order to avoid the additional radiation burden to the young patients, refined software for the optimal analysis of sequential static images would be very helpful.

The use of non-invasive IRC should be preferred over DRC and performed routinely after the non-diuretic renogram, for groups of selected patients with renal disease and/or kidney transplantation, especially when MAG-3 is used. In fact all needed is a specific micturition chair for the female patients. Even if this method cannot detect VUR during the filling phase, the advantage of monitoring the bladder under physiological conditions (with high bladder pressure and monitoring the volume of urine contained) is of importance and could reveal reflux with minimal radiation exposure. As said above, another valuable application of IRC may be the detection of VUR in renal transplants. Vesicoureteral reflux in renal grafts is common (10.5%), although it does not seem to affect renal graft's function and graft's survival [77]. Reflux was also diagnosed in about 40.7% of 646 renal transplant recipients, studied using MCU. The presence of VUR did not affect the survival of death-censored renal grafts (calculated from the date of transplantation to the date of irreversible graft failure). On the contrary VUR was associated with lower estimated glomerular filtration rate (eGFR) levels during the 1st year post-transplantation [78].

In conclusion, various radionuclide techniques related to the diagnosis of VUR have been described. It seems that DRC is a reliable and sensitive diagnostic technique which can detect VUR by continuous monitoring and is also used for the follow-up of children with known VUR. Other radionuclide techniques, such as IRC and CDRC can be used in selected patients. Since there are contradicting views about the diagnosis and treatment of VUR, a standardized diagnostic technique is welcome.

Bibliography

1. Alizadeh F, Shahdoost AA, Zargham M et al. The influence of ureteral orifice configuration on the success rate of endoscopic treatment of vesicoureteral reflux. *Adv Biomed* Res 2013; 2: 1.
2. Shaikh N, Ewing AL, Bhatnagar S, Hoberman A. Risk of renal scarring in children with a first urinary tract infection: a systematic review. *Pediatrics* 2010; 126(6): 1084-91.
3. Silay MS, Spinoit AF, Bogaert G et al. Imaging for Vesicoureteral Reflux and Ureteropelvic Junction Obstruction. *Eur Urol Focus* 2016; 2(2): 130-8.
4. Piscitelli A, Galiano R, Serrao F et al. Which cystography in the diagnosis and grading of vesicoureteral reflux? *Pediatr Nephrol* 2008; 23(1): 107-10.
5. Sükan A, Bayazit AK, Kibar M et al. Comparison of direct radionuclide cystography and voiding direct cystography in the detection of vesicoureteral reflux. *Ann Nucl Med* 2003; 17(7): 549-53.
6. Apperson JW, Atkins H, Fleming R. The value of the isotope cystogram in determining pressure and volume at which ureteral reflux occurs. *J Urol* 1963; 89: 405-13.
7. Winter CC. A new test for vesicoureteral reflux: an external technique using radioisotopes. *J Urol* 1959; 81(1): 105-11.
8. Dodge EA. Vesicoureteric reflux. Diagnosis with iodine-131 sodium ortho-iodohippurate. *Lancet* 1963; 1(7276): 303-4.
9. Braren V, Jones J, Partain L. A Computerized Technique for Radioisotopic Cystography. *J Urol* 1987; 137(6): 345A
10. Conway JJ, Belman AB, King LR, Filmer RB. Direct and indirect radionuclide cystography. *J Urol* 1975; 113(5): 689-93.
11. Fettich J, Colarinha P, Fischer S et al. Guidelines for direct radionuclide cystography in children. *Eur J Nucl Med Mol Imaging* 2003; 30(5): B39-44.

12. Patel CD, Chawla M, Nadig MR et al. Evaluation of dysfunction and malformations of the urinary tract in patients with meningomyelocele, by renal dynamic scintigraphy and direct radionuclide cystography. An Indian perspective. *Hell J Nucl Med* 2007; 10(2): 102-4.
13. Siderias J, Gaudio F, Singer AJ. Comparison of topical anesthetics and lubricants prior to urethral catheterization in males: a randomized controlled trial. *Acad Emerg Med* 2004; 11(6): 703-6. Erratum in: *Acad Emerg Med* 2014; 21(2): 225.
14. Treves ST, Grant FD (2014). Vesicoureteral reflux and Radionuclide Cystography. In: Treves S.T. (Ed.), *Pediat Nucl Med Mol Imag* (4th edn.). Springer Sciences & Business Media New York. pp 335-53.
15. Biassoni L, Gordon I (2016). Vesico-ureteric reflux and urinary tract infection. In: Cook G, Maisey M, Britton K, Chengazi V. (Eds.), *Clin Nucl Med* (4th edn.). Edward Arnold Publishers Ltd London. pp 286-7.
16. Wilkinson AG. Percutaneous direct radionuclide cystography in children: description of technique and early experience. *Pediatr Radiol* 2002; 32(7): 511-7.
17. Lukacz ES, Sampselle C, Gray M et al. A healthy bladder: a consensus statement. *Int J Clin Pract* 2011; 65(10): 1026-36.
18. Mandell GA, Eggli DF, Gilday DL et al. Procedure guideline for radionuclide cystography in children. Society of Nuclear Medicine. *J Nucl Med* 1997; 38(10): 1650-4.
19. AHRQ (US) - Agency for Healthcare Research and Quality. ACR-SPR-SNM practice guideline for the performance of adult and pediatric radionuclide cystography. American College of Radiology. Society for Pediatric Radiology. Society of Nuclear Medicine. United States. 2015.
20. McPherson A, Bennett P (2016). Vesicoureteral Reflux. In: Bennett P, Oza U, Trout A, Mintz A. (Eds). *Diagnostic Imaging. Nuclear Medicine*. Elsevier. Philadelphia. pp 244-7.
21. Mozley PD, Heyman S, Duckett JW, et al. Direct vesicoureteral scintigraphy: quantifying early outcome predictors in children with primary reflux. *J Nucl Med* 1994; 35(10): 1602-8.
22. Barthold JS, Martin-Crespo R, Kryger JV et al. Quantitative nuclear cystography does not predict outcome in patients with primary vesicoureteral reflux. *J Urol* 1999; 162(3 Pt 2): 1193-6.
23. Papachristou F, Printza N, Doumas A, Koliakos G. Urinary bladder volume and pressure at reflux as prognostic factors of vesicoureteral reflux outcome. *Pediatr Radiol* 2004; 34(7): 556-9.
24. Ziessman H, O' Malley J, Thrall J, Fahey F. (2014) Genitourinary System. In: *Nuclear Medicine*. *The Requisites.* Elsevier Saunders. Philadelphia. p. 202
25. De Sadeleer C, De Boe V, Keuppens F et al. How good is technetium-99m mercaptoacetyltriglycine indirect cystography? *Eur J Nucl Med* 1994; 21(3): 223-7.
26. Dikshit MP, Acharya VN, Shikare S et al. Comparison of direct radionuclide cystography with micturating cystourethrography for the diagnosis of vesicoureteric reflux, and its correlation with cystoscopic appearances of the ureteric orifices. *Nephrol Dial Transplant* 1993; 8(7): 600-2.
27. Gordon I. Urinary tract infection in paediatrics: the role of diagnostic imaging. *Br J Radiol* 1990; 63(751): 507-11.
28. Unver T, Alpay H, Biyikli NK et al. Comparison of direct radionuclide cystography and voiding cystourethrography in detecting vesicoureteral reflux. *Pediatr Int* 2006; 48(3): 287-91.
29. Dalirani R, Mahyar A, Sharifian M et al. The value of direct radionuclide cystography in the detection of vesicoureteral reflux in children with normal voiding cystourethrography. *Pediatr Nephrol* 2014; 29(12): 2341-5.
30. Nikibakhsh A A, Mahmoodzadeh H, Hejazi S et al. Comparison of direct radionuclide cystography and voiding cystourethrography in diagnosis of missed vesicoureteral reflux in children with recurrent urinary tract infection. *J Urmia Univ Med Sci* 2014; 25(4): 290-7.
31. McLaren CJ, Simpson ET. Direct comparison of radiology and nuclear medicine cystograms in young infants with vesicoureteric reflux. *BJU Int* 2001; 87(1): 93-7.
32. Blaufox MD, De Palma D, Taylor A et al. The SNMMI and EANM practice guideline for renal scintigraphy in adults. *Eur J Nucl Med Mol Imaging* 2018; 45(12): 2218-28.
33. Piepsz A. Radionuclide studies in paediatric nephro-urology. *Eur J Radiol* 2002; 43(2): 146-53.
34. Gil Salom M, Nuñez F, Hernández R et al. Value of isotopic cystography in the diagnosis of vesicoureteral reflux in childhood. *Actas Urol Esp* 1989; 13(5): 339-42.
35. Chapman SJ, Chantler G, Haycock GB et al. Radionuclide cystography in vesicoureteric reflux. *Arch Dis Child* 1988; 63(6): 650-1.
36. Gordon AC, Thomas DF, Arthur RJ et al. Prenatally diagnosed reflux: a follow-up study. *Br J Urol* 1990; 65(4): 407-12.
37. Majd M. Radionuclide imaging in pediatrics. *Pediatr Clin North Am* 1985; 32(6): 1559-79.
38. Joaquim AI, de Godoy MF, Burdmann EA. Cyclic Direct Radionuclide Cystography in the Diagnosis and Characterization of Vesicoureteral Reflux in Children and Adults. *Clin Nucl Med* 2015; 40(8): 627-31.
39. Fettich JJ, Kenda RB. Cyclic direct radionuclide voiding cystography: increasing reliability in detecting vesicoureteral reflux in children. *Pediatr Radiol* 1992; 22(5): 337-8.
40. Schneider K, Kruger-Stollfub I, Ernst G et al. Pediatric fluoroscopy: a survey of childrens' hospitals in Europe. *Pediatr Radiol* 2001; 1:238-46.
41. Al-Imam OA, Al-Nsour NM, Al-Khulaifat S. Which is the best way of performing a Micturating Cystourethrogram in children? *Saudi J Kidney Dis Transpl* 2008; 19(1): 20-5.
42. American College of Radiology (ACR) and the Society for Pediatric Radiology (SPR) Committees. ACR-SPR Practice Parameter for the Performance of Voiding Cystourethrography in Children. American College of Radiology. Society for Pediatric Radiology. Committee on Practice Parameters-Pediatric Radiology. 2017.
43. Mantovani A, Giroletti E. Evaluation of the dose to pediatric patients undergoing micturating cystourethrography examination and optimization of the examination. *Radiol Med* 2004; 108(3): 283-91.
44. Tarin T, Shinghal R, Linda M. Dairiki S. (2010). Pediatric urinary tract infections. John P. Gearhart, Richard C. Rink, Pierre D.E. Mouriquand (Eds). *Pediatric Urology* (2nd edn.). Elsevier. Philadelphia. pp 180-95.
45. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood: a meta-analysis. *Pediatr Infect Dis J* 2008; 27(4): 302-8.
46. Lebowitz RL. Pediatric uroradiology. *Pediatr Clin North Am* 1985; 32(6): 1353-62.
47. Blumenthal I. Vesicoureteric reflux and urinary tract infection in children. *Postgrad Med J* 2006; 82(963): 31-5.
48. Arlen AM, Kirsch AJ, Leong T, Cooper CS. Validation of the ureteral diameter ratio for predicting early spontaneous resolution of primary vesicoureteral reflux. *J Pediatr Urol* 2017; 13(4): 383.e1-383.e6.
49. Brandström P, Jodal U, Sillén U, Hansson S. The Swedish reflux trial: review of a randomized, controlled trial in children with dilating vesicoureteral reflux. *J Pediatr Urol* 2011; 7(6): 594-600.
50. Mattoo TK, Chesney RW, Carpenter MA. For The RIVUR Trial Investigators. The RIVUR Study: A factual interpretation of our data. *Pediatr Nephrol* 2015; 30(5): 707-12.
51. Chand DH, Rhoades T, Poe SA et al. Incidence and severity of vesicoureteral reflux in children related to age, gender, race and diagnosis. *J Urol* 2003; 170(4 Pt 2): 1548-50.
52. Lee T, Park JM. Vesicoureteral reflux and continuous prophylactic antibiotics. *Investig Clin Urol* 2017; 58 (Suppl 1): S32-S37.
53. Ninoa F, Ilari M, Noviello C et al. Genetics of Vesicoureteral Reflux. *Curr Genomics* 2016; 17(1): 70-9.
54. Bimpaki E, Bitsori M, Choulaki C, Galanakis E. Vascular endothelial growth factor-A gene polymorphism is associated with congenital renal lesions in children with urinary tract infections. *Acta Paediatr* 2017; 106(8): 1348-53.
55. Tombesi MM, Alconcher LF, Lucarelli L, Ciccioli A. Algorithms imaging tests comparison following the first febrile urinary tract infection in children. *Arch Argent Pediatr* 2017; 115(4): 370-3.
56. Hu C, Peng NJ, Lin HS, Chiou YH. Predict the spontaneous resolution of vesicoureteral reflux by direct radionuclide cystography. *Rev Esp Med Nucl Imagen Mol* 2013; 32(2): 65-9.
57. Salo J, Ikäheimo R, Tapiainen T, Uhari M. Childhood urinary tract infections as a cause of chronic kidney disease. *Pediatrics* 2011; 128(5): 840-7.
58. Duran C, Beltrán VP, González A et al. Contrast-enhanced Voiding Urosonography for Vesicoureteral Reflux Diagnosis in Children. *Radiographics* 2017; 37(6): 1854-69.
59. Wong LS, Tse KS, Fan TW et al. Voiding urosonography with second-generation ultrasound contrast versus micturating cystourethrography in the diagnosis of vesicoureteric reflux. *Eur J Pediatr* 2014; 173(8): 1095-101.

60. Tranquart F, Grenier N, Eder V, Pourcelot L. Clinical use of ultrasound tissue harmonic imaging. *Ultrasound Med Biol* 1999; 25(6): 889-94.
61. Darge K. Voiding urosonography with US contrast agent for the diagnosis of vesicoureteric reflux in children: an update. *Pediatr Radiol* 2010; 40(6): 956-62.
62. Papadopoulou F, Anthopoulou A, Siomou E et al. Harmonic voiding urosonography with a second-generation contrast agent for the diagnosis of vesicoureteral reflux. *Pediatr Radiol* 2009; 39(3): 239-44.
63. Takazakura R, Johnin K, Furukawa A et al. Magnetic resonance voiding cystourethrography for vesicoureteral reflux. *J Magn Reson Imaging* 2007; 25(1): 170-4.
64. Hekmatnia A, Merrikhi A, Farghadani M et al. Diagnostic accuracy of magnetic resonance voiding cystourethrography for detecting vesicoureteral reflux in children and adolescents. *J Res Med Sci* 2013; 18(1): 31-6.
65. Johnin K, Takazakura R, Furukawa A et al. Magnetic resonance voiding cystourethrography (MRVCUG): a potential alternative to standard VCUG. *J Magn Reson Imaging* 2013; 38(4): 897-904.
66. Carovac A, Zubovic SV, Carovac M, Pasic IS. Significance of Sonographically Demonstrated Ureteral Dilatation in Evaluation of Vesicoureteral Reflux Verified with Voiding Urosonography in Children with Urinary Tract Infection. *Acta Informatica Medica* 2015; 23(5): 268-72.
67. García-Nieto V, Siverio B, Monge M et al. Urinary calcium excretion in children with vesicoureteral reflux. *Nephrol Dial Transplant* 2003; 18(3): 507-11.
68. Badeli H, Sadeghi M, Shafe O et al. Determination and comparison of mean random urine calcium between children with vesicoureteral reflux and those with improved vesicoureteral reflux. *Saudi J Kidney Dis Transpl* 2011; 22(1): 79-82.
69. Mortazavi F, Ghojazadeh M. Usefulness of serum procalcitonin level for prediction of vesicoureteral reflux in pediatric urinary tract infection. *Iran J Kidney Dis* 2014; 8(1): 37-41.
70. Yousefichaijan P, Kahbazi M, Eghbal A et al. The Mean Platelet Volume in children with Pyelonephritis. *J Pediatr Nephrol* 2016; 4(2): 56-9.
71. Tekgül S, Riedmiller H, Hoebeke P et al. European Association of Urology. EAU guidelines on vesicoureteral reflux in children. *Eur Urol* 2012; 62(3): 534-42.
72. Cooper CS. Diagnosis and management of vesicoureteral reflux in children. *Nat Rev Urol* 2009; 6(9): 481-9.
73. Mattoo TK. Vesicoureteral reflux and reflux nephropathy. *Adv Chronic Kidney Dis* 2011; 18(5): 348-54.
74. Grmek M, Fettick J. The importance of follow-up of children with vesicouretericreflux Grade 1. *Acta Paediatr* 2003; 92: 435-8.
75. Lee H, Lee YS, Im YJ, Han SW. Vesicoureteral reflux and bladder dysfunction. *Trans Androl Urol* 2012; 1(3): 153-9.
76. Gonzalez E, Parazyan J-P, Girardin E. Impact of vesicoureteral reflux on the size of renal lesions after an episode of acute pyelonephritis. *J Urol* 2005; 173(2): 571-5.
77. Molenaar NM, Minnee RC, Bemelman FJ et al. Vesicoureteral Reflux in Kidney Transplantation. *Prog Transplant* 2017; 27(2): 196-9.
78. Margreiter M, Györi GP, Böhmig GA et al. Value of Routine Voiding Cystourethrography After Renal Transplantation. *Amer J Transplan* 2013; 13: 130-5.