Dynamics and modulation of ureteric peristalsis
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CHAPTER 2

FUNCTIONAL ANATOMY OF THE HUMAN URETEROVESICAL JUNCTION

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ABSTRACT

Background: The valve function of the ureterovesical-junction (UVJ) is responsible for protection of the low-pressure upper urinary tract from the refluxing of urine from the bladder. Controversy about the microanatomy of the human ureterovesical-junction persists.

Material and method: Ten (3 male and 7 female) fresh cadaveric bladders (mean age 70 years old) were studied. The bladders were fixed within 24 hours post-mortem, frozen and serially sectioned. Acetyl- and butyryl- (non-specific) cholinesterase activity were visualised as described by Karnovsky and Roots. The three dimensional distribution of the different muscle groups participating in the formation of the UVJ was reconstructed.

Results: Three different muscle groups were identified: 1. The detrusor muscle and the deep trigone were mainly acetylcholinesterase-positive. 2. The inner and outer layer of the ureteric muscle were butyrylcholinesterase-positive. They merge into a single longitudinal layer at the level of the UVJ and form the superficial trigone distally to the ureteric orifices. 3. The muscularis mucosae is a discontinuous butyrylcholinesterase-positive layer in the bladder which is absent from the trigone. No evidence of any muscular connection was found between ureter and bladder musculature.

Conclusions: The anatomy of the UVJ as observed by us, suggests the following model of the ureteric peristalsis. The urine bolus arrives in the ureteric lumen at the UVJ level. The ureter can only shorten its length, slides freely in its tunnel and discharges the urine bolus in the bladder cavity. Ureteric constriction due to the peristalsis and thickening of the contracted portion of the ureter prevents the upstream leakage. Distal spreading of the ureteric “peristalsis” in the superficial trigone increases the submucosal ureteric length and prevents reflux.
INTRODUCTION

The valve of the ureterovesical-junction (UVJ) is responsible for protection of the low-pressure upper urinary tract and the kidneys from the refluxing of urine from the bladder. Vesicoureteral reflux (VUR), in combination with upper urinary tract infection, leads to renal scarring and/or hydroureronephrosis in time, and subsequently to renal failure.

The two main anti-reflux mechanisms that are mentioned in the literature are the passive and the active valve function. The diagonal course and length of the submucosal portion of ureter at the UVJ are the determining factors in passive valve function. These are geometrical properties and will not be discussed further in this article. An active valve function at the UVJ level is reported by several observers. Gil-Vernet (1973) described a prevesical circular sphincter of ureteric origin, which is formed from the most distal circular ureteric muscle fibres. Tanagho and co-workers (1963) presented evidence for a functional contribution of the trigone as an anti-reflux mechanism. According to them, the trigone consists of three different layers. The superficial trigonal layer is the continuation of the longitudinal ureteric muscle fibres. "Waldeyer's sheath" continues distally and forms the middle trigonal layer and the deep trigone is formed by the bladder wall itself. The contraction of the trigonal musculature would occlude the UVJ and prevent the reflux. Blok and co-workers (1985) performed a sophisticated urodynamic study at the UVJ level, which also lends credence to an active occluding function. They distinguished three different segments in the ureterovesical pressure profiles (UVPP). The base line of UVPPs represents the resistance to flow through the UVJ, while the fast and slow waves visualise the ureteric and detrusor activity, respectively.

Our work on topographic anatomy of the UVJ, as studied by functionally oriented staining techniques, reveals its potential as the anatomic substrate for an active anti-reflux mechanism (Thomson et al., 1994). However, controversy about the microanatomy of the human ureterovesical-junction that would support such an active valve function persists. Krause (1876) first reported the extension of the bladder musculature around the distal ureter. Waldeyer (1892) defined this extravesically located structure as the *Ureterscheide* and described an “injectable space” between this sheath and the distal ureter. Many of the early discussions were dominated by (an interpretation of) Waldeyer's *Ureterscheide*. In the past century, a dozen subtly different anatomic interpretations of the muscular
architecture at the level of UVJ in humans have been reported (Elbadawi, 1972). To obtain an insight into the macro-anatomy, Noordzij and Dabhoiwala (1993) performed macroscopic dissections on 8 adult and 2 fetal human bladders and their findings confirmed that the longitudinal muscle of the terminal ureter fans out to form the thin “inner trigonal muscle” or what has been described in the literature as the superficial trigone. The main differences in the descriptions lie in the origins of the contributing structures to the UVJ. Most of these studies have been performed using the dissection microscope or standard staining procedures. Both approaches leave a lot of room for interpretations, because they are unable to reveal the exact identity (e.g. ureteric or vesical origin) of representative structures. The cholinesterase-staining method, on the other hand, has proved to be a useful technique to distinguish between the different components of the musculature of ureter and bladder (Gosling et al, 1984; Gearhart et al, 1993; Thomson et al, 1994). We therefore decided to study the microscopic anatomy of the adult human UVJ with this technique.

MATERIAL AND METHODS

**Human cadaveric true-pelvis blocs.** Ten (3 male and 7 female) fresh cadaveric “true-pelvis” blocs (containing the bladder, distal ureters, proximal vagina in females, or rectum and prostates in males) were obtained for study. None of the specimens in the studied cadaver material had a previous history of vesicoureteric reflux. The median age of the group was 70 years (range of 28-92). From the ten, only one was from a 28 year old male, while the other nine were from patients above the age of 57. A second inclusion criterion was a post-mortem autopsy interval of less than 24 hours because of a substantial decrease in cholinesterase-enzyme activity after that period. Every bladder was opened mid-sagittally anteriorly, and inspected for pathology of the mucosa, ureteric orifices and for the degree of trabeculation. After careful dissection of the bladder from the surrounding tissues, the specimens were pinned on a plastic slab in their normal anatomical position.

**Enzyme histochemistry.** Four percent formaldehyde/ 0.22 M sucrose/ 0.1 M sodium phosphate (pH 7.3) was used as fixation fluid. The fixation was for 16 hours at 4 °C. The fixed bladder wall was rinsed twice in bidistilled water. The bladder was cut across the following lines to get a four-angled piece of posterior bladder wall that was small enough to permit the subsequent sectioning with the cryostat. Superiorly the specimen was cut approximately 1 cm above the level of juxtavesical ureter and inferiorly through the bladder
The medial margin of the tissue bloc was at the parasagittal plane through the contralateral ureter orifice, and the lateral margin was some millimetres lateral to the ipsilateral ureter (Fig. 1). The resulting posterior bladder wall was frozen in isopentane, precooled in liquid nitrogen and stored at -70 °C until further use. Serial cryostat sections (40μm) were prepared with a motor-driven cryostat (Adamas, Leersum, The Netherlands).

Fig. 1: Section lines (solid) in posterior bladder wall to prepare tissue bloc. PSP: Para-sagittal plane, MSP: Mid-sagittal plane, UO: Ureteric orifice, LSL: Lateral section line, SE: Serosal exit of the ureter

Cryostat sections were processed to demonstrate tissue acetylcholinesterase (AChE) or butyrylcholinesterase (BChE, non-specific cholinesterase) using the ‘direct-colouring thiocholine method’ (Karnovsky and Roots, 1964; Thomson et al., 1994). The optimum pH for staining human bladder smooth muscle was found to be 5.6 after testing a range from 4.5 to 6.5. The incubation medium was made in the following sequence while continuously stirring: 25 mg acetylthiocholine (or 35 mg butyrylthiocholine) was dissolved in 25 ml of a solution containing 120 mM sodium acetate, 40 mM sodium citrate and 6 mM copper sulphate. To this solution was added 5 ml 5 mM potassium ferrocyanide and, if AChE was studied, 2.5 ml 0.01 mM tetra-isopropylpyrophosphamid (iso-OMPA), a non-specific cholinesterase inhibitor. The sections were incubated at room temperature. The optimal incubation times were found to be 16 h for AChE and 8 h for BChE. The stained sections were mounted in Entellan (Merck, Darmstadt, Germany) after rinsing twice in bidistilled water.
**Connective tissue analysis.** Connective tissue was stained using picrosirius red, eosine van Gieson and Periodic acid-Schiff staining techniques to visualise collagen, elastin and glycoproteins, respectively.

**3D-reconstruction technique.** The contours of different structures recognised at the UVJ were drawn on an A4 format paper after proper magnification. From a series of consecutive sections, all relevant contours in the spatial configurations were stored in an input database. Two reference points per section were included for proper realignment of different sections in order to generate a 3D-reconstructed model. The 3D-reconstruction software was developed in our laboratory (Verbeeck et al., 1995). The input software ran 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 (Verbeeck et al., 1993). Volume rendering was used to produce a visualisation of the volume model (Fig. 7).

**RESULTS**

The differential staining intensity for AChE and BChE of the smooth muscle cells of bladder and ureter allowed us to distinguish structures of ureteric origin from the surrounding detrusor muscle. Despite the strict inclusion criteria, sections stained to demonstrate both cholinesterase isoenzymes showed a colour density variation, which was related to the post-mortem autopsy interval. This was especially evident for BChE. To deal with this problem, only ten “well-staining specimens” were included in this study. On the basis of the expression of both isoenzymes, we have identified three different muscular structures (Table 1).

<table>
<thead>
<tr>
<th>Structures</th>
<th>AChE</th>
<th>BChE</th>
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<tbody>
<tr>
<td>Detrusor musculature including deep trigone</td>
<td>++</td>
<td>+ / ++</td>
</tr>
<tr>
<td>Ureteral smooth muscle fibres and superficial trigone</td>
<td>- / +</td>
<td>+++</td>
</tr>
<tr>
<td>Muscularis mucosae</td>
<td>+</td>
<td>++</td>
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</table>

**Table 1:** Cholinesterase isoenzyme expression in structures forming the human ureterovesical junction. +++: strongly positive, ++: intermediately positive, +: weakly positive, -: negative.

The detrusor muscle is mainly AChE-positive. The deep trigone, which is that part of the detrusor that covers the trigone dorsally which is characterised by a circular orientation of the muscle fibres, accompanies the ureter a few millimeters upwards and forms an incomplete ventromedial
sheath around the juxta- and part of the transmural ureter. This periureteral sheath is present mainly around the ventral part of the ureter (Fig. 2, 5). The deep trigone gets bulkier and more vertically oriented towards the bladder neck. In 3D reconstruction, the deep trigone makes the impression to be diamond shaped with only the caudal part being covered by the superficial trigone. At other locations of the detrusor muscle, bundles appear to be randomly oriented.

Fig. 2: Expression of acetylcholinesterase is observed in the circularly oriented detrusor bundles (deep trigone: DT) at juxtavesical ureter level. BL: bladder lumen, U: ureter. The ureter is acetylcholinesterase negative. (magnification: X7, bar: 1mm, specimen was from 72 years old female)

In contrast to the detrusor muscle, the ureteric smooth muscle fibres are mainly BChE-positive (Fig. 3). The extramural ureter consists of a circular outer and a longitudinal inner layer (Fig. 3). In the bladder wall the ureteric fibres all continue as longitudinally oriented “cables” which continue distally to form the superficial trigone (Fig. 4). The ureter lumen decentralises in its submucosal course and opens at the ureteric orifice.
Fig. 3: Extramural ureter, stained to demonstrate acetylcholinesterase (panel A) and butyrylcholinesterase (panel B). At this level, the ureter consists of a circular outer and a longitudinal inner layer. The difference in enzyme activity pattern is evident. (magnification: X7, bar: 1mm, specimen was from 72 years old female)

Finally, the muscularis mucosae can be visualised as islands of BChE activity directly under the bladder mucosa. These islands are absent in the trigone.

The intramural ureter is surrounded by a periureteral connective tissue cylinder which is directly continuous with the adventitia ureterae externally and the lamina propria vesicae internally (Fig. 4).

Fig. 4: Longitudinal section of the entire human ureterovesical junction, stained to visualise butyrylcholinesterase. U: ureter, BN: superficial trigone muscle fibres are running to the bladder neck level. D: detrusor muscle bundles, ST: superficial trigone, BL: bladder lumen. (magnification: X3.5, bar: 1mm, specimen was from 60 years old female)
Analysis of connective tissue reveals the presence of collagen, elastin, and PAS-positive material, which allows the ureter its proper mobility characteristics in this trajectory.

**Fig. 5:** Transverse sections through the human ureterovesical junction. Panel A: section at juxtavesical level. The deep trigone (DT) and ventromedially oriented periureteral (less butyrylcholinesterase-positive: arrows) muscle fibres are observed. Panel B: transverse section at intramural level. The deep trigone (DT) and the periureteral muscle layer portion of the detrusor are located ventrally (arrows) to the ureter. There is no muscular connection between ureter and the detrusor. BL: bladder lumen. (magnification: X8, bar: 1mm, specimen was from 72 years old female)

This periureteral connective tissue cylinder can thus directly interact with the connective tissue matrix of the detrusor muscle. No muscular connections can
be observed between either the ureter and detrusor muscle or between their representative derivatives, the superficial and deep trigone (Fig. 4). However, even though the periureteric loose connective tissue layer at the juxtavesical and transdetrusor level allows the creation of an injectable space, we want to stress that there is no pre-existing anatomical space (Fig. 5). A specific study to quantify the amount of connective tissue present was not undertaken by us.

**DISCUSSION**

The interplay between the contractile and connective tissue components determines the behaviour of most physiological processes. The UVJ is a chimerical structure, which arises from the merging of the ureter and the bladder muscle. The structure of UVJ has to fulfil certain functional requirements. Firstly, it forms the boundary between the low-pressure upper urinary tract and the lower urinary tract with large alterations in urinary pressure. Secondly, it must allow the urine bolus to pass antegrade while still preventing vesicoureteral reflux (VUR) to the upper urinary tract. According to both our study and a similar study by Gearhart and co-workers (1993), the ureter has no muscular connections with the detrusor and is surrounded by a periureteral connective tissue sheath along its entire intramural trajectory. This muscular (contractile) independence allows the ureter to slide relatively freely in its transvesical channel.

*The ureteric architecture and the anti-reflux mechanism.* The exclusively longitudinal orientation of the ureteric muscle fibres in the intramural trajectory suggests that this part of the ureter does not contribute much to the peristaltic wave, but it seems to be able to decrease its length by a progressive telescopic mechanism. Most urologists have observed this ureteric activity against a quiet bladder (detrusor) background during cystoscopy. A VUR-preventing factor may be the ureteric peristalsis (constriction) itself (Tsuchida et al 1963, 1967). Due to the shortening of the intramural ureteric segment, the bladder lumen is actually displaced toward the urine bolus awaiting in the UVJ to be discharged. A favourable side effect of the intramural ureter shortening is that the contracted portion of the intramural ureter becomes thicker and can (partially) obstruct the ureter lumen upstream of the urine bolus, thus blocking retrograde leakage. After the passage of the peristaltic wave (spreading into the superficial trigone), the shortened intramural and submucosal ureter segment are pulled back by the trigonal extension of the ureteric muscle fibres to their resting position and possibly even farther towards the bladder neck, thereby
temporarily increasing the length of the submucosal portion of the ureter. By such a mechanism, it should be possible to transport a urine bolus even against a higher bladder pressure. This mechanism is similar to the hydrodynamic principles used in the construction of water locks which are used to protect low-lying areas from flooding and simultaneously permit transportation of boats.

Gearhart and co-workers (1993) published the result of an enzyme-histochemical study of the UVJ in children using the AChE- and BChE-staining technique. Our findings concur with theirs with respect to the cholinesterase expression pattern of the ureteric and detrusor muscle bundles. Gearhart and co-workers (1993) describe, in addition to the longitudinal muscle layer of the ureter, an “intermediate component” which surrounds the longitudinal layer at the UVJ and which continues towards the bladder neck to merge with the superficial trigone. The muscle bundles described by these authors as intermediate component were also seen by us (see Figs. 4 and 5), but considered to belong to the detrusor and therefore not representing a separate entity. Especially Fig. 4 strongly supports our viewpoint. We assume that the intermediate component of Gearhart and co-workers (1993) is identical to what we describe as the incomplete ventromedial sheath of detrusor origin, which arises from the deep trigone (Fig 7).

**Bladder contribution to the anti-reflux mechanism.** Tanagho and co-workers (1963-1965) emphasised the contribution of the deep and superficial trigone in the UVJ closure mechanism in their description of the functional implications of the topographic anatomy of the UVJ. Based on electro-stimulation of the trigone and on the ureteric perfusion resistance registration in the dog they concluded that the stimulation and the contraction of the deep and superficial trigone occludes the ureteric orifice and acts as an anti-reflux mechanism. Our results also suggest that the ureter does play an active role in this valve construction. We, however, do not find that the anatomical substrate of the deep trigone is such that it can play an important role in the UVJ closure mechanism. Gearhart and co-workers (1993) concur with this opinion. The deep trigone portion of the detrusor which is of vesical origin (it shows the same cholinesterase isoenzyme activity as the detrusor) has no muscular connection to the ureter. De relatively tiny connection of the deep trigone to the incomplete ventromedial sheath (Fig 7) makes it implausible for it to play an important active role in the ureteric closure mechanism or to be directly co-ordinated
with functional ureteric activity. We therefore do not recognise these fibres as a “middle trigonal layer” (Tanagho et al, 1963). This interpretation is supported by the observation that the anti-reflux mechanism in pigs functions well in spite of the fact that they have no “Waldeyer’s sheath” and that they lack the deep trigone (Thomson et al., 1994). In our opinion, the ureteric functional autonomy, its internal fibre organisation and the asymmetry of its lumen position in its submucosal trajectory are important factors in controlling its own anti-reflux valve function. The firm muscular tunnel through the detrusor is the important functional bed, which allows the ureter to perform its anti-reflux function. The hypertrophy of the ureteric smooth muscle fibres that is seen in conjunction with an infravesical obstruction is perhaps a compensatory factor to prevent VUR, also lending further support to this active mechanism (Fig. 6).

![Image](image_url)

**Fig. 6**: Transverse section through the human ureterovesical junction at its submucosal level in a trabeculated bladder. The section is stained for butyrylcholinesterase. Note the hypertrophied thick ureteral muscle bundles within the hypertrophied detrusor. D: detrusor, U: ureter. (magnification: X7, bar: 1mm, specimen was from 75 years old male)

The species difference between the pig and the human UVJ. The 3D models generated from the cholinesterase-stained specimens, reveals the topographic relationships between the different structures (Fig. 7).
Fig. 7: Three-dimensional reconstruction of human ureterovesical junction based on cholinesterase isoenzyme activities. Panel A is a full colour reconstruction. In panel B, the lateral part of the reconstructed block is cut and the detrusor muscle is made more transparent to show the topographic relationship between the ureter and the detrusor. The asterisk (*) indicate incomplete muscle sheath which is continuous with the deep trigone. Note that this periureteral layer is present mainly around the ventromedial part of the ureter. Ureter and superficial trigone (ST): green, detrusor (Det): red, deep trigone (DT): blue. The bladder lumen is uppermost in the photograph.

In the pig, the longitudinal inner layer of the ureteric muscle is clearly AChE-positive, whereas the circular outer layer is BChE-positive (Thomson et al, 1994). In man, the entire ureteric muscle is BChE-positive (Fig. 3).

<table>
<thead>
<tr>
<th>Localisation of the ureteric orifice</th>
<th>Pig</th>
<th>Human</th>
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<tbody>
<tr>
<td></td>
<td>Bladder neck</td>
<td>Posterior aspect of bladder wall</td>
</tr>
<tr>
<td>Shape of Trigone at urethrocystoscopy</td>
<td>Difficult to recognise</td>
<td>Triangular</td>
</tr>
<tr>
<td>Periureteral muscle sheath</td>
<td>Absent</td>
<td>Present</td>
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Table 2: Species differences between the pig and human ureterovesical junction.

The ureter orifices are macroscopically located at approximately the bladder neck in the pig. Upon inspection during urethrocystoscopy, we observed that the porcine trigone is less developed compared to that in the human and is located very close to the posterior aspect of the bladder neck. According to our enzyme histochemical study, the pig also lacks a
periureteral muscle sheath (Tab. 2). Investigations are in progress to study the urodynamics and the mechanism of the anti-reflux function of the UVJ in the pig in vivo.

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