CHAPTER 3

ANATOMY OF URETEROVESICAL JUNCTION AND DISTAL URETER STUDIED BY ENDOLUMINAL ULTRASONOGRAPHY IN VITRO

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ABSTRACT

Purpose: The emerging technique of endoluminal ultrasonography (ELUS) provides a new modality for endoscopic visualisation of the urinary tract, which needs to be further, evaluated. We studied the normal anatomy of distal ureter and ureterovesical junction using ELUS.

Material and Methods: An assessment of in vitro ELUS ureteric images undertaken at 1 mm intervals from 8 fresh human cadaver pelvis blocs of bladder and distal ureter were compared to findings of serial histological sections of the same specimens (stained for cholinesterase iso-enzymes) to assess the degree of correlation. Computer-assisted 3D reconstructions were made.

Results: The different components (ureteric, detrusor and periureteric tissue) of the UVJ could be identified on the basis of echogenicity and form, but differentiation between the respective muscle layers in the wall of the ureter or of the detrusor was not possible. Nevertheless, ureteric volume measurements and an assessment of transmural ureteric length and the angle of passage through the bladder wall were possible.

Conclusions: ELUS is able to differentiate between the ureteric and detrusor muscle and the UVJ gross anatomy can be reconstructed. ELUS technology, however, fails to differentiate between individual muscular layers of the ureter or the detrusor. Further improvement in ELUS is mandatory.
INTRODUCTION
The anatomy of the ureterovesical junction has been studied extensively by different authors because of its significance as an anatomical substrate in the prevention of vesicoureteral reflux (VUR)\textsuperscript{1-7}. Endoluminal (ureteral) ultrasonography (ELUS) using a miniature flexible (4.1 F), high frequency (30 MHz) transducer near the catheter tip, enables one to obtain high-resolution 360° cross-sectional images at short penetration depths ($\pm 1$ cm). Lower frequency catheters (20, 15 or 10 MHz) are commercially available for deeper penetration depths when required. These flexible probes using the endoluminal imaging technique were designed in the late 1980s to investigate atherosclerotic plaques in peripheral and coronary vessels. Goldberg and co-workers have also reported satisfactory experimental results in animals with non-vascular applications of ELUS, including those of the urethra, urinary bladder, ureter and renal pelvis\textsuperscript{11}. The technical improvements in the field of transducer technology, miniaturisation, and the hard- and software used help greatly to minimise artifacts. This experimental study was undertaken to study the potential use of ELUS in urology and to ascertain whether detailed images of the UVJ could be obtained using ELUS, and to compare the results thus obtained to the histological findings from the same UVJ. The study was undertaken in vitro because: 1. The commercially available ELUS catheters are not designed for use in the urinary tract, are relatively stiff, very long, and uncalibrated, as well as having a relatively sharp open end-point. These facts make endoscopic manipulation and orientation difficult and may pose some hazards for the epithelial lining of the urethra, bladder and ureter. 2. An in vitro study permits us to localise the transducer visually so that interpretation of the topographic anatomy of the different images is very reliable. 3. The in vitro study is necessary to establish the advantages and the limitations of investigation of different layers and their echogenic properties in fresh human material and to be able to establish the morphological correlates. 4. From an imaging in vitro study it is possible to obtain morphometric data of ureteric volume measurements and bladder wall thickness. 5. With suitably adapted catheter an in vivo ELUS investigation in the urethra and ureter may in future be possible for volume measurements.
MATERIAL AND METHODS

*ELUS catheter and equipment.* Commercially available Du-MED® equipment with 4.1 F ELUS catheters (140 cm long), and a 30 MHz miniature rotating transducer tip were used on loan from the cardiologists. The transducer generates 360° cross-sectional images at a velocity of 16 frames per second. Bidistilled water was used to flush the space between the core and body of the catheter occasionally to eliminate air bubbles. No balloon dilatation was used. The ultrasound catheter was in direct continuous contact with the ureteric wall. The images were simultaneously visualised on the monitor and recorded on VHS videotape for later analysis and digitisation. The catheter displacement was measured accurately using an electronic device especially designed for this purpose. The device can detect displacements of 0.1 mm (Fig. 1).

![Diagram](image)

**Figure 1:** Schematic representation of the experimental set-up. BN: bladder neck, U: ureter with ELUS catheter inside. Rendered wedge represents a hemitrigone.

*Human true-pelvis blocs:* Eight human true-pelvis blocs (mean age 74, range 63-86 years) containing bladder, distal 1/3 of the ureters and vagina in females or rectum and prostate in males were obtained at post-mortem conducted within 24 hours of death. The vagina or rectum was dissected and removed very carefully from the bladder avoiding any damage to the posterior bladder wall or distal ureters. Perivesical fat especially around the ureters was not manipulated to avoid any preparation artifact. The anterior bladder wall was opened mid-sagittally and the specimen fixed with pins on a plastic slab in the anatomic position. The mounted specimen was positioned in a physiological saline bath. The ELUS catheter was introduced antegradely into the cut distal ureter and advanced manually.
towards the bladder lumen until its tip appeared at the ureteric orifice. By retrograde intermittent displacement of the catheter through the ureter lumen, a series of ELUS images at 1 mm intervals of the UVJ were obtained and recorded on VHS videotape.

These images were later digitised using an analogue-digital converter and stored on a hard disk. On completion of these studies, the posterior bladder wall and ureters were carefully dissected free from the rest of the bladder for further microscopic study.

**Histological studies.** Four percent formaldehyde/0.22 M sucrose/0.1 M sodium phosphate (pH 7.3) was used as fixative. The entire posterior bladder wall was fixed for 16 hours at 4 °C. After fixation, the specimen was rinsed twice in bidistilled water and frozen in isopentane, precooled in liquid nitrogen, and later sectioned longitudinally or transversely at 40μm. Cryostat sections were stained with haematoxyline-azophloxine (HA) or with the ‘direct-colouring thiocholine method’ as described by Karnovsky and Roots to demonstrate tissue acetylcholinesterase (AChE) or butyrylcholinesterase (BChE, non-specific cholinesterase) activity. The optimum pH for staining human smooth muscle was established to be 5.6, after testing a range from 4.5 to 7. A staining period of 16 h for AChE and 8 h for BChE gave the best staining effect. The stained sections were mounted in Entellan (Merck, Darmstadt, Germany).

**Three-dimensional computer-assisted reconstruction.** The contours of different ultrasound textures recognised in the ELUS images were drawn manually. From the series of consecutive sections, the contours in spatial configurations were all serially stored in an input database. Two reference points per section were included for proper realignment of different sections in order to generate a reconstructed three-dimensional (3D) model. The 3D reconstruction software was developed in our laboratory. The input software was run on an IBM compatible PC-486 equipped with a high-resolution video adapter and a colour monitor. From the contour model a volume model was generated using the 3D-base format. Volume rendering was used to produce visualisation of the volume model (Fig. 4).
RESULTS

Ultrasound properties of the UVJ. Despite the moderate quality of imaging acquired with this older generation of commercially available ELUS device, our study revealed the bladder and ureteric mucosa as one contiguous hyperechogenic layer. Direct contact between catheter and urothelium must be avoided to prevent the down-ring artifact (repetitive visualisation of the body of the catheter) (Figs. 2a, f). Muscle tissue including ureteric and detrusor muscle bundles are indistinguishable from each other based on the echogenicity criterion alone. However, the ureteric muscle fibres (as seen in our in vitro specimens) had a fine-punctuate structure, in comparison to the detrusor, which appeared to be circularly organised and had a rough texture (Figs. 2d,e). ELUS does not enable us to differentiate between the different layers of the bladder muscle e.g. the detrusor muscle, trigonal layer and the periureteric muscular sheath, suggesting equal acoustic impedance. The results of our analysis of the echogenic properties of the UVJ are summarised in Table 1.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Ultrasound characteristic</th>
</tr>
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<tbody>
<tr>
<td>Mucosa (bladder/ ureter)</td>
<td>Hyperechogenic</td>
</tr>
<tr>
<td>Ureteric muscle</td>
<td>Fine-punctuate in shape, hypoechochogenic</td>
</tr>
<tr>
<td>Detrusor</td>
<td>Rough and circular in shape, hypoechochogenic</td>
</tr>
<tr>
<td>Ureteric orifice</td>
<td>An inverted hyperechogenic Ω</td>
</tr>
<tr>
<td>Serosa of the bladder</td>
<td>Thick hyperechogenic line</td>
</tr>
<tr>
<td>Periureteric tissue</td>
<td>Hypoechogenic, located posterior to the ureter</td>
</tr>
</tbody>
</table>

Table 1: Echogenicity and US texture of contributing structures at UVJ level.

The topographic anatomy of the US images. The topography of the different parts of the distal ureter (juxtavesical, intramural and submucosal) can be visualised well with ELUS. Characteristic ELUS images are obtained at the junctions of: 1) the juxtavesical and trans-detrusor portion of the ureter; 2) the trans-detrusor and submucosal portion of the ureter; and 3) at the ureteric orifice, which is the border between the bladder lumen and the ureter. The ureteric orifice is visualised as a rotated (depending on the position of the transducer tip relative to the body of the catheter), hyperechogenic Ω-shaped structure (Fig. 2e). The detrusor muscle (rough US texture) is seen to surround completely the ureteric tube from the level at which the submucosal ureter ends and the trans-detrusor ureter begins (Fig. 2d). The exit of the ureter from the posterior bladder wall is also characterised by an
unmistakably thick tangential hyperechogenic layer, representing the acoustic impedance difference between the ureteric muscle and the periureteric tissue (Fig. 2c). Posterior to the serosa of the bladder wall the periureteric (fat) tissue is imaged as a hypoechoic field.

Figure 2: ELUS images (left) and corresponding histological sections (right) of the upper urinary tract. Magnification: Bar: 1 mm. Panel a: ELUS image of the ureteric mucosa (renal pyelum lumen: PL) which is hyperechogenic. Histological section of pyelum is not relevant for this article. Panel b: ELUS image of the pelvic ureter (surrounding tissue has been removed carefully under the dissection microscope). Ureteric muscle has a fine “punctate” US-texture. Histological section (BChE) shows the same thickness and muscular pattern. The mucosa can not be visualised separately because of the down-ring artifact. Ureteric lumen: UL. Panel c: ELUS image of the ureter at its exit from the bladder wall. The ureter serosal surface and the periureteric tissue (PUT) is delineated as a hyperechogenic circle. Histological section (BChE) at this level reveals that PUT is composed mainly of non-muscular tissue. For the topographic position of this image see also Fig. 3, D: detrusor muscle. Panel d: ELUS image of the submucosal segment of the ureter. The ureter is recognisable centrally by its punctuate muscle pattern and covered posteriorly by the circularly organised detrusor of “rough” echo-texture. Histology (BChE) reveals
the same data and also shows that the border between the ureter and the detrusor (D) is only a thin non-muscular adventitial layer. See also Fig. 4 for the topographic position of this image. Bladder lumen: BL. Panel e: ELUS image of the ureteric orifice (O) is hyperechogenic and has an rotated (Ω) shape. In a histological section (AChE stained) 1 mm. proximal to the orifice, one can see the detrusor located posteriorly in the ELUS image. For the topographic position of this image see also Fig. 4. Bladder lumen: BL. Panel f: The mucosa of the bladder is also hyperechogenic. Histological section of the bladder wall is omitted for clarity of discussion. Bladder lumen: BL.

The length of the transmural ureter was determined by measuring the displacement distance (23±0.6 mm in this study) of the catheter through the ureter from the Ω-shaped ureteric orifice to the point of visualisation of the hyperechogenic serosal layer. The bladder wall thickness (4±0.2 mm in this study) at the transmural level can also be measured by ELUS at the same session.

Using these two parameters, one can calculate the sinus of the diagonal angle (α = 11±0.5 degrees in this study) at which the ureter passes through the posterior bladder wall by employing the mathematical formula:

\[
\sin \alpha = \frac{\text{bladder thickness}}{\text{transvesical ureter length}}
\]

The length of the transmural ureter and the backing provided by the thickness of the detrusor may play an important role in preventing VUR and in maintenance urinary bolus transport.

3D reconstruction of the ELUS images and volume measurements. The 3D reconstructed model obtained from analysis of serial ELUS images reveals a cross-sectional view of underlying morphology. It reveals a straight cylindrical form because of two pre-marked reference points that were used (Fig. 4). The cylindrical form correlates well with histological findings. The detrusor-muscle bed supporting the ureter is well visualised by this method. Using this form of reconstruction (Table 2 and Fig. 4) ureteric volume measurements obtained by adding together data from five consecutive images at different segmental levels reveals that ureteric volume at the UVJ remains practically constant throughout its transmural and submucosal passage.
Table 2: Volume measurements (mm$^3$) of serial 5 mm segments of eight left ureters measured from the ureteric orifice to the juxtavesical ureter (distal to proximal). SEM: standard error of the mean.

<table>
<thead>
<tr>
<th>Ureter segment in millimetres (distal to proximal)</th>
<th>01-05</th>
<th>05-10</th>
<th>10-15</th>
<th>15-20</th>
<th>20-25</th>
<th>25-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean volume (mm$^3$)</td>
<td>43</td>
<td>45</td>
<td>48</td>
<td>47</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>SEM</td>
<td>1.4</td>
<td>2.8</td>
<td>2.5</td>
<td>2.8</td>
<td>1.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Figure 3: The volume of the ureter at different levels within the UVJ. The error bars represent the standard deviation (SD) at different segments.

This data also supports the histological finding of reorientation of ureteric muscle fibres from two layers (an outer circular and an inner longitudinal) proximal to the juxtavesical level into a single longitudinal layer in the intramural tract. A simple loss of the outer circular layer would entail a progressive decrease in the ureteric volume throughout the UVJ tract.

Comparison between ELUS and histology. ELUS generates characteristic reproducible images of specific sections of the ureter at the UVJ. The length of the different segments can be determined. These lengths correlate well with histological measurements (Table 3). The average UVJ length as measured by retrograde ELUS catheter movement was 23 ±0.6 mm as compared to 22 ±0.7 mm by in vitro measurement of histological material from the same ureter. The mean ratio of the ELUS to histological measurements is 1.05 with a range of 0.8 to 1.2. The average diagonal angle
of passage of the ureter through the bladder wall as measured by ELUS and histology is $11 \pm 0.5$ and $13 \pm 1.2$ degrees, respectively.

Figure 4: 3-D reconstruction of the human UVJ based on ELUS images. The constant diameter of the ureter and backing provided by the detrusor bed to the ureter are shown in this reconstructed model. SMU: submucosal ureter. The position of Figs. 2e,d and c is indicated.

**Histological studies.** Longitudinal sections were stained and studied as described to measure accurately the ureteric length at the UVJ and provide control data for the analysis of ELUS images (Table 3). The ureteric muscle fibres were only longitudinally oriented at the UVJ. “Shrinkage” of tissue after fixation is calculated as a percentage change in length of a piece of ureter (from the same cadaver), before and after fixation. This enables us to determine a correction factor for the histological study. Shrinkage of a strip of bladder wall leads to deformity of the specimen and made measurement of detrusor shrinkage unreliable and is thus omitted. The
mean length measured of the transmural segment of the ureter at the UVJ was 22 ±0.7 mm and the diagonal angle of passage of the ureter through the bladder wall was measured to be 13 ±1.2 degrees.

<table>
<thead>
<tr>
<th></th>
<th>Length UVJ (mm)</th>
<th>Thickness bladder (mm)</th>
<th>Calculated angle (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELUS</td>
<td>Histology</td>
<td>ELUS</td>
</tr>
<tr>
<td>Mean</td>
<td>23</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>SEM</td>
<td>0.6</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>ELUS / Histology</td>
<td>1.05</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Comparison between histological and Endo Luminal UltraSonographic observations. SEM: standard error of the mean. Mean of shrinkage of tissue due to fixation for histological processing: 15%

DISCUSSION

ELUS and the UVJ anatomy. Reflux is an important pathological entity in paediatric and early adult urology. The incidence of VUR is approximately one percent and increases to 50 % in children with urinary tract infection. Eight percent of adults with bacteriuria reveal VUR as the underlying pathology. The complications of untreated, high grade VUR are serious for renal growth in children and can lead to renal insufficiency, end-stage renal failure and hypertension. Standard clinical evaluation of patients with VUR includes upper urinary tract radiography using intravenous urography (IVU), assessment of renal function by dimercapsosuccinic acid renal scan (DMSA) and evaluation of ureterovesical junction competence by videocystourethrography (VCUG). Only a VUR of a grade higher than 3 includes per definition a degree of hydroureteronephrosis and is detectable by body surface ultrasound according to the international classification of vesicoureteral reflux. Our study establishes that ELUS can be used to determine a) the length of the transmural ureter segment (Fig. 3) and b) the angle of passage of the ureter through the detrusor (table 3). Also the anatomical relationships between the ureter and its detrusor bed can be studied. These facts are of importance in the study of the passive valvular mechanism of the UVJ. The importance of the angular transmural passage of the ureter and the presence of a good detrusor backing for the prevention of VUR are well known. ELUS provides more accurate method of investigating these two properties than abdominal ultrasound. Instrumentation does affect the diagonal angle. Currently available catheters have, however, to be
modified and miniaturised further before they can be used as a routine in office urological practice for these static studies as well as for dynamic motility studies of ureter dynamics and urinary bolus transport. ELUS holds thus the promise also of becoming a new clinical tool to study local UVJ pathology.

Conventional ultrasonography is today a well-established imaging modality. Endoluminal ultrasonography is, however, relatively new. In urology transrectal endoluminal ultrasonography imaging of the prostate is a well established procedure and an important diagnostic tool. The endoluminal route could provide an important new avenue for investigation of the upper and lower urinary tracts provided suitable instruments are tested and developed by urologists in collaboration with industry. Currently, conventional ultrasonography in infancy and early childhood permits the clinician to visualise the bladder lying intrabdominaly and only indirectly to assess the effect of incompetence of the UVJ mechanism through the presence of distal ureteric dilatation. However, this form of imaging is useless as soon as the bladder descends into the pelvis during normal growth of a child. ELUS as an emerging technology needs good evaluation in the urological field to be able to assess its true potential. There have been incidental reports of its use in the uro-oncology literature as a diagnostic modality \[15-20\], but a systematic evaluation study of its potential for specific target regions has yet not been reported.

**Current limitations of ELUS and resulting artifacts.** The short penetration depth of commercially available probes is responsible for the inability of ELUS to study structures positioned more than 1 cm from the US probe. The (lateral) resolution also decreases with the distance from a probe. Both these factors affect negatively the quality of imaging of peripheral structures. In the case of VUR with ureterohydronephrosis or "megaureter" the diameter of the ureter could exceed the penetration depth of the probe and thus difficulties in study of the ureteric wall could ensue. In such cases switching down to lower frequencies (20, 15 or 10 MHz) would ensure sufficient imaging penetration. This may, however, negatively affect the resolution of the entire image. Furthermore, catheters with lower frequencies are at present thicker and less flexible, which in turn produces difficulties for endoscopic manipulation and introduction. This problem needs the urgent attention of bio-engineers.

Ring-down artefacts are imaged by the software because of the re-reflection of the catheter's body echo as concentric rings around the US
These artefacts interfere with good imaging of structures like mucosa and lamina properia.

The ureter at the UVJ and in the pelvis is not a straight tube. The ELUS catheter should thus be able to pass up curvatures in the ureter freely and still provide accurate imaging. Marked bending of an ELUS catheter can at present interfere with the rotatory mechanism of the probe causing non-uniform rotation of the core of the catheter and thus producing “pie-slice artefacts”. This artefact arises because the data obtained from a small segment of the imaging circle is spread out over a much larger segment. The manufacture of more flexible probes e.g. containing a double central mechanism rotating against each other to eliminate this non-uniformity of rotation is a promising perspective in this respect.

The software of the ELUS equipment is adjusted to image a cross-sectional plane of tissue perpendicular to the longitudinal axis of the probe and, hence, of the ureter. If this co-axiality is disturbed due to curvature or an acute bend of the ureter or due to a larger diameter of the ureter, the imaging quality and especially the measurements based on such images will be distorted and inaccurate. Also rotation of the ELUS catheter in relation to the tissue under study as a possible solution could result in rotation of the ultrasound image on the monitor and loss of orientation for the investigator especially when the penetrating depths are limited so that no peripheral anatomical structure can be used as an orientation point.

**ELUS in urology.** Currently available commercial ELUS probes are neither patient nor user friendly. Certain important adaptations of the currently available commercial ELUS probes are urgently required to suit urological needs. Mandatory adaptations should be: 1) calibration markings on the length of the catheter similar to those on a standard ureteric catheter. 2) a multi-frequency probe tip to be able to examine tissue at various depths without having to change catheters repeatedly. 3) a more flexible catheter which can negotiate curvatures and bends atraumatically. 4) adaptation of the catheter to minimise pie shaped artefacts. 5) A shorter catheter length (±75 cm) to minimise catheter kinking and facilitate endoscopic manipulation and handling. 6) catheters should also be modified for easy endoscopic retrograde and antegrade manipulation.

ELUS being a real time ultrasound modality could prove useful in motility studies in the upper urinary tract. In an “in vivo” study of ureter motility in the pig model, we have found the images obtained to be promising. Ultrasound has a proven track record of safety and does not have
the drawbacks of either X-ray investigation or of radioisotopes. This is an additional major advantage. Advances in miniaturisation in the foreseeable future should make it possible to have catheters suitable for the paediatric age group and office practice too. The availability of a urologically oriented commercial catheter would further reduce instrumentation trauma and tissue damage, as well as permitting a systematic evaluation of this technology in improving diagnosis and investigation of space occupying lesions within the lumen e.g. calculi or wall tumours of the upper urinary tract. Multi-frequency analysis could also play an important role in assessing and measuring periureteral pathology. Just as trans-oesophageal ultrasonography is used in cardiology to assess cardiac function, multi-frequency ELUS could be useful in the evaluation of periureteral or perivascular lymph-node and retroperitoneal pathology, especially in the pelvic region.

CONCLUSION
ELUS is a technique, which provides the clinician with a new diagnostic modality, which could in future prove useful in the diagnosis and evaluation of upper tract pathophysiology. Our “in vitro” study of the ureterovesical junction proves that histology correlates well with US imaging of the normal anatomical structure. The different muscular layers of the detrusor are not separately imaged. Using this technique one can accurately measure the length of the transmural ureter and the angle of passage through the bladder wall. The backing provided by the detrusor muscle can be visualised well, especially in the transdetrusor segment of the ureter. Changes of ureteric volume of the transmural ureter can be studied. Using this technology, any weakness or defect in the detrusor layer would be identifiable and future therapy could thus be better-planned or tailored to existing pathology in an individual patient. In our opinion, ELUS is a valuable new diagnostic tool whose full potential needs to be evaluated further. The manufacture of a modified ELUS catheter incorporating the mandatory adaptations and suggestions offered by us is urgently required.
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