Detrusor ultrastructural studies in human lower urinary tract dysfunction: correlation of structural features and function.

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Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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Dr Amanda Shu Jun Chung

31st December 2022

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Professor Lewis Chan, 22nd Jan 2023

Associate Professor Vincent Tse, 26th Jan 2023

ABSTRACT

Introduction:

Lower urinary tract symptoms (LUTS), voiding dysfunction (VD) and urinary retention are common in the aging population, associated with significant morbidity.(1) LUTS can be due to overactive bladder (OAB), underactive bladder (UAB), bladder outflow obstruction (BOO), or a combination. Diagnosis is key to management, whilst avoiding harm of inappropriate therapy. Due to challenges in evaluation, there may be a role for detrusor ultrastructural analyses in diagnosis and prognosis of VD.(2,3,4)

Objectives:

To investigate detrusor ultrastructural changes in VD in the older population and potential clinical applications. Specifically:

1. females with bladder outflow obstruction (fBOO).

2. males with detrusor underactivity (mDU), with long term functional-structural correlation.

3. older males with coexistent overactive underactive bladders (mCOUB).

Methodology and patient cohort:

Patients were recruited from the Urodynamic Clinic at Concord Hospital. Patients with fBOO, mDU and mCOUB on urodynamic study who were undergoing cystoscopy as part of their clinical management were recruited. Detrusor biopsies were obtained and examined by electron microscopy. Ultrastructural analyses were performed using a standardised protocol and correlated with functional outcomes.

Results

Detrusor ultrastructural features of ‘myohypertrophy’ were similar in fBOO and male BOO; severity correlated with BOO duration and severity. In mDU, severe features of myohyertrophy and degeneration predicted poor long term voiding outcomes. Older mCOUB had concomitant features of myohypertrophy and dysjunctional patterns. Our standardised protocol allowed analyses of all 3 VD.

Conclusions

Detrusor ultrastructural features in patients with fBOO, mDU and mCOUB were described with correlations found between detrusor ultrastructure and lower urinary tract function. Detrusor ultrastructure studies not only improve understanding of VD, but may also assist in diagnosis, prognosis, and management.

References:

1. Taylor JA, 3rd, Kuchel GA. Detrusor underactivity: Clinical features and pathogenesis of an underdiagnosed geriatric condition. J Am Geriatr Soc. 2006;54(12):1920-32.
2. VW. N. Pressure flow urodynamic studies: the gold standard for diagnosing bladder outlet obstruction. . Rev Urol 2005 ;7 Suppl 6(Suppl 6):S14-21. 2005.
3. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
4. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.

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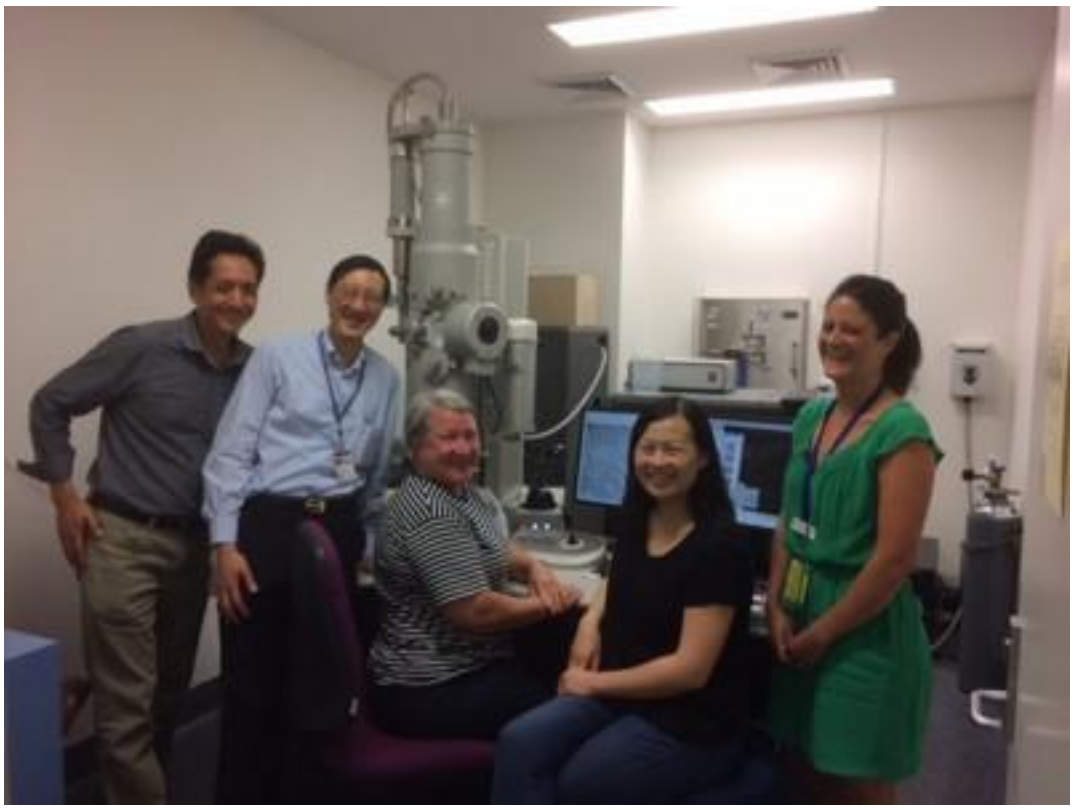
Figure 1.1: Photograph of Electron Microscrope with A/Prof Charles Chan (EM Pathologist), Lewis Chan, Susan Brammah, Amanda Chung, Stephanie Sampedro (left to right).



ABBREVIATIONS

AC	Amanda Chung
Agg Global	aggregate global impression score
AGN	Abram-Griffiths Number
AUA	American Urological Association
BCI	Bladder Contractility Index
BOO	Bladder Outlet Obstruction
BOOI	Bladder Outlet Obstruction Index
BOOIf	Bladder Outlet Obstruction Index for females
BPE	Benign Prostatic Enlargement
BPO	Benign Prostatic Obstruction
Cell	cell size and irregularity
cm	centimetre
CMG	CystoMetroGram
Coll	increase in intercellular collagen
COUB	Co-existing Overactive-Underactive Bladder
Degen	Degeneration category global impression score
DO	Detrusor Overactivity
DODU	Detrusor Overactivity Detrusor Underactivity
DU	Detrusor Underactivity
Elas	hyperelastosis
EM	Electron Microscopy
EMG	ElectroMyoGraphy
F	Female
fBOO	Female Bladder Outlet Obstruction
Fr	French
Frag	fragmentation and exclusion
H2O	Water
HC	HypoContractile
HoLEP	holmium laser enucleation of the prostate
IBS	Increased Bladder Sensation
ICS	International Continence Society
IPSS	International Prostate Symptom Score
IQR	InterQuartile Range
LC	Lewis Chan
LUT	Lower Urinary Tract
LUTD	Lower Urinary Tract Dysfunction
LUTS	Lower Urinary Tract Symptoms
M	Male
mBOO	Male Bladder Outlet Obstruction
min	minute
mL	millilitre
mL/s	millilitres per second
MSU MCS	MidStream Urine Microscopy Culture and Sensitivities
Myoh Global	myohypertrophy category global impression score
N	No
nm	nanometre
OAB	OverActive Bladder
Pabdo	Abdominal pressure
PC	Poor Compliance
PCB	Poorly Compliant Bladder
Pdet	Detrusor pressure
PFS	Pressure-Flow Study
PGII	patient global impression of improvement score
PhD	Doctor of Philosophy
PTNS	Percutaneous Tibial Nerve Stimulation
Pves	Intravesical pressure

PVR	PostVoid Residual
Qmax	Peak flow
Sep	increased separation of cells
SGN	Solomon-Greenwell Nomogram
SOAB	Sensory OverActive Bladder
SNM	Sacral NeuroModulation
SU	Sensory Urgency
SUI	Stress Urinary Incontience
TURP	TransUrethral Resection of Prostate
UAB	Underactive Bladder
UDS	UroDynamic Study
um	micrometre
UPG	Urethral Pressure Gradient
UTI	Urinary Tract Infection
UUU	Urodynamic Urinary Urgency
Vac	vacuolation and dense bodies
Var	electron density variation
VE	Voiding Efficiency
VT	Vincent Tse
VV	Voided Volume
Y	Yes

CHAPTER 1: INTRODUCTION

Epidemiology of lower urinary tract symptoms

Lower urinary tract symptoms (LUTS) such as frequency, urgency, nocturia, urgency incontinence and poor urinary flow, affect up to 1 in 4 persons in our population and can cause significant morbidity in older individuals, affecting their health and independence.(1) Owing to the aging population both globally and within Australia, the prevalence of bothersome LUTS is anticipated to increase, with increased morbidity and pressure on the health care system to manage lower urinary tract dysfunction.(2)

LUTS is a collective term for lower urinary tract symptoms. These may be broadly classified as 'storage' symptoms (urinary frequency, nocturia and urgency) and 'voiding' symptoms (weak stream, intermittency, straining to void, and sensation of incomplete emptying). LUTS can be caused by a variety of lower urinary tract dysfunctions including bladder outlet obstruction (BOO, in males often due to prostatomegaly), overactive bladder, underactive bladder, or a combination of these conditions. Neurological conditions such as stroke, extrapyramidal disorders and multiple sclerosis also commonly result in LUT dysfunction. Apart from their impact on patients' quality of life, LUT dysfunction can result in complications such as increased risk of falls, urinary tract infections, and urinary retention. LUTS are listed in the International Prostate Symptom Score (IPSS) system, with an additional question reflecting the IPSS-Quality of Life score (See Appendix 1, baus.org.uk). The IPSS has been validated for use in males with bladder outlet obstruction due to prostatomegaly, however the symptoms listed in the IPSS often overlap with other voiding dysfunctions such as urethral stricture disease, overactive or underactive bladder.

Urinary retention (the inability to pass urine) is a bothersome condition as it can cause significant distress and require medical intervention for treatment and management. These interventions consist of medication, surgery, and retention appliance such as catheterisation (self-catheterisation, indwelling urethral catheterisation, suprapubic catheter). Sequelae of urinary retention includes recurrent urinary tract infections, overflow incontinence, bladder stones, renal impairment due to obstructive nephropathy, and bothersome symptoms such as urinary frequency, urgency or retention pain. Complications of chronic straining to void - such as herniae, pelvic organ prolapse and haemorrhoids – are also included. The two main causes of urinary retention are either bladder outflow obstruction or underactive bladder, and both may be present. Bladder outflow obstruction is most commonly due to prostatomegaly – about 50% of men aged 50 years and 80% of men aged 80 years have prostatomegaly. Bladder outflow obstruction can also be caused by urethral stricture disease. Underactive bladder (UAB) (see Chapters 5 and 6) is a poorly understood condition, and is often misdiagnosed as bladder outflow obstruction or overactive bladder as both voiding and storage symptoms can be present in patients with UAB. The prevalence of detrusor underactivity (DU) is about 9-23% in men <50 years of age and increases to about 48% in men >70 years of age. The prevalence of DU in older women is 12-45%.(3)

Clinical assessment of lower urinary tract symptoms

In the assessment and management of lower urinary tract dysfunction and urinary incontinence, the concept of the bladder as "an unreliable witness" – that is, that LUTS do not always correlate with underlying pathology – is well accepted.(4) Patients with storage and voiding dysfunction often describe such overlapping symptomatology that it is difficult and sometimes even misleading to try to make a clinical diagnosis based on symptomatology alone.(5)

Assessment of voiding dysfunction comprises of history and examination as well as basic initial investigations. When taking history, a clinician enquires about details of LUTS and other symptomatology regarding nearby organs, including gynaecological areas (such as bulge symptoms

of pelvic organ prolapse) and bowel function (such as constipation). They also ask questions about potential causative mechanisms, such as for a potential neurological source. Other urological signs and symptoms are elicited, such as haematuria, recurrent urinary tract infection (UTI), and pain. Other aspects of medical history, such as cancer, pelvic radiotherapy, surgeries, and travel history, are also important.

Physical examination involves the abdomen and genitalia, looking at the urethral meatus, any obvious anatomical issues such as vaginal prolapse, digital rectal examination for males, and pelvic examination for females.

Basic investigation of lower urinary tract symptoms

Initial investigations include midstream urine microscopy, culture and sensitivities (MSU MCS) for UTI, electrolytes urea creatinine (EUC) for renal function, urinary flow study with postvoid residual bladder scan - which can rule out the possibility of urinary retention and screen for voiding dysfunction (low amplitude flow pattern, intermittent flow pattern) - and ultrasound of the urinary tract to screen for upper urinary tract abnormality (such as hydronephrosis or stone disease).

Advanced investigation of lower urinary tract symptoms

When bothersome LUTS persist despite initial therapies (such as dietary modification, fluid management, pelvic floor physiotherapy and bladder retraining, timed voiding, and medication), or if there are any red flags in the initial workup (such as microhaematuria), then the patient will usually be recommended further investigations. These include cystoscopic examination and urodynamic studies (UDS), which are more advanced and involved methods of investigation.

Cystoscopic examination is endoscopy of the lower urinary tract (urethra, prostate in males, and urinary bladder). In Australia, it is generally performed under local anaesthesia or sedation with a flexible cystoscope, or under general anaesthesia with a rigid cystoscope. It allows visualisation of the lumen of the urethra, prostate, bladder neck, and interior of the bladder, including ureteric orifices and trigone.

Urodynamic study is a test of the functional aspects of the lower urinary tract, including storage and voiding function assessments. The aim of urodynamic studies is to reproduce symptoms whilst making precise measurements in order to identify the underlying causes for the symptoms, and hence quantify the related pathophysiological processes.(6) The test commonly involves placement of catheters that measure filling and bladder pressure into the bladder via the urethra, as well a rectal balloon transducer to measure abdominal pressure. During the filling phase (filling cystometrogram/CMG), the storage function of the bladder is assessed. At this stage, information on functional bladder capacity, bladder sensation, presence or absence of detrusor overactivity, and bladder compliance are obtained. Urinary incontinence assessment, including leak point pressures, can be determined. In the voiding phase of UDS, simultaneous measurement of voiding pressures and urinary flow rate allows for assessment of contractility of the detrusor and bladder outlet obstruction.

The simultaneous measurement of detrusor pressure and urinary flow parameters (that is, the pressure-flow study (PFS)) will generally identify three potential voiding states:

1) Low detrusor pressure and high flow rate (normal, no voiding dysfunction)
2) High detrusor pressure and low flow rate (bladder outlet obstruction)
3) Low detrusor pressure and low flow rate (hypocontractile bladder).

However, there are some limitations to this categorisation, and borderline cases also exist.(7)

Challenges and limitations of urodynamic study in assessment of voiding dysfunction

Urodynamic study is invasive, involving placement of vesical and rectal catheters and performed with the patient awake under local anaesthetic. As a result, some patients find the experience quite uncomfortable. A urodynamic study is also limited in its assessment of LUT function, as the test generally only evaluates a single episode of bladder filling and emptying. If patients have a 'bashful bladder' or cannot void on cue during the study, the voiding function cannot be assessed, and it may not be representative of their day-to-day lower urinary tract function or dysfunction. Studies have reported that UDS may miss approximately 30% of overactive bladder (OAB) diagnoses. One reason for this is that during the one fill test phase, the UDS may not show DO when it may exist at other times during that patient's day-to-day life, therefore making diagnosis difficult. For this reason, some have advocated for the use of ambulatory UDS to increase the sensitivity and detection rate of lower urinary tract dysfunction (LUTD).(8)

Another limitation of UDS is in the diagnosis of BOO in the setting of the failing detrusor. Diagnosis of BOO on UDS requires high pressure and low flow state. The bladder outlet obstruction index (BOOI) is Pdet at Qmax minus 2.Qmax , whereby BOOI > 40 is considered in the obstructive range. On the other hand, detrusor underactivity (DU) is defined by the International Continence Society (ICS) as "a contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or failure to achieve complete bladder emptying within a normal time span".(9) When there is impaired detrusor function, such as hypocontractility or acontractility, the urodynamic-based calculation is technically not able to define whether BOO is present. Therefore, the clinician may be left to wonder if BOO surgery and treatments are the appropriate course of action. Clinicians may still reasonably recommend BOO surgery and treatments in the setting of hypocontractile detrusor for the purpose of reducing outlet resistance in the hope that the patient's ability to spontaneously empty their bladder will improve.

Proposed role for detrusor ultrastructural analysis in assessment of voiding dysfunction

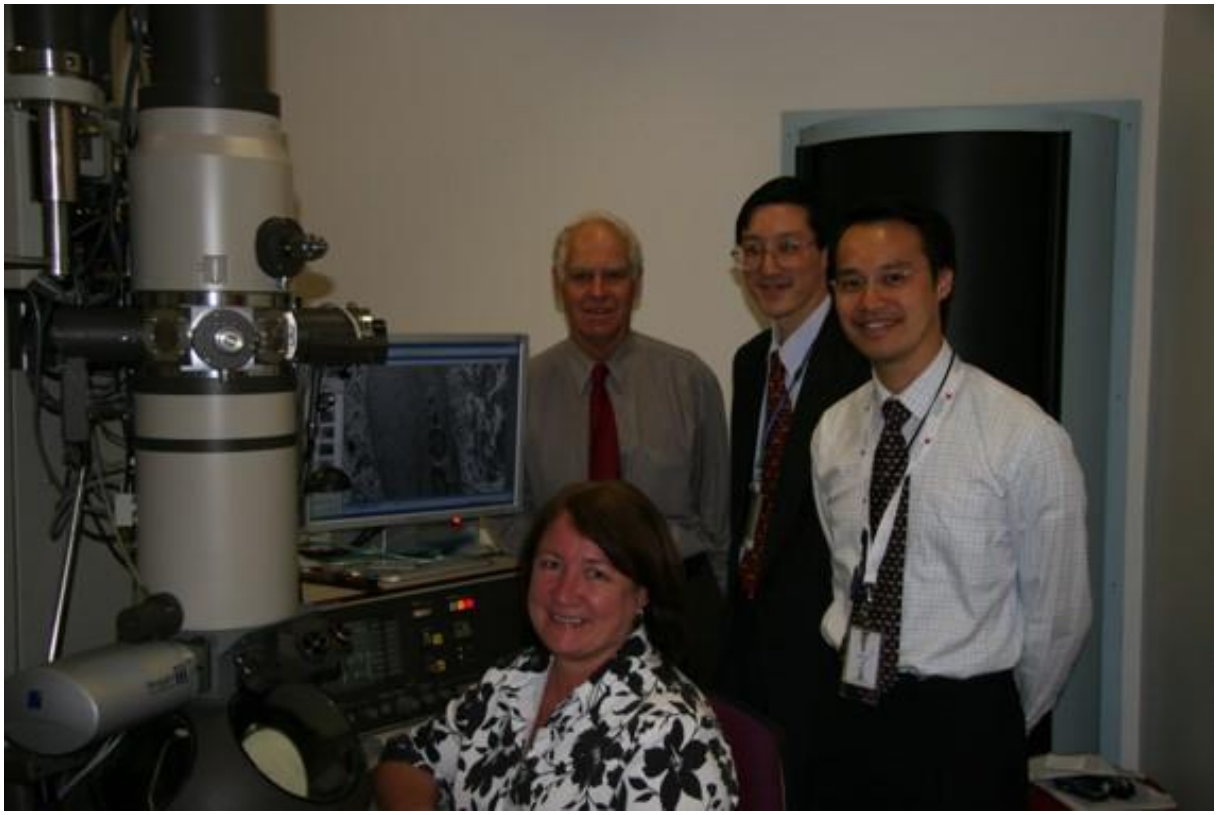
The detrusor can be divided macroscopically into the body and base, with the line of division being at the level of the two ureteric orifices.(10, 11) This division is derived from adrenergic innervation confirmed both anatomically and physiologically.(11) The detrusor body is the distensible unit responsible for storage and expulsion with muscle fibres which are an interwoven, non-layered network.(12) The detrusor base (also called the trigone) is non-distensible with muscle bundles arranged in loops and rings, to create a funnel configuration during the act of voiding.(13, 14)

In a series of seminal papers, Elbadawi et al. demonstrated specific ultrastructural changes on electron microscopy (EM) in the detrusor muscle of patients with lower urinary tract dysfunction (see Chapter 2).(12) At Concord Repatriation General Hospital, collaborative research by the Department of Urology and Department of Pathology confirmed the initial results of Elbadawi in demonstrating specific ultrastructural changes on electron microscopy in the detrusor muscle of patients with a variety of voiding dysfunctions. (15, 16) Furthermore, these previous studies in our institution demonstrated an increased number of abnormal junctions between muscle cells in patients with the overactive bladder.(15) These junctions are postulated to be "gap junctions", which allow electrical activity to spread from cell to cell in generating involuntary detrusor contractions, leading to symptoms of urgency and incontinence. Similar ultrastructural changes were demonstrated in both neurogenic and non-neurogenic overactive bladders, suggesting a possible 'final common pathway' in the development of detrusor overactivity.(17, 18) In her Master of Surgery Thesis, Blatt et al. demonstrated that specific detrusor ultrastructural features (the 'myohypertrophy' pattern consisting of increased variation of myocyte size and appearance, separation of muscle fascicles) in patients with bladder outflow obstruction and hypocontractile bladder correlated with poor voiding outcomes following prostate surgery (transurethral resection of prostate, TURP), and that preoperative detrusor ultrastructure analysis may be a useful diagnostic and prognostic tool.(19)

The Concord Repatriation General Hospital detrusor ultrastructure research group

The Concord Repatriation General Hospital detrusor ultrastructure research group is a collaboration between the Urology and Pathology departments with over 25 years' experience (commencing in 1997 with Dr. Edward Wills and Ms. Susan Brammah from the Electron Microscopy Unit, and urologists Associate Professor Vincent Tse and Professor Lewis Chan) in ultrastructural studies of the human detrusor. The Concord detrusor ultrastructure tissue bank now has over 100 specimens from patients with lower urinary tract dysfunction over a range of ages and lower urinary tract dysfunctions. Today, this unit is possibly one of the only active research groups with such experience in the world, and has already seen two previous Master of Surgery candidates successfully completing their degrees based on detrusor ultrastructure at the University of Sydney.

Figure 1.2: Photograph of Electron Microscope with Susan Brammah (front, centre), Edward Wills (back, left), Lewis Chan (back, centre), Vincent Tse (back, right).



Gaps in detrusor ultrastructural knowledge and rationale for thesis

We note that there are significant gaps in the detrusor ultrastructure knowledge and literature. The work in my PhD builds upon the work that was previously completed. There is a specific focus on the theme of voiding dysfunction in the older adult, covering females with BOO, males with hypocontractile detrusor, and older patients with overactive bladder and concurrent detrusor underactivity.

Whilst there are a number of studies describing detrusor ultrastructural features of male bladder outlet obstruction, there are no studies describing the detrusor ultrastructural features of female bladder outlet obstruction. This is an area of interest in functional urology with the use of sling procedures to treat female stress urinary incontinence and potential complication of obstructive voiding.

The reported studies on hypocontractile detrusors are small in sample size and provide limited clinical outcome information, with none providing long term clinical outcome information. With the long history of our detrusor ultrastructure research group, we are able to provide long term data correlating detrusor ultrastructural features and long term clinical outcomes. This is the largest hypocontractile bladder patient group with the longest follow-up data.

There is also increasing interest in detrusor overactivity during bladder filling in conjunction with detrusor underactivity during voiding (DODU, previously called detrusor overactivity with impaired contractility). This type of voiding dysfunction is generally found to affect older patients and presents significant treatment challenges (see Chapter 6). As there is no ultrastructure literature on this group, it would be worthwhile to examine older patients with overactive bladders to assess whether their detrusors may possess occult features of detrusor underactivity and degenerative features on ultrastructure.

The aims and objectives of this PhD project

The aims of this PhD project are as follows:

1. To investigate ultrastructural features in the detrusor muscle of females with bladder outflow obstruction. (See Chapter 4)

2. To investigate ultrastructural features in the detrusor muscle of males with underactive bladders, with functional-structural correlation in both the short and long term. (See Chapter 5)

3. To investigate ultrastructural features in the detrusor muscle of older patients with overactive bladders, examining for presence of occult features of detrusor underactivity and degenerative ultrastructural pattern. (See Chapter 6)

4. To discuss clinical applications and future directions for detrusor ultrastructure research. (See chapter 7).

References

1. Taylor JA, 3rd, Kuchel GA. Detrusor underactivity: Clinical features and pathogenesis of an underdiagnosed geriatric condition. J Am Geriatr Soc. 2006;54(12):1920-32.
2. Organisation WH. 2022.
3. Osman NI, Chapple CR, Abrams P, Dmochowski R, Haab F, Nitti V, et al. Detrusor underactivity and the underactive bladder: a new clinical entity? A review of current terminology, definitions, epidemiology, aetiology, and diagnosis. Eur Urol. 2014;65(2):389-98.
4. Blaivas JG. The bladder is an unreliable witness. Neurourology and Urodynamics. 1996;15(5):443-5.
5. Bromage SJ, Dorkin TJ, Chan L, Tse V. Urodynamics in the octogenarian female: is it worthwhile? Int Urogynecol J. 2010;21(9):1117-21.
6. Schafer W, Abrams P, Liao L, Mattiasson A, Pesce F, Spangberg A, et al. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn. 2002;21(3):261-74.
7. VW. N. Pressure flow urodynamic studies: the gold standard for diagnosing bladder outlet obstruction. . Rev Urol 2005 ;7 Suppl 6(Suppl 6):S14-21. 2005.
8. van Waalwijk van Doorn E, Anders K, Khullar V, Kulseng-Hanssen S, Pesce F, Robertson A, et al. Standardisation of ambulatory urodynamic monitoring: Report of the Standardisation Sub-Committee of the International Continence Society for Ambulatory Urodynamic Studies. Neurourol Urodyn. 2000;19(2):113-25.
9. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.
10. Holm NR, Horn T, Elbadawi A, Skjoldby B, Nordling J. A new technique for detrusor biopsy and its applicability in the ultrastructural study and diagnosis of voiding dysfunction. Br J Urol. 1996;77(6):785-91.
11. Hailemariam S, Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. V. Standardized protocols for routine ultrastructural study and diagnosis of endoscopic detrusor biopsies. J Urol. 1997;157(5):1783-801.
12. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. I. Methods of a prospective ultrastructural/urodynamic study and an overview of the findings. J Urol. 1993;150(5 Pt 2):1650-6.
13. Tanagho EA, Miller ER, Meyers FH, Corbett RK. Observations on the dynamics of the bladder neck. Br J Urol. 1966;38(1):72-84.
14. Tanagho EA, Smith DR. The anatomy and function of the bladder neck. Br J Urol. 1966;38(1):54-71.
15. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
16. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. J Urol. 1993;150(5 Pt 2):1668-80.
17. Brammah S CL, Tse V, Wills EJ. . An ultrastructural study of detrusor muscle junctions in patients with poorly compliant bladders. . J Urol 2005; 173(4) Suppl 325. 2005.
18. Yin JL CL, Tse V, Brammah S, Wills E. . Gap junctional protein expression in the unstable human detrusor. . J Urol 2003; 169(4) Suppl 317. 2003.
19. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.

CHAPTER 2: LITERATURE REVIEW - DETRUSOR ULTRASTRUCTURAL STUDIES IN HUMAN BLADDERS

Objective of this chapter

This chapter reviews the literature on current knowledge of detrusor ultrastructure in both normal and abnormal human bladders.

Literature review methodology

A PUBMED search was performed using key words “detrusor” AND “ultrastructur*” AND “human” in May 2022. This generated 106 articles which were reviewed for inclusion into this manuscript. A PUBMED search was performed using key words “detrusor” AND “ultrastructur*” AND “patients” in June 2022. This generated 13 articles which were reviewed for inclusion into this manuscript. Only an additional 10 were unique. From this number, non-English language publications were excluded. The remaining English language publications were reviewed for relevance and those considered relevant were included and referenced in the literature review; a total 35 publications were included and referenced in the final literature review. The body of literature was categorised into subheadings for ease of synthesis and understanding.

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ULTRASTRUCTURE OF THE HUMAN DETRUSOR

The predominant ultrastructural features studied are those of the muscle, interstitium, and intercellular junctions. See Table 1.1 for a summary of detrusor ultrastructural features.

Part 1. Normal detrusor ultrastructural features

19.1 Light microscopy features

The detrusor is seen to consist of spindle shaped smooth muscle cells with a single nucleus. The detrusor architecture is an interwoven non-layered network of muscle bundles. The arrangement is such that the myocytes are grouped into fascicles with minimal interstitium and these are further grouped into muscle bundles with collagen and elastin between them, as seen on light microscopy and electron microscopy on low power magnification (x 1000-3000).(1)

19.2 Electron microscopy features

On electron microscopy, the detrusor is seen to consist of smooth muscle, interstitium and intrinsic nerves.(2)

19.2.1 Detrusor smooth muscle cells

The electron density of detrusor myocytes can range from light to dark. The myocytes contain multiple mitochondria, occasional endoplasmic reticulum, and Golgi apparatus. Ribosomes and dense bodies may be seen throughout the cytoplasm (or “sarcoplasm”).(3) Actin myofilaments (6nm) are seen arranged parallel to the myocytes’ long axis. However, myosin filaments (15nm) are rarely preserved in the transmission electron microscopy preparations, and intermediate filaments (vimentin and desmin) are also rarely visible. Fusiform “dense bodies” are distributed among the filaments.(4) Nucleoli and mitotic figures are rarely seen in the nucleus. The ”sarcolemma” is a continuous layer cell membrane which envelopes the perimeter of the cell, and consists of alternating dense and thin bands. The dense bands are due to areas of myofilament attachment and may be widespread or patchy. Rows of caveolae (flask-shaped surface vesicles) may appear at the thin zones. Cisternae of endoplasmic reticulum may be beneath the caveolae.(4)

19.2.2 Detrusor ultrastructural arrangement

A “muscle fascicle” is a compact unit of muscle grouping consisting of 4-12 cells closely aligned (less than 0.2um spaces) and unidirectional. Within a fascicle, individual myocytes

are only separated by thing microsepta of interstitium made of collagen fibres with some elastic fibres and rare fibroblasts.(5)
A “muscle bundle” is an aggregate of “muscle fascicles” surrounded and outlined by thick macrosepta of interstitium containing fibroblasts.(5) It can be seen on light microscopy or electron microscopy on lower power magnification (x 1000-3000).

19.2.3 Intercellular junctions

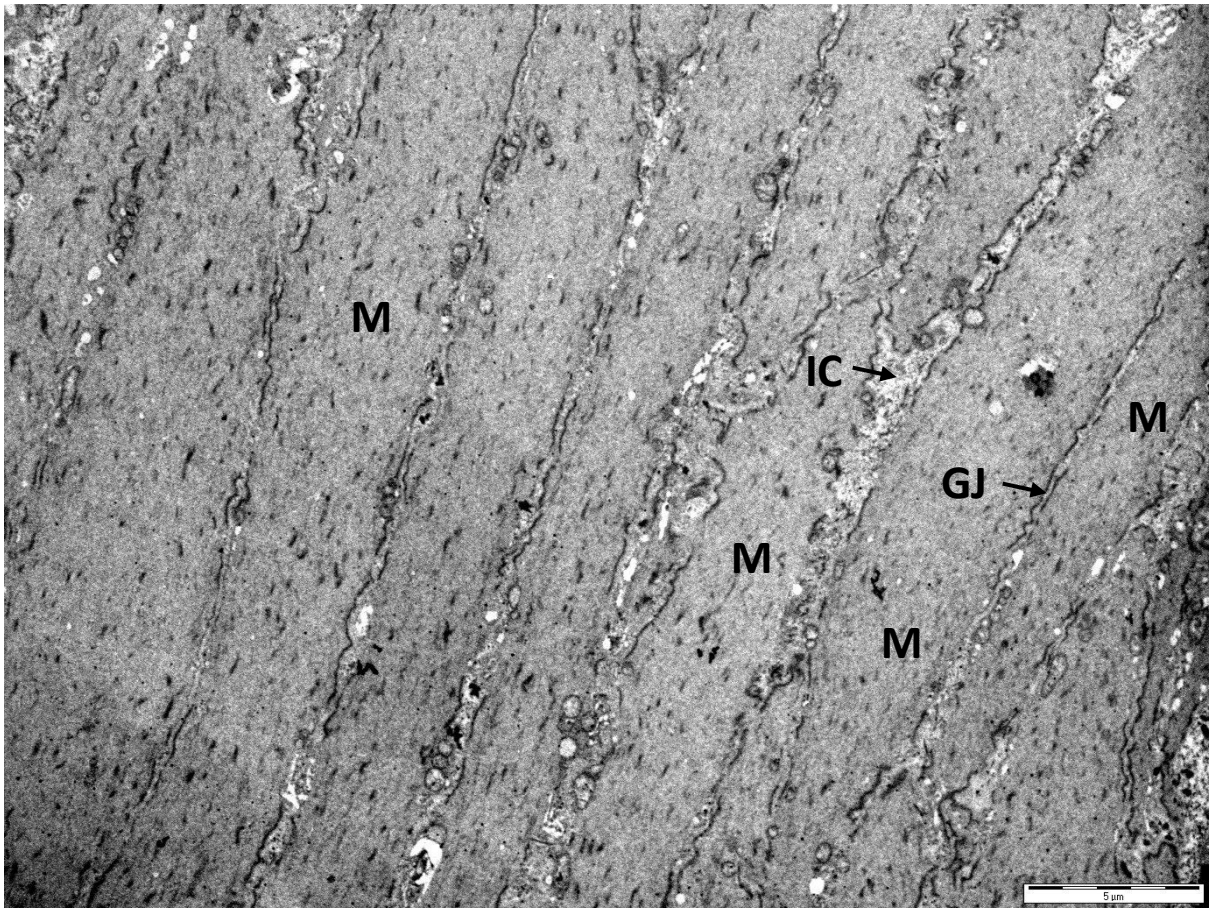
The “intermediate cell junction” (ICJ or “zonulae adherents”) is the most common type of intercellular junction. This consists of two closely apposed (25-70nm space) sarcolemma aligned parallel to one another for a length of up to 10um with paired symmetrical dense plaques.(6) “Gap junctions” are less common in normal detrusors. They include “protrusion junctions” as slender finger like projections between cells which contact one-another with a narrow-spaced contact zone. These projections are short (0.3-1.5um long), slender (75-300nm thick) and have a contact zone of 40-100nm. “Ultraclose abutments” are another type of gap junction, characterised by the tight apposition of parallel surfaces of two myocytes in a shallow bump-impression configuration. The contact zone is up to 0.8um long.(6) Cell junctions are visible with medium magnification (x 4000-7000) and contact zone details are best seen at high power magnification (x 40,000).(5, 6)

19.2.4 Detrusor interstitium and nerves

The interstitium contains amorphous ground substance, collagen, elastin and nerve fibres. The collagen fibril is a 20-120um diameter cylindrical unit structure with a banded cross-striated “pine needle” appearance.(2) A group of collagen fibrils of various sizes forms a collagen fibre. An elastin fibre consists of a light to moderately electron dense amorphous structure of various shapes, with unevenly distributed aggregates of highly electron dense filaments on its surface and inside it. Free filaments are often present next to the elastin fibre.(2)

Axon bundles are sparse in interstitium and may be difficult to identify on electron microscopy. The intrinsic nerves travel through the major interstitial divisions within the detrusor. It is understood that only a proportion of myocytes are directly stimulated by neuroeffector junctions and that the majority receive stimulus to contract via mechanical coupling or electrical coupling via intercellular junctions.(3)

Figure 2.1: Micrograph of normal detrusor ultrastructural features in female patient.



M = myocyte ; IC = intercellular collagen ; GJ = gap junction.

Part 2. Abnormal detrusor ultrastructural features

Abnormal ultrastructural features are categorised as changes to overall architecture, myocyte, interstitium or cell junctions, associated with lower urinary tract storage or voiding dysfunctions.

2.1 Artifact

Prior to describing abnormal ultrastructural features, it is important to recognise artifact. Empty-appearing mitochondria with lost cisternae are generally considered artifactual, as mitochondria are particularly susceptible to artifactual distortion in specimen processing.(5)

2.2 Abnormal architecture

Detrusor fascicle arrangement consisting of uniform and close apposition of adjacent myocytes within fascicles can be qualitatively described and graded using grading systems proposed by Hailemariam and Elbadawi as:

1) “Compact” – a fascicle-bundle unit with only mild uneven myocyte separation,
2) “Intermediate” – a mixture of uniform units and occasional intermediate or loose fascicles, and
3) “Loose” – mostly moderate to marked myocyte separation of indistinct arrangement with rarely seen uniform units.(5)

2.3 Myocyte changes

Myocyte shape and content can indicate cellular degeneration. A cell may contain vacuoles and debris, be fragmented, or be a shrivelled dense cell without internal structure. These

disrupted cells may be the cause or effect of bladder dysjunction, presumably impairing contractility. They may otherwise be the result of bladder overdistension and patchy hypoxia in an overstretched muscle or a poorly contractile bladder. Myocyte shape can be abnormal, such as branched (long processes extending in the same direction as “fork prongs”), braided, intertwined, or bizarre shapes such as “cannelloni”.(5) Degenerative vacuoles are not membrane-bound, and when sarcoplasm has a number of these degenerative vacuoles, it has a “moth-eaten” appearance.(4, 5, 7, 8)

Hypertrophic myocytes have been correlated with a history of bladder outlet obstruction.(9) It has been proposed that hypertrophy can be suspected with a high level of certainty when extreme variation in diameters exceeding the rand of 3 times the smallest is observed in the same microscopic field at low to medium magnification (x 1000-3000).(5)
Elbadawi et al. noted increased protrusion junctions and ultra-close abutments in patients with detrusor overactivity and considered them abnormal.(6) However, our own studies showed that these gap junctions are not pathognomonic of detrusor overactivity. Rather, the ratio of normal to abnormal gap junctions increases with overactive bladder.(3) The syncytium pattern of indiscernible gaps between cell processes linking up to 10 cells or greater may change the overall summative nature of the detrusor contraction. This may be associated with a lower resistance pathway mediating rapid electrical coupling, thereby implicating them in the pathophysiology of unstable detrusor contractions seen on urodynamic studies in overactive bladders.(3)

2.4 Abnormal interstitium

Abnormal architecture is often associated with abnormal interstitial content. As the amount of collagen and elastin increase, there are also increased numbers of distorted fascicle arrangements. In abnormal amounts, collagen reinforces mechanical cell coupling, and plays a key role in the eventual summated unitary contraction of the detrusor. However, excess collagen is thought to dissipate the force achieved by the myocytes.(10) Additionally, collagen is a stiff biomaterial, and may be responsible for the increased rigidity and reduced distensibility of the bladder, leading to a poorly compliant bladder. Elastin is a very stretchable biomaterial and may promote distensibility and allow bladder capacity to increase dramatically in chronic retention with overdistension. The number of fibroblasts may also give some clues to activity such as production of collagen fibrils. Interstitial debris may be increased as a ‘degenerative’ feature.(10)

Table 2.1: Summary of ultrastructural features of the detrusor.

Ultrastructural Feature	Normal	Abnormal
Muscle fascicle arrangement:	Uniform (compact, some mild/uneven smooth muscle separation)	Slightly variable to widely variable or vague (smooth muscle cell separation, loose or indistinct fascicle arrangement)
Muscle cell profiles:		
Size	Comparable (similar sized cell profiles)	Variable (ratio between largest and smallest aligned cells >3:1)
Shape	Normal (spindles)	Bizarre (branched, hugging, fork-prongs, braided/intertwined, cannelloni)
Electron density	Uniform	Variable density
Muscle cell content:		
Myofilament alignment	Regular spacing and parallel orientation	Disorientation and myofilament stacking
Degenerative cells	Rarely seen	Abnormal cellular content common (vacuoles, multilaminate bodies, disrupted mitochondria & endoplasmic reticulum, sequestration,

		blebbing, fragmentation, shrivelled dense cell without internal structure)
Dense bodies	Normal regular distribution	Clumping and streaming
Interstitium content:		
Collagen	Collagen normal (fibrils only within fascicles, fibres surround fascicles)	Collagenosis/fibrosis (fibres commonly seen and present within fascicles
Elastin	Elastin normal (fibrils predominate)	Hyperelastosis (fibres/clumps common)
Fibroblasts	Normal (none within fascicles)	Increased/active (prominent through rough endoplasmic reticulum, producing collagen fibres, presence within fascicles
Nerves	Normal (if seen at all)	Degenerative (vacuolated, collapsed Schwann's cells)
Interstitial debris	Absent	Present
Intracellular junctions:	Intermediate cell junctions predominate with a ratio of gap junctions <2:1	Gap junctions predominate with a ratio of 2-3:1 or >3:1
Syncytium	Absent	Present (indiscernible gaps between cell processes, chain of linked cells >5-10)

Part 3. Detrusor ultrastructural patterns

The first publications to show a clear relationship between lower urinary tract storage and voiding dysfunction, and detrusor ultrastructural features were authored by Elbadawi's research group.(2, 4, 6, 10) The original ultrastructural Elbadawi categories and their clinical correlates are summarised in Table 3.1.

Table 2.2: Elbadawi's detrusor ultrastructural categories

Ultrastructure Pattern	Clinical Correlate
Dense band pattern	Normal variant/Aging
Myohypertrophy pattern	Bladder outlet obstruction
Degeneration pattern	Detrusor underactivity
Hyperelastosis	Detrusor overdistension
Dysjunction pattern	Overactive bladder

These categories were based on urodynamic definitions which have since evolved and are a source of discrepancy between ultrastructural researchers. These ultrastructural definitions have since been refined to increase objectivity in reporting. The patterns are considered to be additive when multiple abnormalities of lower urinary tract dysfunction exist.

Clinical studies showed some discrepancies in findings. Mastropietro conducted a study aiming to assess all of Elbadawi's originally described ultrastructural patterns, but was unable to significantly correlate any of the patterns with clinical diagnoses in 24 women.(11) The overall agreement in detrusor ultrastructural pattern and clinical diagnosis was only 30%. There is marked variation between studies in definitions used for lower urinary tract dysfunction diagnoses, including defining controls, use of urodynamic studies, specimen processing technique, and ultrastructural analysis technique.

Ultrastructural categories which remain in usage in the literature currently include:
1) myohypertrophy pattern of bladder outflow obstruction (with hypertrophied, contorted myocytes and loose fascicles),
2) dysjunction pattern of detrusor overactivity (with gap junctions and intermediate fascicles), and

3) degenerative pattern of detrusor failure (with vacuolated lytic cells with intracellular and extracellular debris).

Hyperelastosis appears to correlate with chronic bladder overdistention.(12) However, not all studies agree.(13)

The “dense band pattern” which was organically described as a feature of aging despite lack of healthy young controls for comparison (4) has not been confirmed by other researchers and is rarely reported.

Table 3.2: Outline of major studies on detrusor ultrastructure with clinical diagnoses.

Reference	Number of Patients	Disease Definition	Pattern Findings
All Patterns			
Mastropietro, 2001 (11)	24 various	HC = PVR>50	30% correlation of patterns with urodynamic diagnosis
Dense Band			
Elbadawi, 1997b (14)	23 various	Normal PVR<50 Severe HC PVR >250 BOO = UPG >10	Varies in patient re-biopsied over time No correlating clinical diagnosis
Myohypertrophy			
Brierly, 2003b (7)	12 BOO (includes 6 DO) 17 controls	AGN >40	Myohypertrophy in 66% Full degeneration in 50% (highest PVRs) Normal ultrastructure in 17% Limited features in 18% of controls
Holm, 2003 (15)	25 BOO 6 controls	AGN>40	No myohypertrophy in patients or controls Significant correlation between amount of elastin and AGN
Degeneration			
Collins, 2005 (16)	19 HC	PVR>250	Mild-moderate degeneration in 58%, of whom 45% able to void Severe degeneration in 42%, of whom 13% are able to void
Brierly, 2003 (8)	14 HC 17 controls	PVR>300, AGN<40 Schafer weak/v. weak	Patients had significantly higher “disrupted cell ratios” than controls
Holm, 2002 (13)	15 HC 6 controls	PVR >500	Degeneration in 100% of patients Degeneration in 83% of controls
Hindley, 2002 (12)	21 HC 6 controls	PVR>300, AGN<40 Schafer weak/v. weak	Degeneration in all patients (65% full, 35% focal) Degenerative axons too rare to be reliable Nondisruptive Degen in all patients and controls
Dysjunction			
Tse, Willis, 2000 (3)	21 OAB (17 DO, 4 SU) 6 controls	BOO = AGN >40	Ratio of normal to abnormal junctions significantly rises from control to SU to DO Ratio 4:1 has specificity of 96% for diagnosis of DO

Carey, 2000 (17)	13 DO 11 controls		No dysjunction pattern seen
Hyperelastosis			
Collins, 2005 (16)	19 HC	PVR>250	63% of pts with PVR>1L No correlation with voiding outcome
Holm, 2003 (15)	25 BOO 6 controls	AGN>40	Morphometric amount of elastin correlated with AGN
Holm, 2002 (13)	15 HC 6 controls	PVR>500	No hyperelastosis
Hindley, 2002 (12)	21 HC 6 controls	PVR>300, AGN<40 Schafer weak/v. weak	Hyperelastosis in 35% of patients correlates with severe BOO and PVR>1L

HC = hypocontractile bladder; UPG = urethral pressure gradient in cmH20; SU = sensory urgency; DO = detrusor overactivity; BOO = bladder outlet obstruction; PVR = post void residual; Schafer = nomogram for defining impaired detrusor contractility as low pressure-low flow; AGN = Abrams-Griffith Number where >40 corresponds to BOO.

3.1 Dense band pattern

Elbadawi et al. described the dense band pattern in a group of 11 women and 2 men out of a total of 35 subjects, in whom there was no evidence of detrusor overactivity or bladder outlet obstruction on urodynamic studies, and were asymptomatic except for 2 subjects with minimal stress urinary incontinence. It was proposed that the dense band pattern therefore represented the structural norm of an ageing detrusor and may affect the exchange and storage of ions involved in the excitation-contractions coupling mechanism of myocytes through depletion of caveolae.(4, 18) However, 10 patients' urodynamic studies displayed "impaired detrusor contractility", defined as a post void residual over 50ml, which nowadays would be considered a normal post void residual measurement. Notwithstanding, Elbadawi et al. described the dense band pattern as characterised by overall normal configuration of myocytes and cell junctions, sarcolemma dominated by dense bands with depleted caveolae in interposed zones, and slight widening of spaces with sparsity of collagen between myocytes. Widespread degeneration of muscle cells, superimposed on the dense band pattern, was proposed as the structural correlate of impaired detrusor contractility in the aging detrusor.(4) However, there was a lack of healthy young controls for comparison.

Further studies corroborated these findings and additionally identified new features of the normally aging detrusor. Ultrastructural correlates distinguish moderate and severe from mild or borderline, but not moderate from severe impairment of detrusor contractility.(19) It is not determined what the temporal sequence of changes in detrusor ultrastructure and function is. However, the ultrastructure does not appear to be necessarily a result of long-standing change in function.(14)

The dense band pattern is not confirmed by contemporary ultrastructural investigators, is rarely reported in recent literature, and can be considered a normal variant.

3.2 Myohypertrophy pattern

The myohypertrophy pattern is characterised by widely separated muscle cells with reduction of intermediate cell junctions, collagenosis (abundant collagen plus some elastic fibres) in very widened spaces between individual muscle cells, and abundant profiles characteristic of enlarged, hypertrophic muscle cells. This was initially reported by Elbadawi in 6 males and 1 female out of the 35 patient bladder biopsies examined. Bladder outlet obstruction was defined with urethral pressure profilometry as a supramembranous urethral (distal urethra in female) pressure greater than 10cmH2O during voiding, in some cases corroborated with pressure-flow studies.(10) Some of the patients had ultrastructural features suggestive of superimposed degeneration. Three patients also had detrusor

overactivity, with abundant protrusion junctions and ultraclose abutments on ultrastructure study. It was concluded that myohypertrophy pattern, with or without superimposed degeneration, can explain overall weakness of the obstructed detrusor despite hypertrophy of its cells. This is because although hypertrophic muscle cells may be able to generate a strong contraction, it does not necessarily translate to a stronger overall detrusor body contraction, which requires a coordinated summative contraction of adequate strength and duration of the unit as a whole.

It was difficult for an independent pathologist to apply the original criteria of the myohypertrophy pattern, as several features remained very subjective. An example of this is when identifying the variability versus uniformity of fascicular structure and defining hypertrophic cell profiles. To help with this, three structural types were described:

1) normal compact (correlated with normal lower urinary tract function),
2) intermediate (correlated with detrusor overactivity),
3) loose fascicle (correlated with bladder outlet obstruction).(19)

The normal fascicle has closely coapted myocytes, frequent intermediate cell junctions, a narrow empty intercellular compartment with limited amorphous material, and occasional collagen fibrils. The intermediate fascicle consists of unevenly separated myocytes with sporadic intermediate junctions, more collagen fibrils, and rare elastin microfibrils within slightly widened intercellular spaces. The loose fascicle has moderate to marked cell separation, sparce intermediate junctions, abundant collagen fibrils, collagen fibres, and even aggregated of elastin fibrils or fibres.(5)

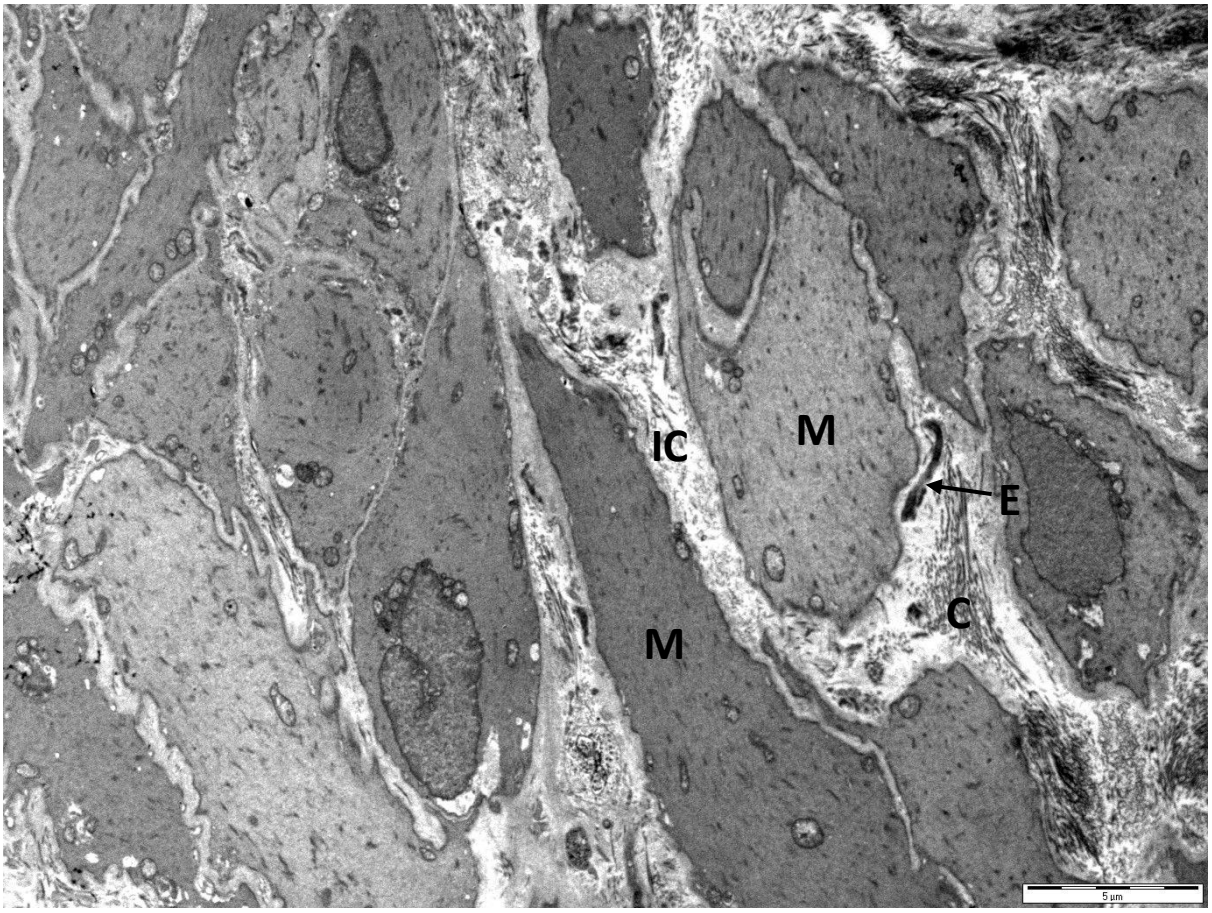
A later study of 12 men with BOO showed that 8 of the 12 had myohypertrophy pattern, two were normal, and two had degenerative pattern alone. Therefore, it was concluded that although there are interesting qualitative ultrastructural changes in the obstructed detrusor, they were not consistent enough to provide a reliable diagnostic tool. However, there was a relationship found between degeneration pattern and post void residual.(7)

Another study of 25 men with BOO on urodynamic study showed that the only parameter which was found to correlate with degree of obstruction was an increase in intra- and interfascicular elastin. All other correlations, including myohypertrophy pattern, did not reach significance. Therefore, the authors concluded that their study does not confirm specific relationships between ultrastructural detrusor smooth muscle features and various types of BOO, and that ultrastructural analysis cannot replace urodynamic evaluation in the classification of LUT function.(15)

Mirone et al. described that epithelial and smooth muscle cells in the bladder wall are mechanosensitive and undergo modifications of gene expression and protein synthesis in response to mechanical stretch stress caused by BOO. This process involves several transduction mechanisms and finally alter the ultrastructure and physiology of cell membranes, cytoskeleton, contractile proteins, mitochondria, extracellular matrix, and neuronal networks.(20)

Another study showed that bladder outflow obstruction due to BPE was associated with ultrastructural changes in the detrusor muscle and that these changes persisted post TURP. Additionally, nerve hypertrophy, which was not thoroughly discussed in previous studies, was a prominent feature in this study.(21)

Figure 2.2: Micrograph of detrusor ultrastructure showing myohypertrophy pattern in male patient with bladder outlet obstruction



M = myocyte ; IC = intercellular collagen ; E = elastin ; C = collagen

3.3 Degeneration pattern and hyperelastosis

The definition of the degeneration pattern has evolved over time, with its initial description being very qualitative and difficult to recognise unless all components were present. The original description was reported in 10 patients with impaired detrusor contractility, defined by a post void residual measurement of greater than 50ml, a definition which has also changed over time. In a subsequent paper, this is defined as a post void residual over 250ml (less than 50ml is now considered normal).(4, 5) Nowadays, the definition of a normal post void residual is still controversial, although it is agreed that a consistently “significantly” raised post void residual is often an indication of relative detrusor failure.

Ultrastructural features of degeneration are seen in the cells and interstitium. The cells show malalignments, disarray and stacking of myofilaments, clumping or streaming of dense bodies in the sarcoplasm, aggregates of glycogen particles, vacuoles, and multilaminate whorled bodies in the sarcoplasm. The mitochondria and endoplasmic reticulum were disrupted. Occasional cells were shrivelled and highly electron-dense with no discernible internal structure, and ultimately, cell lysis and fragmentation was seen. The interstitial changes were irregular, highly electron-dense masses of cellular debris and an exaggeration of intercellular spaces (up to 2.5um). Degenerating axons found in the interstitium were often retracted from muscle cells with 100 or greater wide separation gaps and showed depletion of synaptic vesicle with bloated clear axoplasm, disrupted mitochondria with axoplasmic dense patches, and fragmentation or lysis of axoplasm. Schwann cells could be seen containing either remnants of axons or were collapsed with redundant basal laminae. No degenerating axons were noted, unlike the degeneration seen in neurogenic detrusor degeneration.(22)

Degeneration pattern findings can be qualitatively subcategorised into early, moderate, and advanced groups. Early features consist of disarray of myofilaments, streaming or coalescence of sarcoplasmic dense bodies in muscle cells, and bloating of vesicle depletion of axons. Moderate features included disruption of endoplasmic reticulum, vacuolation, condensation or sequestration of sarcoplasm, and axonal dense patches and multivesicular bodies. Advanced features were sarcoplasmic extrusion and fragmentation or lysis of muscle cells, axonal breakdown, loss of axons and neuroeffector junctions, and redundant Schwann cells.(4)

In attempting to apply these criteria with an independent pathologist blinded to the clinical diagnosis, there were challenges as degenerative features were also seen in normal specimens. Therefore, disruptive vs nondisruptive degeneration features were described. Disruptive features included sarcoplasmic vacuolation, sequestration and blebbing, multilaminate whorled bodies, cell shrivelling, fragmentation, extreme variability of muscle cell electron density, cell debris in intercellular spaces, and degenerating axons. Nondisruptive features included myofilament disarray, stacking, malalignment, dense patches, and exaggeration of intercellular spaces up to 2.5um.(12)

“Full” degeneration is classified as disrupted changes being present in more than one muscle cell profile in all of 12 random fields at x1000 to x1800 magnification. “Limited” degeneration is either “focal”, with disruptive changes in at least half the field, or “rare”, with less than half the fields affected. However, this categorisation is considered rather arbitrary, since in reality, it is likely a spectrum of disease.(8, 12) Another suggestion is to redefine the definition based on the actual number of degenerative profiles per cell studies, reported as the “disrupted cell ratio”, which is calculated as the number of disrupted cells per 500 cells at x12000-24000 magnification.(12)

Jhang et al. more recently described detrusor ultrastructural characteristics in patients with detrusor underactivity of various aetiologies. The bladders showed total epithelial denudation in 52%. In the remainder, there were apical cell uroplakins (44.4%) and tight junction complexes (77.8%). The lamina propria had loose extracellular connective tissue (48%) and a lack of nerve terminals (76%). Smooth muscle shrinkage and a loss of regular spindle shape (91.6%) was seen. Patients with DU with intact epithelial cell layers had significantly larger void volumes and maximal flow rates than those with mild or severe epithelial denudation. Patients with nerve terminals remaining in the lamina propria had a strong first sensation of filling and smaller post void residual urine measurement than those without nerve terminals present.(23)

The pathophysiology of degenerative changes is poorly understood. It was proposed that disruptive cells would impair the contractile ability of the detrusor body as a unit. Alternatively, a dysfunctional bladder may cause the ultrastructural changes by insult arising from overdistension or increased bladder wall tension and hypoxia in overstretched muscle.(12)

Blatt et al. reported that failure to void after transurethral prostatectomy was significantly associated with the ultrastructural features of variation in muscle cell size, muscle cell shape, collagenosis, and abnormal fascicles. For failure to void after transurethral prostatectomy in patients with urinary retention, the sensitivity, specificity, and positive and negative predictive values of all 4 features together were 60%, 91%, 75% and 84% respectively. Three or four features on detrusor biopsy predicted voiding failure. In this study, it was proposed that patients with ultrastructural features previously described as part of the myohypertrophy pattern do not have just have a primary diagnosis of bladder outlet obstruction, but rather represent detrusor failure secondary to bladder outlet obstruction.(24)

3.4 Dysjunction pattern

The dysjunction pattern was initially described in 12 females and 3 males with detrusor overactivity, and consists of slightly widened intercellular spaces and an alteration in the pattern of muscle cell junctions, with the normal intercellular junction largely replaced by

protrusion junctions and ultraclose abutments.(2, 6, 10) In this dysjunction pattern, each protrusion junction of ultraclose abutment adjoined two myocytes, with three cell profiles involved on occasion. Four to eight muscle cell profiles were frequently adjoined in an open or closed chain-like arrangement. Alterations of the dysjunction pattern may change the summation of the detrusor body activity. Subsequent studies have reported mixed findings.

Smooth muscle cells of the human detrusor muscle are electrically coupled through classical gap junctions, forming limited local functional syncytia.(25, 26) In a later validation study, patients with normal urodynamic studies also displayed atypical junctions and a quasi-syncytium appearance at low magnification.(5) Furthermore, a different qualitative ultrastructural study of 13 women with idiopathic detrusor overactivity did not show any atypical junctions.(17) Another study was unable to demonstrate a clinical to ultrastructural correlation in 26 women, with the overall percentage agreement among diagnoses being only 30%.(11) The semi-quantitative study from our unit performed by Tse et al. showed a significant stepwise increase in the ratio of atypical junctions to normal intercellular junctions in four groups respectively: controls, idiopathic sensory urgency, bladder outlet obstruction with detrusor overactivity, and detrusor overactivity only.(3)

Remodelling of the detrusor in overactive bladders associated with bladder outlet obstruction has been described, with degenerative and atrophic changes and elimination of smooth muscle cells, compensatory hypertrophy of remaining cells, and diffuse or focal-diffuse replacement of fibrosis. Nepomnyashchikh et al. described the infiltration (focal or diffuse) of all layers of the detrusor with lymphocytes and plasma cells to be an important pathological feature of overactive bladder in patients with benign prostatic hyperplasia.(34)

Haferkamp et al. examined the detrusor ultrastructure in patients with long-standing neurogenic bladder dysfunction and reported that ultrastructural complete dysjunction pattern is a feature of neurogenic detrusor overactivity as well as non-neuropathic DO. They reported that more than 2 intimate cell apposition-to-intermediate junction ratios had 98% sensitivity, although the specificity was yet to be determined. No particular changes were associated with functional BOO due to detrusor sphincter dyssynergia, and there was a lack of relationship between myocyte degeneration and detrusor contractility.(28) Further study showed combined axonal degeneration and regeneration in intrinsic nerves in neurogenic detrusor dysfunction. This was not seen in nonneurogenic detrusor dysfunction, which only showed axonal degeneration without regeneration.(29)

A phenotypic shift towards a fibroblastic phenotype was seen in both neurogenic detrusor overactivity (NDO) and bladder pain syndrome bladders, with more pronounced changes noted in the NDO group. In both groups, there was also an increased presence in upper lamina propria lymphocytes.(30)

3.4.1 Interstitial cells

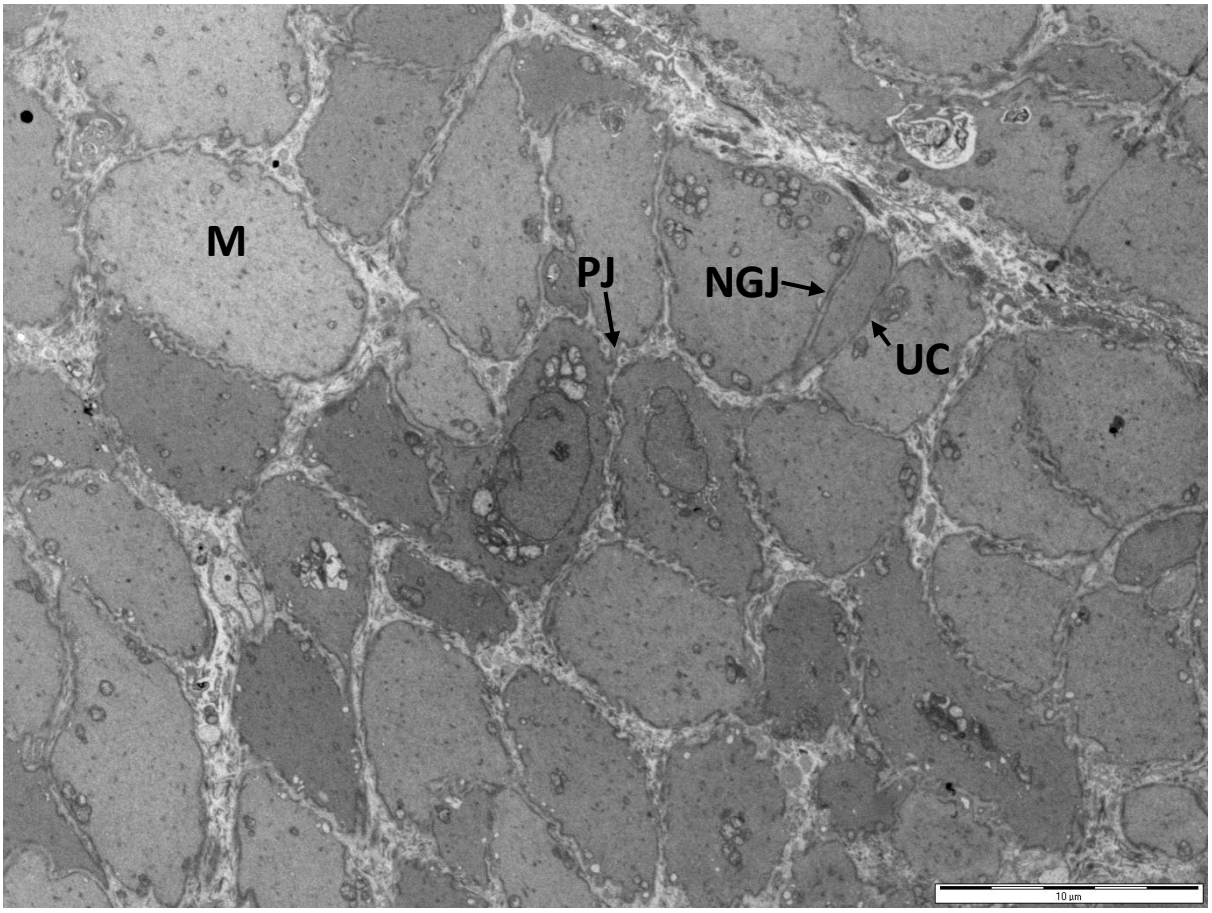
Bladder lamina propria myofibroblasts were observed to have close contact with nerves containing both small clear and small clear with dense-cored vesicles, implying they have both efferent and afferent nerve supply and possibly function as bladder stretch receptors. They also have similarities to the interstitial cells of Cajal in the gut.(31) There is emerging evidence that the expression of interstitial cells (or interstitial cells of Cajal) are present in the urinary bladder, making important associations with other cells that make up the bladder wall and possessing physiological properties consistent with a role of bladder activity modulation. They appear to be upregulated in pathophysiological conditions, including the overactive bladder.(32-34)

Johnston et al. described that the human bladder contains a network of KIT positive interstitial cells of Cajal in the lamina propria, which make frequent connections with a cholinergic nerve plexus. Novel perivascular interstitial cells of Cajal were discovered close to vascular smooth muscle cells, suggesting interstitial cells of Cajal-vascular coupling in the bladder. KIT positive detrusor interstitial cells of Cajal tracked smooth muscle bundles and were associated with nerves, perhaps showing a functional tri-unit controlling bladder

contractility. Detrusor interstitial cells of Cajal were spindle-shaped, branched cells tracking the smooth muscle bundles, closely associated with smooth muscle cells and vesicular acetylcholine transferase nerves.(35)

Studies on neurogenic detrusor overactivity described the phenomenon of abnormal handling of afferent signals by the urothelium and lamina propria. There are three types of telocytes in the lamina propria and detrusor. These telocytes are stromal cells that form a 3D network scaffold which contains and organises the connective components, acting as a guide for tissue (re)-modelling, producing trophic and/or regulatory molecules, sharing contacts with immune cells. A study showed that neurogenic detrusor overactivity was associated with changes in all three telocyte types, including signs of telocyte activation. Furthermore, a cell infiltrate mainly composed of plasma cells was present in the vicinity of or making contact with the telocytes.(36)

Figure 2.3: Micrograph of detrusor ultrastructure showing dysjunction pattern in male patient with overactive bladder.



M = myocyte, PJ = protrusion junction, UC = ultraclose abutment, NGJ = normal gap junction.

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Comments

Whilst there have been a small number of studies on detrusor ultrastructure and attempts to correlate these features with clinical functional diagnoses, there are still many areas of controversy and inconsistency, while the current knowledge provides little justification for clinical applications. The major abnormal ultrastructural categories of myohypertrophy, degeneration, and dysjunctions are largely agreed upon, but there are inconsistencies and challenges in correlating these features with

clinical functional information. This project therefore aims to explore some of the potential areas for clinical applications of ultrastuctural analyses in voiding dysfunction.

References

1. Gosling JA, Dixon JS. Structure of trabeculated detrusor smooth muscle in cases of prostatic hypertrophy. Urol Int. 1980;35(5):351-5.
2. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. I. Methods of a prospective ultrastructural/urodynamic study and an overview of the findings. J Urol. 1993;150(5 Pt 2):1650-6.
3. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
4. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. J Urol. 1993;150(5 Pt 2):1657-67.
5. Hailemariam S, Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. V. Standardized protocols for routine ultrastructural study and diagnosis of endoscopic detrusor biopsies. J Urol. 1997;157(5):1783-801.
6. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. J Urol. 1993;150(5 Pt 2):1668-80.
7. Brierly RD, Hindley RG, McLarty E, Harding DM, Thomas PJ. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU Int. 2003;91(4):360-4.
8. Brierly RD, Hindley RG, McLarty E, Harding DM, Thomas PJ. A prospective controlled quantitative study of ultrastructural changes in the underactive detrusor. J Urol. 2003;169(4):1374-8.
9. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.
10. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. IV. Bladder outlet obstruction. J Urol. 1993;150(5 Pt 2):1681-95.
11. Mastropietro MA, Geary W, Fuller E, Benson JT. Detrusor biopsy as a potential clinical tool. Int Urogynecol J Pelvic Floor Dysfunct. 2001;12(6):355-60.
12. Hindley RG, Brierly RD, McLarty E, Harding DM, Thomas PJ. A qualitative ultrastructural study of the hypocontractile detrusor. J Urol. 2002;168(1):126-31.
13. Holm NR, Horn T, Smedts F, Nordling J, de la Rossette J. Does ultrastructural morphology of human detrusor smooth muscle cells characterize acute urinary retention? J Urol. 2002;167(4):1705-9.
14. Elbadawi A, Hailemariam S, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. VII. Prospective ultrastructural/urodynamic evaluation of its natural evolution. J Urol. 1997;157(5):1814-22.
15. Holm NR, Horn T, Smedts F, Nordling J, de la Rossette J. The detrusor muscle cell in bladder outlet obstruction--ultrastructural and morphometric findings. Scand J Urol Nephrol. 2003;37(4):309-15.
16. Collins R, Chan L, Tse V, Brammah S, Wills E. Ultrastructural detrusor changes and clinical outcome in patients with detrusor hypocontractility. AUA Conference Abstract number 1203. The Journal of Urology supplement 2005,174(4):326
17. Carey MP, De Jong S, Friedhuber A, Moran PA, Dwyer PL, Scurry J. A prospective evaluation of the pathogenesis of detrusor instability in women, using electron microscopy and immunohistochemistry. BJU Int. 2000;86(9):970-6.
18. Zhu Q, Resnick NM, Elbadawi A, Kuchel GA. Estrogen and postnatal maturation increase caveolar number and caveolin-1 protein in bladder smooth muscle cells. J Urol. 2004;171(1):467-71.
19. Elbadawi A, Hailemariam S, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. VI. Validation and update of diagnostic criteria in 71 detrusor biopsies. J Urol. 1997;157(5):1802-13.
20. Mirone V, Imbimbo C, Longo N, Fusco F. The detrusor muscle: an innocent victim of bladder outlet obstruction. Eur Urol. 2007;51(1):57-66.
21. Yadav SS, Bhattar R, Sharma L, Banga G, Sadasukhi TC. Electron microscopic changes of detrusor in benign enlargement of prostate and its clinical correlation. Int Braz J Urol. 2017;43(6):1092-101.
22. Elbadawi A, Resnick NM, Dorsam J, Yalla SV, Haferkamp A. Structural basis of neurogenic bladder dysfunction. I. Methods of prospective ultrastructural study and overview of the findings. J Urol. 2003;169(2):540-6.
23. Jhang JF, Ho HC, Jiang YH, Hsu YH, Kuo HC. Clinical Correlation of Bladder Electron Microscopic Characteristics in Patients with Detrusor Underactivity of Various Etiologies. Biomedicines. 2022;10(5).

24. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.
25. John H, Wang X, Wehrli E, Hauri D, Maake C. Evidence of gap junctions in the stable nonobstructed human bladder. J Urol. 2003;169(2):745-9.
26. Neuhaus J, Pfeiffer F, Wolburg H, Horn LC, Dorschner W. Alterations in connexin expression in the bladder of patients with urge symptoms. BJU Int. 2005;96(4):670-6.
27. Nepomnyashchikh LM, Lushnikova EL, Neimark AI. Remodeling of the muscle layer (detrusor muscle) of hyperactive bladder disease in patients with benign prostatic hyperplasia. Bull Exp Biol Med. 2012;153(5):778-83.
28. Haferkamp A, Dorsam J, Resnick NM, Yalla SV, Elbadawi A. Structural basis of neurogenic bladder dysfunction. II. Myogenic basis of detrusor hyperreflexia. J Urol. 2003;169(2):547-54.
29. Haferkamp A, Dorsam J, Resnick NM, Yalla SV, Elbadawi A. Structural basis of neurogenic bladder dysfunction. III. Intrinsic detrusor innervation. J Urol. 2003;169(2):555-62.
30. Gevaert T, De Vos R, Everaerts W, Libbrecht L, Van Der Aa F, van den Oord J, et al. Characterization of upper lamina propria interstitial cells in bladders from patients with neurogenic detrusor overactivity and bladder pain syndrome. J Cell Mol Med. 2011;15(12):2586-93.
31. Wiseman OJ, Fowler CJ, Landon DN. The role of the human bladder lamina propria myofibroblast. BJU Int. 2003;91(1):89-93.
32. McCloskey KD. Interstitial cells in the urinary bladder--localization and function. Neurourol Urodyn. 2010;29(1):82-7.
33. Rasmussen H, Hansen A, Smedts F, Rumessen JJ, Horn T. CD34-positive interstitial cells of the human detrusor. APMIS. 2007;115(11):1260-6.
34. Rasmussen H, Rumessen JJ, Hansen A, Smedts F, Horn T. Ultrastructure of Cajal-like interstitial cells in the human detrusor. Cell Tissue Res. 2009;335(3):517-27.
35. Johnston L, Woolsey S, Cunningham RM, O'Kane H, Duggan B, Keane P, et al. Morphological expression of KIT positive interstitial cells of Cajal in human bladder. J Urol. 2010;184(1):370-7.
36. Traini C, Fausssone-Pellegrini MS, Guasti D, Del Popolo G, Frizzi J, Serni S, et al. Adaptive changes of telocytes in the urinary bladder of patients affected by neurogenic detrusor overactivity. J Cell Mol Med. 2018;22(1):195-206.

CHAPTER 3: METHODOLOGY

Ethics approval

The protocol used in this study was approved by the Human Research Ethics Committee of Sydney Local Health District prior to commencement (2019 / ETH 07866). Approval was granted for obtaining detrusor biopsy specimens from patients with voiding dysfunction who underwent cystoscopic examination under general anaesthesia as part of their clinical care. Patients who were not otherwise scheduled to undergo a cystoscopy were not invited into the study. A “Patient Information Statement” was provided to eligible patients, explaining in lay terms the significance of the study, what it involved, the possible side-effects, and patient confidentiality (see Appendix 2). Signed consent forms were obtained from patients willing to participate in the study.

Patient recruitment

Patients were recruited from the Urodynamic Clinic at Concord Repatriation General Hospital. Patients with female bladder outflow obstruction, detrusor underactivity, and/or detrusor overactivity as demonstrated on urodynamic study who were going to undergo cystoscopy as part of their clinical management were invited for recruitment into the study. A control group was also recruited consisting of patients with stress urinary incontinence without known bladder dysfunction who underwent cystoscopy as part of their clinical management.

Exclusion criteria;
1) Patients who have not had urodynamic study (that is, no definitive diagnosis of their lower urinary tract dysfunction)
2) Patients who need to remain on anticoagulation at time of cystoscopy or in the opinion of the treating urologist had significant risks of complications from bladder biopsy.

Baseline data

Patient age and sex were recorded, as well as lower urinary tract symptomatology and relevant urological and medical history.

Urodynamic study technique

Standard multichannel video urodynamic study was performed according to International Continence Society standards using a Laborie Urodynamic System.(1) An initial uroflowmetry study was conducted, with patients sitting or standing (depending on patient preference and usual posture of urination) and urinating into a uroflowmeter to record flow pattern and parameters such as voided volume and peak flow rate.

The patient was then placed in supine position for insertion of a 5Fr vesical pressure measurement line and 10Fr Nelaton filling catheter via the urethra into the bladder under local anaesthesia. The residual volume was recorded.

The 12F rectal balloon pressure measurement line was then placed into the rectum.

Calibration was performed, and cystometrogram recorded with saline at a filling rate of 50ml/min. Filling was stopped when patient felt at capacity, or when filling reached 1L, depending on whichever occurred first.

The 10Fr filling catheter was removed at the end of filling and the patient was positioned upright. If clinically indicated, cough and Valsalva testing was then carried out to assess for stress urinary incontinence. The patient was then permitted to urinate in either the standing or sitting position, depending on patient preference and usual posture for urination. Imaging of the bladder, prostate (including intravesical prostatic protrusion measurement in males) and kidneys was performed during the filling phase using a Philips EPIQ 7 ultrasound system and C9-2 curved transducer.

Urodynamic parameters in the storage and voiding phase were analysed and reported. Storage phase parameters included bladder sensation (increased, decreased or normal), the presence of detrusor overactivity (present or absent), compliance (poor or normal), and any urinary incontinence. Voiding phase parameters included flow pattern, detrusor pressure during peak flow, peak urinary flow rate, and post void residual urine measurement.

In particular for females, the Solomon-Greenwell nomogram and its associated number, the Solomon-Greenwell Number (SGN), was used to define female bladder outflow obstruction. SGN = Pdet at Qmax minus 2.2 times Qmax. If SGN >18, then the patient was considered to have female bladder outflow obstruction to an almost certain degree (>90%), and if SGN >5, the patient was likely obstructed (>50%).(2)

For males, the Abrams-Griffiths nomogram and its associated Bladder Outflow Obstruction Index (BOOI) was used to define male bladder outflow obstruction. BOOI = Pdet at Qmax minus 2xQmax. If BOOI > 40, then the patient was considered to have bladder outflow obstruction. BOOI < 20 indicated no obstruction, and BOOI 20-40 was equivocal for obstruction.(3, 4)

For males with underactive bladder, the Bladder Contractility Index (BCI) derived from the Schaefer nomogram was used to define male detrusor underactivity. BCI = Pdet at Qmax + 5*Qmax. A BCI <100 cmH2O is considered to be weak, BCI of 100-150 cmH2O is considered to be normal contractility, and BCI >150 cmH2O is considered to be strong.(4)

Bladder Voiding Efficiency (VE) was calculated as voided volume (VV) * 100 / pre-void bladder volume and expressed as a percentage. Pre-void bladder volume can also be quantified as VV plus post void residual (PVR) volume.(4)

Cystoscopy and detrusor biopsy technique

Eligible patients underwent detrusor muscle biopsy at the time of cystoscopy. Cystoscopy was performed under general anaesthesia with the patient in dorsal lithotomy position. A 22Fr cystoscope sheath was introduced via the urethral meatus with 30 degree lens. The urethra, prostate and bladder were examined, and the ureteric orifices identified (Figure 1). Cold cup bladder biopsy forceps were used to obtain two 1mm specimens from the wall of the bladder approximately 2cm superolateral to the ureteric orifice, as per standard technique. The biopsy was taken by cold cup forceps to preserve tissue integrity. The urothelium was first lifted and a small portion removed using the biopsy forceps. This allowed exposure of the underlying detrusor muscle bundles, and the detrusor muscle biopsies were obtained with the cold cup biopsy forceps for analysis (Figure 2). The biopsy site was then cauterised with a monopolar Bugbee electrode to secure haemostasis, as per our Institution's previous protocol.(5) There were no complications related to the detrusor muscle biopsies in this study.

Figure 3.1: Cystoscopic identification of the right ureteric orifice.

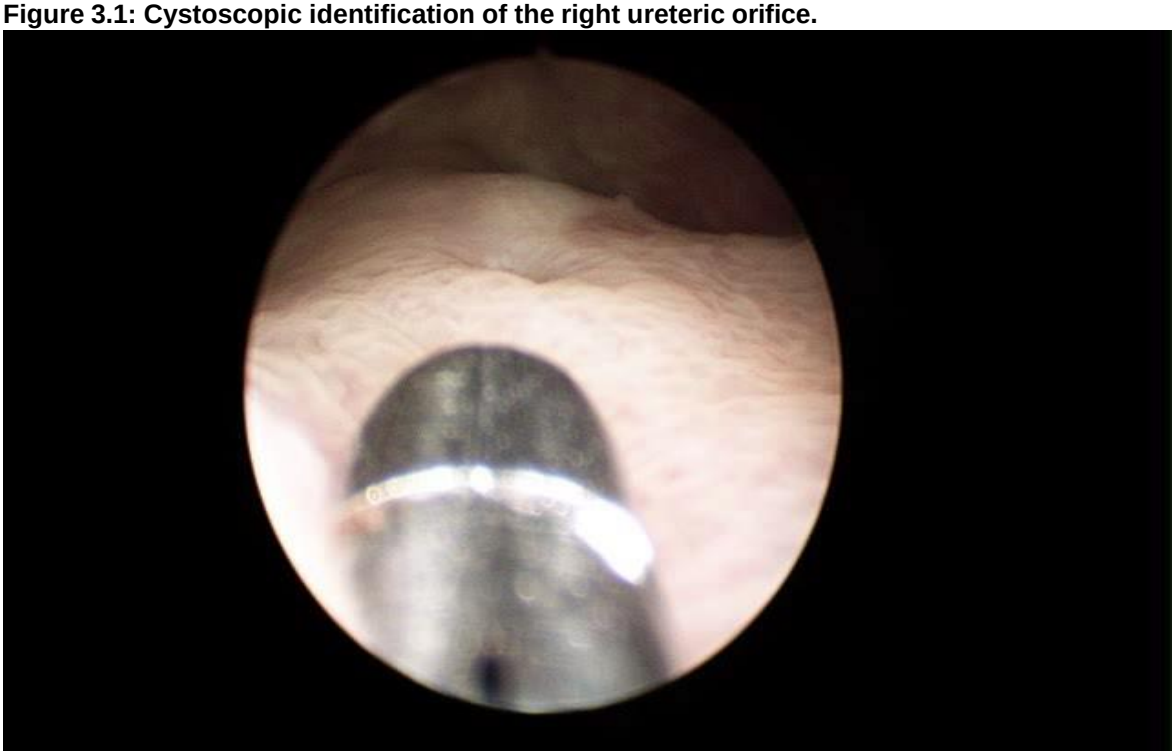
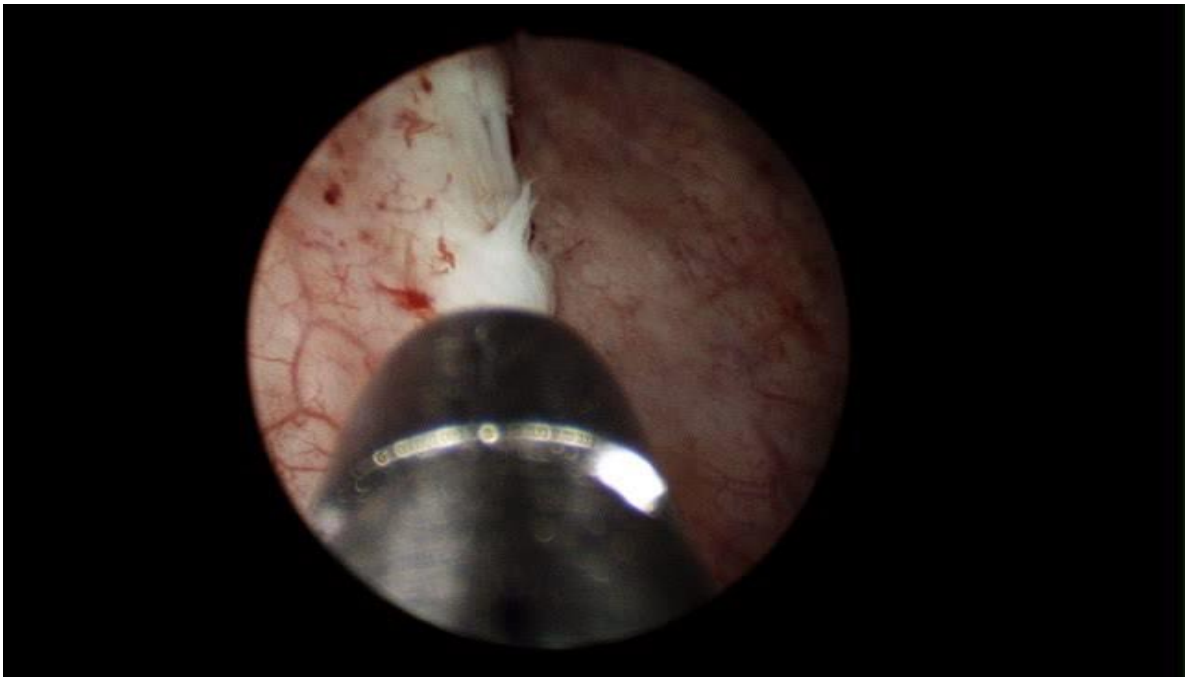


Figure 3.2: Cystoscopic view of biopsy forceps pointed to obtain detrusor biopsy, having already lifted overlying urothelium to expose the detrusor muscle.



Electron Microscopy Unit at Concord Repatriation General Hospital

All detrusor biopsy specimens were processed in the Concord Repatriation General Hospital Department of Pathology Electron Microscopy Unit on the Ground Level of the Main Hospital Building. The Unit was initially established for processing of renal biopsy ultrastructural analysis, but merged with the unit from Royal Prince Alfred Hospital Camperdown in 1996 to form one of the leading Electron Microscopy Units in Australia and New Zealand. The unit performs a significant number of

ultrastructural diagnostic pathology services for New South Wales and also receives specimens for analysis from New Zealand. There are two transmission electron microscopes in the department.

Over the course of this PhD work, the Unit has had some changes in personnel. The Unit was run by one senior staff specialist, two senior scientists (who sequentially retired throughout the course of the past 5 years), and three scientists. Currently, there is one senior staff specialist, one staff specialist, one senior EM scientist, and four EM scientists. It is a very busy unit and processes and analyses over 1800 specimens annually, including renal and muscle biopsies.

With much gratitude to the generous sharing of expertise by the urologists, senior scientists and other scientists in the Unit, I learnt how to process and analyse the detrusor biopsies, from harvesting at time of cystoscopy to visualisation under the electron microscope and scoring of ultrastructural features. The process is described as follows:

Detrusor biopsy processing technique

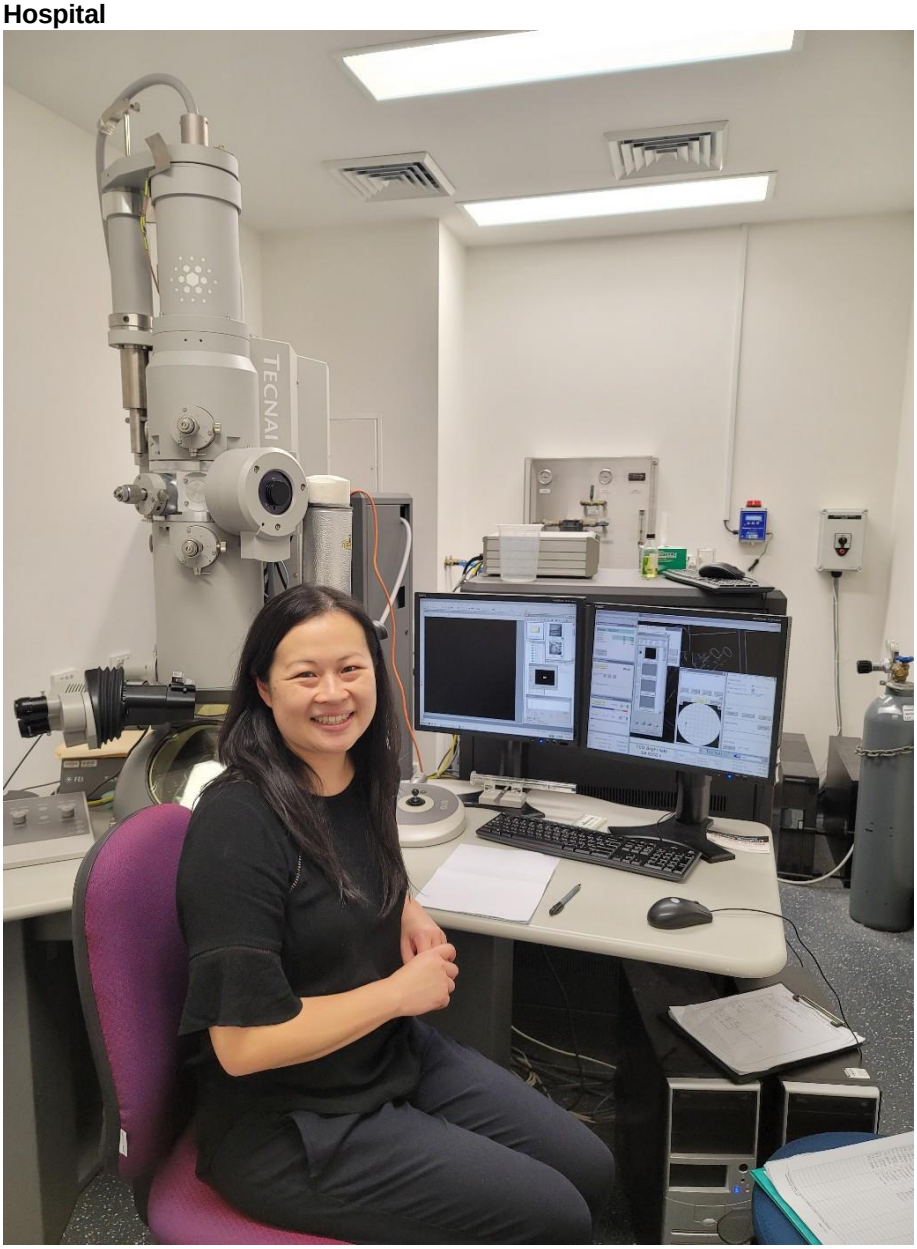
The detrusor biopsy tissue was first placed into vials containing pre-chilled fixative (2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.3 at 4 degrees Celsius) and refrigerated. Specimens were assigned an identification code number and cut to $1mm^3$ sized cubes for processing. The specimen then underwent processing as follows;

1) Washed with cacodylate buffer
2) Postfixed with 2% osmium tetroxide
3) En bloc staining with uranyl acetate
4) Dehydration with increasing concentrations of ethanol and acetone
5) Embedded in Spurrs resin, placed in capsules and polymerized in preparation for cutting.

The specimens in resin blocks were then cut using a microtome into semithin (0.5µm) sections, mounted on a glass slide, stained with toluidine blue, and examined under light microscopy to ensure there was adequate smooth muscle present for ultrastructural study.

An ultratome with diamond knife was used to cut the ultrathin (90-100nm) sections, which were then mounted on 3.0nm copper grides and stained with uranyl acetate and lead citrate for ultrastructural examination. The specimens were viewed using a Philips FEI Tecnai Spirit Biotwin transmission electron microscope. Digital photographs were taken electronically using the transmission electron microscope software.

Figure 3.3: Photograph of Amanda Chung and the Philips FEI Tecnai Transmission Electron Microscope at the Electron Microscopy Unit in Concord Repatriation General Hospital



Detrusor ultrastructure analysis technique

Ultrastructural examination was performed using a standardised protocol whereby at least 10 low magnification and 10 high magnification images were captured for ultrastructural analysis. Ultrastructural analysis for specific detrusor ultrastructural features of lower urinary tract dysfunction was performed in similar fashion to our previously established diagnostic criteria.(5, 6) Features such as myohypertrophy features global impression scores, myocyte size and irregularity, myocyte cell separation, intercellular collagen, degenerative features global impression scores, electron density variation, vacuolation and dense bodies, fragmentation and exclusion bodies, and hyperelastosis were identified and scored on a visual scoring scale (0 to 3, where 0 is normal, 1 is mild changes, 2 is moderate changes, and 3 is severe changes). Scores were recorded on Excel spreadsheets and/or scoring sheets (see Appendix 3).

In this PhD work, we have used a simplified standardized protocol, similar to our Institution's established protocol for analysis of male bladder outlet obstruction, a tool that can be applied in a diagnostic pathology setting. We are cognizant of the fact that our Institution's previous protocol for examination of overactive bladders was more complex, time consuming, and unlikely to be applicable outside of a purely research setting. Previously, for male bladder outlet obstruction, micrographs were scored 1 to 3 according to the following features: muscle fascicle, degeneration, collagen, cell size variation, muscle shape, electron density, elastin, interstitial debris, fibroblasts, and nerves.(6) In our simplified standardized protocol for this project, some of these features are grouped into global impression subheadings. There is also no specific analysis of nerves, as this was not a significant feature in our previous studies.

Previously, for overactive bladders, normal and abnormal intercellular junctions were counted in at least 20 photographic images per specimen at magnifications between 10,000 and 17,000x for examination of at least 200 myocyte profiles, and the junction counts recorded and expressed as a ratio of abnormal:normal.(5) For this study, the simplified protocol for overactive bladders includes at least 10 low and 10 high magnification images obtained for scoring of the aforementioned features. We expected that this qualitative and semi-quantitative analysis protocol would be less time-consuming and able to be used as a standardized protocol for detrusor ultrastructure analysis across a range of lower urinary tract dysfunctions (that is, same protocol for BOO, overactive and underactive bladders), allowing for more standardized comparisons and contrasts.

Each detrusor ultrastructure specimen was assessed by two independent observers (AC and either LC or VT) who remained blinded to the clinical and urodynamic diagnosis of the patient. Where there was disagreement in scores attributed, the final score was decided by consensus discussion or arbitration by a third observer (either LC or VT).

Categorization of functional and structural data for evaluation

Patients with specific urodynamic diagnoses of female bladder outlet obstruction (see Chapter 4), male detrusor underactivity (see Chapter 5), and older patients (>65 years) with overactive bladder syndrome/detrusor overactivity with impaired contractility (see Chapter 6) were identified for more detailed analysis of their functional and detrusor ultrastructural data, and correlations examined and reported. Methodology for data analysis of specific patient cohorts will be described in the relevant following chapters.

Statistical analysis

The majority of this PhD work involves qualitative ultrastructural study. For the components of the study that are semi-quantitative, the statistical analyses were performed using SPSS® Version 28. Multivariate analyses were performed using binary logistic regression to test for ultrastructural and functional variables relating to clinical outcome. Non-parametric tests were used to analyse continuous variables. Chi-squared tests and Spearman's correlation were used for categorical variables. Fisher's Exact Test was used for univariate analysis between categorical variables when cell count was less than 5. $p<0.05$ was considered statistically significant.

References

1. Schafer W, Abrams P, Liao L, Mattiasson A, Pesce F, Spangberg A, et al. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn. 2002;21(3):261-74.
2. Solomon E, Yasmin H, Duffy M, Rashid T, Akinluyi E, Greenwell TJ. Developing and validating a new nomogram for diagnosing bladder outlet obstruction in women. Neurourol Urodyn. 2018;37(1):368-78.
3. Griffiths D, Hofner K, van Mastrigt R, Rollema HJ, Spangberg A, Gleason D. Standardization of terminology of lower urinary tract function: pressure-flow studies of voiding, urethral resistance, and urethral obstruction. International Continence Society Subcommittee on Standardization of Terminology of Pressure-Flow Studies. Neurourol Urodyn. 1997;16(1):1-18.
4. Abrams P. Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: three simple indices to define bladder voiding function. BJU Int. 1999;84(1):14-5.
5. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
6. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.

CHAPTER 4: ULTRASTRUCTURAL STUDIES IN FEMALES WITH BLADDER OUTLET OBSTRUCTION

Introduction and objectives

Bladder outlet obstruction as a voiding dysfunction

In males, bladder outlet obstruction due to benign prostatic obstruction is a common voiding dysfunction. There is a large amount of literature describing features of male bladder outlet obstruction from clinical and pathophysiological viewpoints, as well as some work on detrusor ultrastructure. In contrast, there is relatively little published on female bladder outlet obstruction, including a comparatively small number of clinical studies and no ultrastructural studies specifically examining this group. BOO occurs more commonly in males than females due to prostatomegaly as the main causative factor.

Epidemiological studies of LUTS in older men have reported that 20% of men over 60 years of age had a history of prostatic surgery, and annual incidence rates were 2.6% and 3.3% over the next two years respectively.(1) Additionally, the male urethra is much longer than the female urethra, and is therefore more likely to acquire urethral stricture disease, which is the second most common cause for male bladder outlet obstruction.

In general, female bladder outlet obstruction is poorly understood, under-recognised and undertreated. Female patients with voiding dysfunction can suffer symptoms for long durations, with visits to multiple doctors - including primary care physicians, urologists, and gynaecologists - before their condition is properly diagnosed and treated. Morbidity of underdiagnosed and undertreated female bladder outlet obstruction includes distressing symptoms such as difficulty voiding, slow urinary flow, feeling of incomplete emptying, urinary frequency, catheterisation including self-catheterisation and suprapubic catheterisation, and secondary medical conditions which include recurrent urinary infections, bladder stones and renal impairment due to obstructive nephropathy.

Female bladder outlet obstruction aetiology and relevance

Aetiology of female BOO may include urethral stricture, primary bladder neck obstruction, sphincter active voiding, or as a complication of continence surgery such as urethral slings. Female stress urinary incontinence is relatively common, and many women undergo surgery to restore urinary continence. This commonly occurs as a sling procedure using either synthetic mesh or native tissue such as rectus fascia. A known potential complication of continence sling surgery is voiding dysfunction and bladder outlet obstruction. This has become particularly relevant over the past decade, as mesh complications have been highlighted in public and medical media. Complications associated with transvaginal mesh for vaginal prolapse and slings have become high profile cases, highlighting the morbidity many women suffer in relation to mesh implants. As a result of issues surrounding the use of transvaginal mesh in women, vaginally placed mesh has since been banned in Australia, and many continence mesh slings have also been taken off market over the past few years following a Senate Inquiry and Royal Commission investigation and recommendations. A similar response has also occurred in many other countries, including the United Kingdom and Canada.

Detrusor ultrastructure literature

Elbadawi described a ‘myohypertrophy’ pattern of detrusor ultrastructure which correlated with bladder outlet obstruction in his cohort of older male patients. The myohypertrophy pattern is characterised by widely separated muscle cells with reduction of intermediate cell junctions, collagenosis (abundant collagen plus some elastic fibres) in very widened spaces between individual muscle cells, and abundant profiles characteristic of enlarged, hypertrophic muscle cells.(2) Other features include variation in size of myocytes.(3) Some of the features of the original criteria were difficult for independent pathologists to apply given that they were quite subjective.(4)

Further studies showed that the myohypertrophy pattern features were not consistent enough to provide a reliable diagnostic tool for obstruction, but there was a relationship between degeneration pattern and post void residual.(5) Another study showed that the only parameter found to correlate with degree of obstruction was an increase in intra- and inter-fascicular elastin; all other correlations,

including myohypertrophy pattern, did not reach significance.(6) A more recent study by Yadav showed that bladder outflow obstruction due to benign prostatic enlargement was associated with detrusor ultrastructural changes which persisted post TURP. Additionally, nerve hypertrophy, which was not thoroughly discussed in previous studies, was a prominent feature in this study.(7)

All the studies reported to date were performed in the male bladder outlet obstruction population with sometimes only 1-2 females in the cohort. There are still no published ultrastructural studies specifically examining the detrusor in female humans with bladder outlet obstruction. The previous ultrastructural studies in our institution have consistently identified myohypertrophy changes in male patients with bladder outlet obstruction, with some of these features overlapping into patients with the failing detrusor.(8,9)

Aim of this chapter

This chapter aims to report on our study of ultrastructural features in females with bladder outlet obstruction, and to compare findings with male bladder outlet obstruction using a standardized protocol.

Methodology and patient cohort

14 patients (8 female, 6 male) with known BOO on urodynamic study (except two female patients where BOO was diagnosed on anatomical finding of significant urethral stricture disease) and one control patient (1 female) were included in this part of the PhD project. The control patient had known clinical and urodynamic stress urinary incontinence, but otherwise normal storage and voiding function on urodynamic studies.

As detailed in the Chapter 3, for the male patients, BOO was defined as BOOI >40 using the AGN, whereby BOOI = Pdet at Qmax – 2Qmax. Many of these patients also had elevated post void residual urine measurements. In most cases for female patients (all except two patients), BOO was defined as BOOIf >5, based on the Solomon-Greenwell nomogram whereby the Solomon-Greenwell Number (SGN) is calculated by Pdet at Qmax minus 2.2*Qmax.(10) BOOIf>5 represents likely obstructed (>50%), and BOOIf>18 represents almost certainly obstructed (>90%). The BOOIf and SGN essentially also acknowledge high detrusor voiding pressure with low or no flow voiding. Many of these female patients also had elevated post void residual urine measurements. In the additional two patients, BOO was diagnosed based on the anatomical finding of very narrow urethral stricture in the context of bothersome voiding LUTS.

All patients underwent cystoscopy and detrusor muscle biopsy. The detrusor muscle biopsy specimens were processed for transmission electron microscopy examination and electronic photographs taken of the detrusor ultrastructure. Ultrastructure analysis was performed according to our established protocol (see Chapter 3). Two observers blinded to the urodynamic diagnosis assessed the detrusor ultrastructure on electron micrographs and scored the features on a scoring sheet. Key features of myohypertrophy (myocyte size and irregularity, increased separation of cells, and increase in intercellular collagen) were scored 0,1,2 or 3, and the category of myohypertrophy features was given a global impression score 0,1,2 or 3. Features of degeneration (electron density variation, vacuolation, dense bodies, fragmentation, and extrusion) were scored 0,1,2 or 3, and the category of degeneration features was given a global impression score 0,1,2, or 3. Features of hyperelastosis was given a score of 0,1,2 or 3. An aggregate global impression score was also given. Scores were tabulated and analysed and correlated with clinical and functional outcomes.

Results

Patient clinical outcome data

Control patient

One female patient was recruited as a control (see Table 4). Her age was 52 years. She had a diagnosis of stress urinary incontinence on clinical history, as well as demonstrated on urodynamic study. She had normal bladder sensation, normal compliance, and no detrusor overactivity during the storage phase of urodynamic study. During the voiding phase, she had no evidence of bladder outlet obstruction as calculated by the SGN (<18). She voided to completion.

The control patient was going to undergo cystoscopy as part of her course of treatment at the time of surgical treatment for stress urinary incontinence, and hence was recruited for inclusion into the study. The control patient had bladder biopsy performed for the study at the time of her surgery for stress urinary incontinence.

Table 4.1: Control's demographic and urodynamic study data

Control	Age	Sex	Qmax	pDet	BOO	SUI	DO	PVR
1	52	F	53	20	N	Y	N	0

Age in years. Sex F = female, M = male. Qmax (ml/s) = peak urinary flow rate. pDet (cmH2O) = detrusor pressure at Qmax. BOO defined as BOOI>40 based on Abram-Griffiths number (AGN) for males, BOO defined as Solomon-Greenwell number (SGN) >18 for females, Y = yes, N = no. SUI = stress urinary incontinence. DO = detrusor overactivity. PVR (ml) = post void residual urine measurement.

Figure 4.1: Scanned copy of cystometrogram (CMG) of urodynamic study of control patient 1 showing stable bladder and no evidence of bladder outlet obstruction.

Male bladder outflow obstruction patients

Six male patients with urodynamic confirmed bladder outlet obstruction were included in this study. Their demographic and urodynamic data is displayed in the table below.

Table 4.2: Male bladder outflow obstruction patients' demographic and urodynamic study data

mBOO patient	Age	Sex	Qmax	pDet	BOOI (AGN)	DO	PVR
1	74	M	7	70	56	N	0
2	68	M	9	89	70	N	50
3	64	M	5	70	60	N	100
4	68	M	3	100	94	N	300
5	78	M	0	100	100	Y	750
6	89	M	0	55	55	Y	1000

mBOO = male bladder outlet obstruction. Age in years. Sex M = male. Qmax (ml/s) = peak urinary flow rate. pDet (cmH2O) = detrusor pressure at Qmax. BOO defined as BOOI>40 based on Abram-Griffiths number (AGN) for males; >40 represents bladder outflow obstruction. DO = detrusor overactivity, N = no. PVR (ml) = post void residual urine measurement.

Female bladder outflow obstruction patients

Eight female patients with bladder outflow obstruction were included in this study.

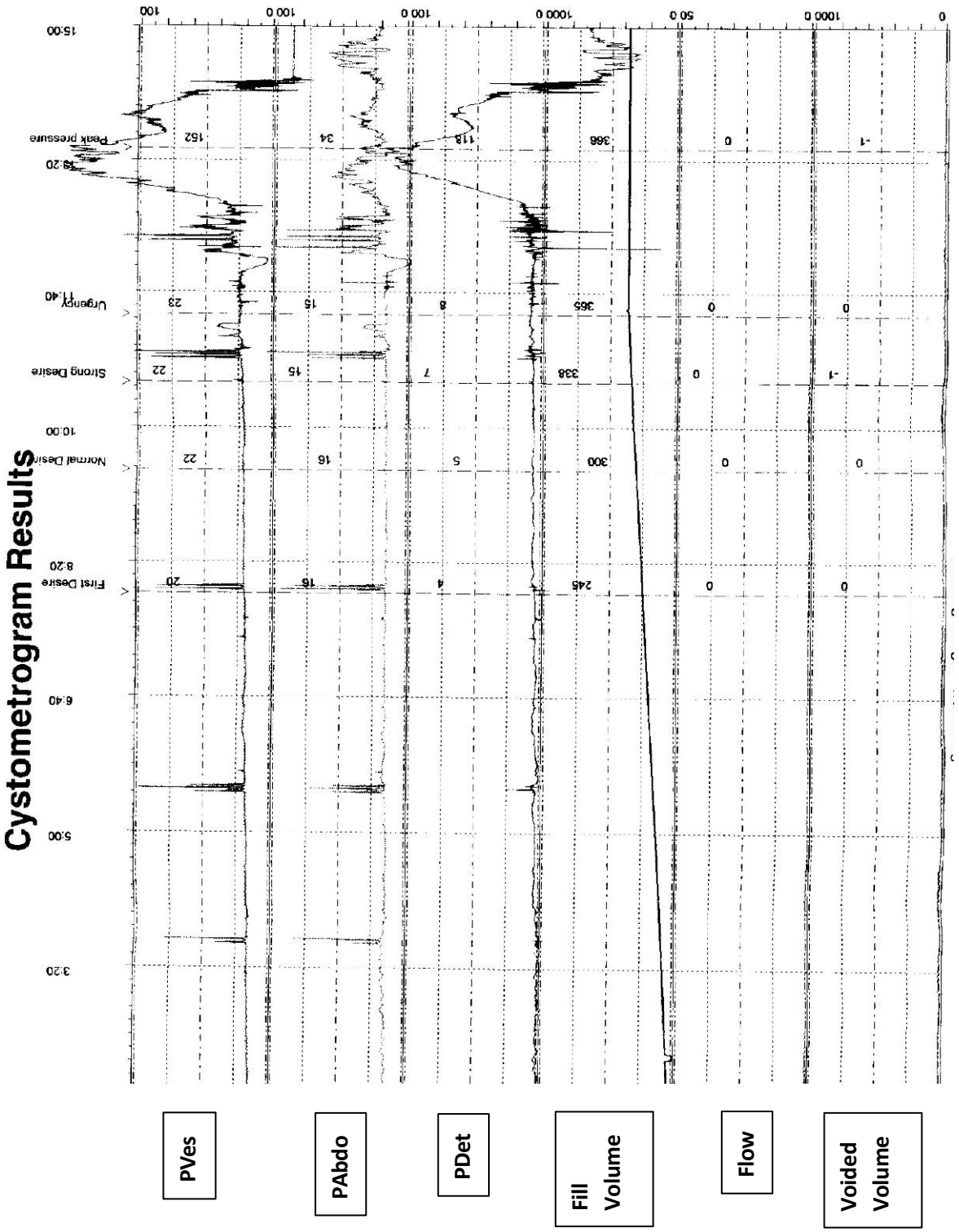
The cause of female bladder outflow obstruction was post midurethral sling in 1 patient (fBOO patient 1), post colposuspension in 1 patient (fBOO patient 2), sphincter active voiding in 3 patients (fBOO patients 3 5, 6), and primary female bladder outflow obstruction in 1 patient (fBOO patient 4). Additionally, two patients had urethral stricture as their cause for bladder outlet obstruction. Their bladder outlet obstruction was diagnosed on severe anatomical pathology, and urodynamic studies were not performed on these patients (fBOO patient 7 and 8). The urethral filling catheters and vesical sensor line needed to conduct urodynamic studies would not have been able to be introduced without causing urethral trauma or dilating the stricture in these two patients.

Table 4.3: Female bladder outflow obstruction patients' demographic and urodynamic study data

fBOO patient	Age	Sex	Voided Volume (ml)	Qmax (ml/s)	pDet (cmH2O)	BOOIf (SGN)	DO	PVR
1	67	F	38	4	23	14.2	N	320
2	66	F	40	1	46	43.8	N	460
3	65	F	90	4	71	62.2	N	400
4	27	F	0	0	118	150	N	390
5	69	F	0	0	150	150	N	300
6	52	F	120	9	UK	Y	UK	700
7	47	F	48	6.4	UK	Urethral stricture	UK	153
8	57	F	UK	UK	UK	Urethral stricture	UK	UK

fBOO = female bladder outlet obstruction. Age in years. Sex F = male. Qmax (ml/s) = peak urinary flow rate. pDet (cmH2O) = detrusor pressure at Qmax. BOO defined as BOOIf, also known as Solomon-Greenwell number (SGN) >5 for females is likely obstructed (>50%), SGN>18 for females is almost certainly obstructed (>90%); SGN>18 represents bladder outlet obstruction, Y=yes, N=no. DO = detrusor overactivity, N = no. PVR (ml) = postvoid residual urine measurement. UK = unknown.

Figure 4.2: Scanned copy of CMG of fBOO patient 4 showing high pressure low flow voiding.



Detrusor ultrastructural analysis results

Control

The detrusor ultrastructural features were recorded and tabulated. The control patient's detrusor ultrastructure was moderate in myohypertrophy global impression score, mild degeneration, and there was little elastosis present. Aggregate global impression score was 1. The features are displayed in the table below.

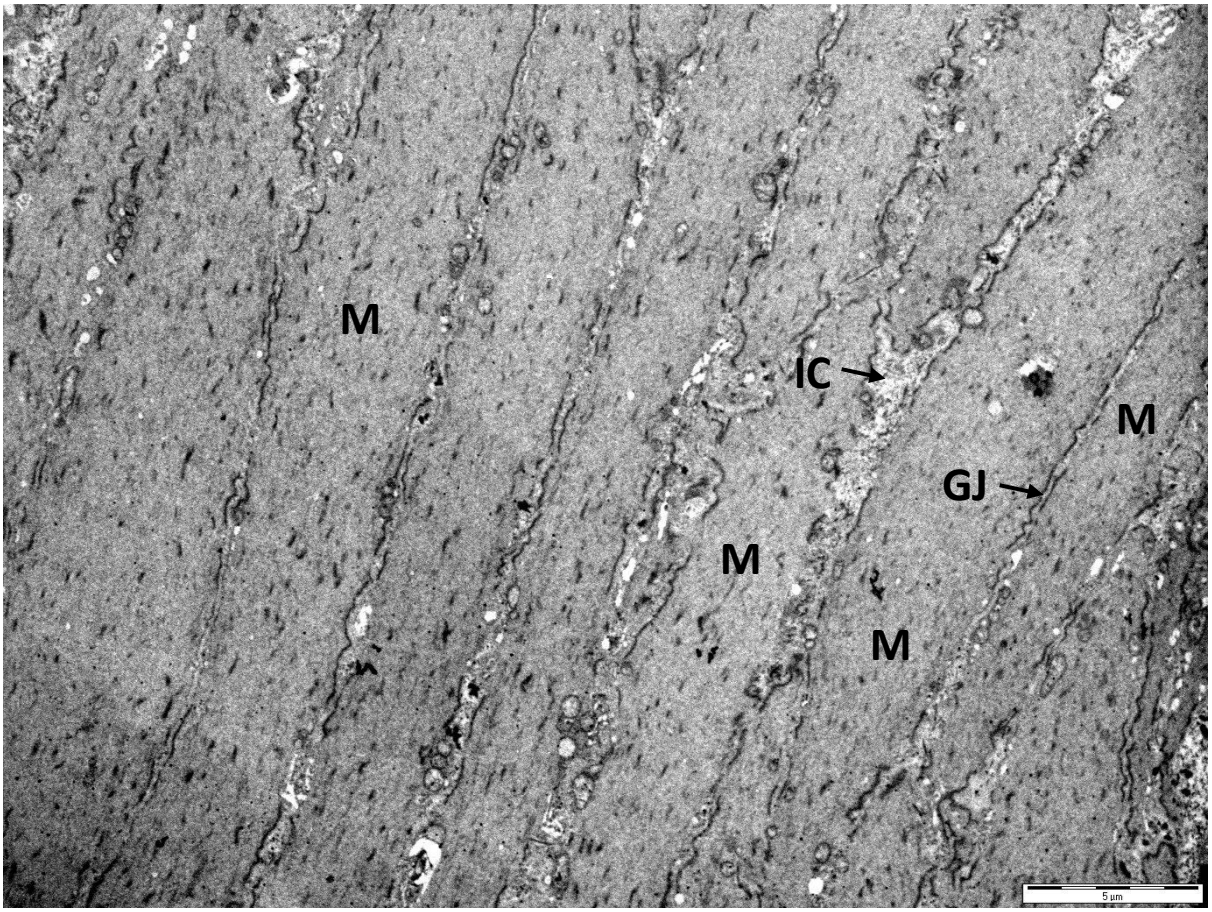
Table 4.4: Detrusor ultrastructural features of control patient.

Control patient	Agg Global	Myoh Global	Cell	Sep	Coll	Degen	Var	Vac	Frag	Elas
1	1	2	2	1	2	1	1	1	1	1

Agg Global = aggregate global impression score. Myoh Global = myohypertrophy category global impression score. Cell = cell size and irregularity. Sep = increased separation of cells. Coll = increase in intercellular collagen. Degen = Degeneration category global impression score. Var = electron density variation. Vac = vacuolation and dense bodies. Frag = fragmentation and exclusion. Elas = hyperelastosis.

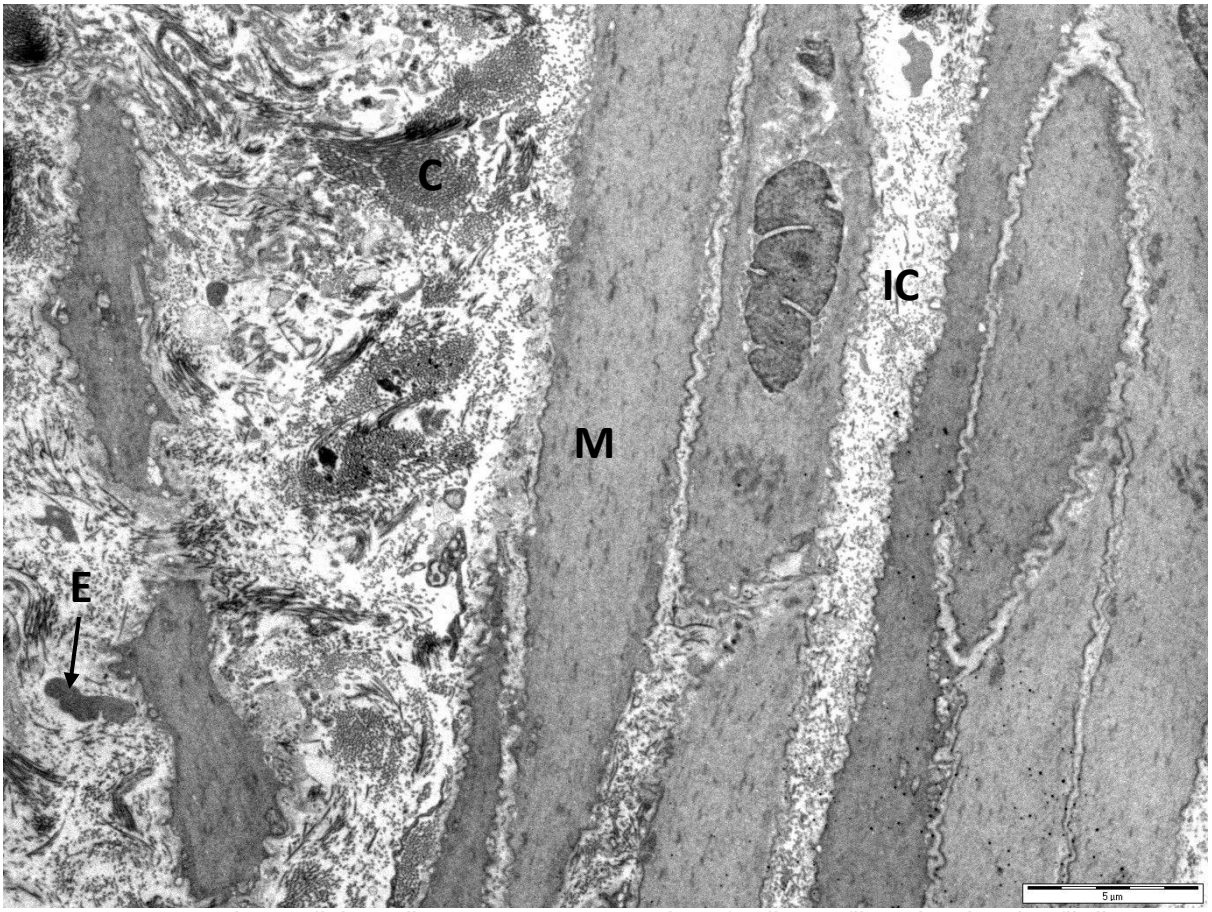
Figure 4.3.1: Micrograph of detrusor ultrastructure of female control patient 1.

There is regular fascicular arrangement of myocytes and relatively uniform density and size of myocytes.



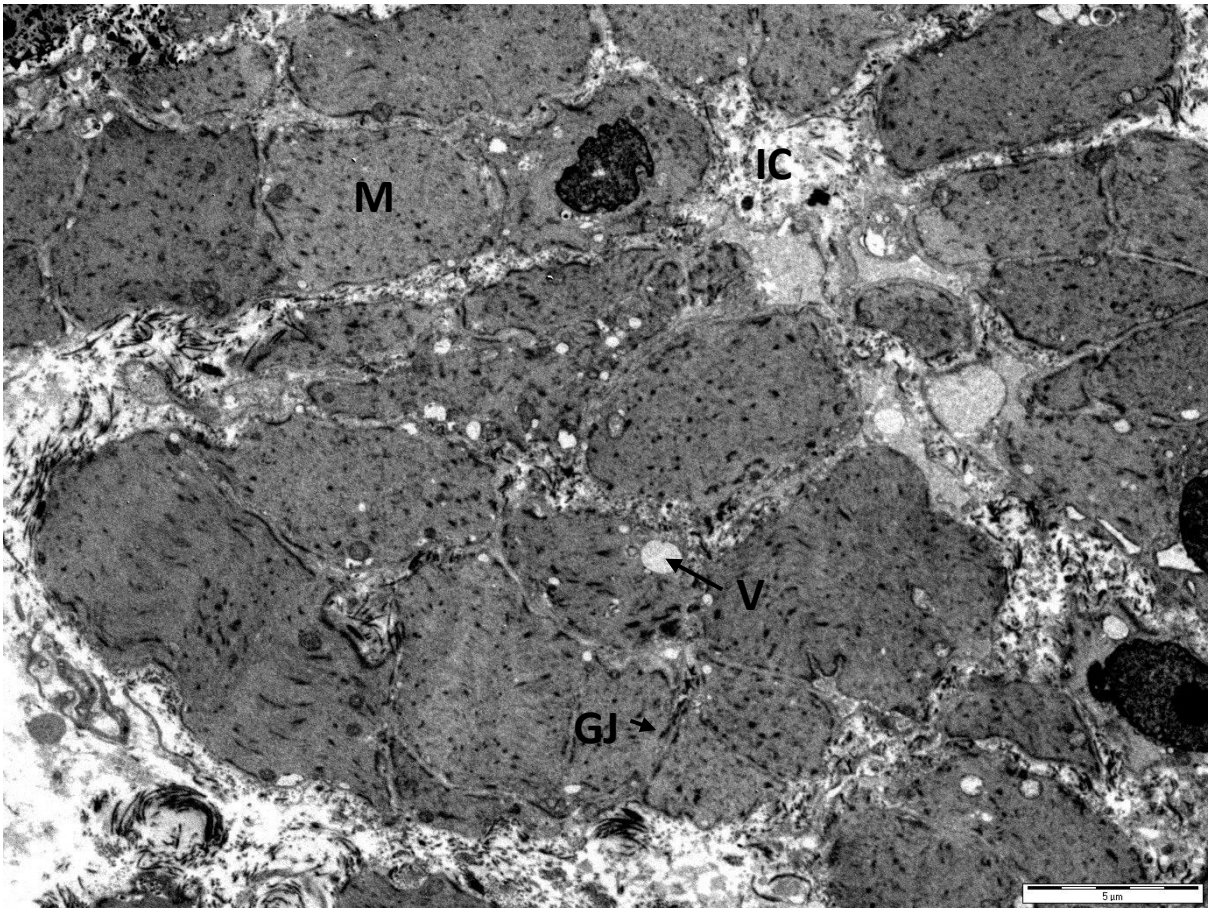
M = myocyte ; IC = intercellular collagen ; GJ = gap junction.

Figure 4.3.48: Micrograph of detrusor ultrastructure of female control patient



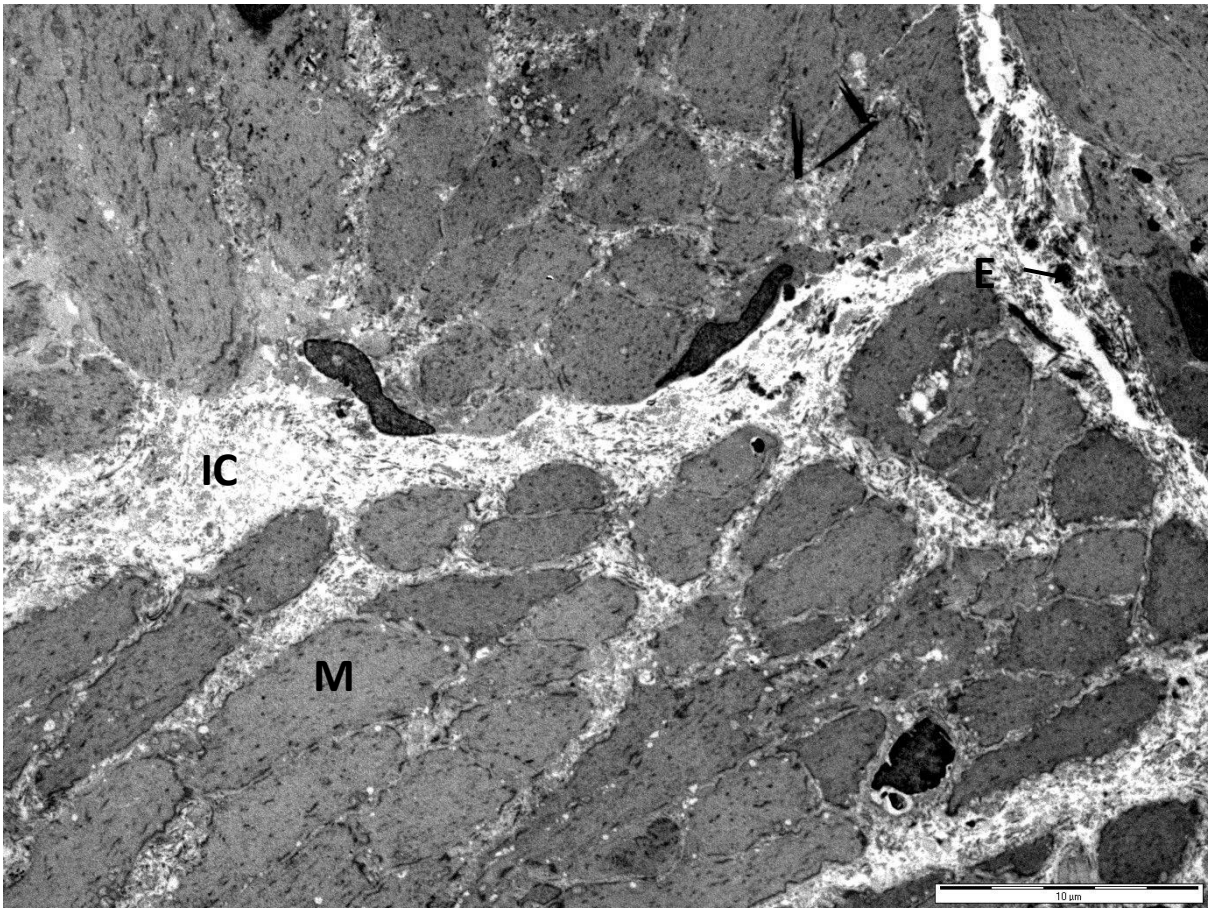
M = myocyte ; IC = intercellular collagen ; C = cross section of collagen fibre showing tiny fibrils ; E = electron dense chunks of elastin cut in tangential section.

Figure 4.3.49: Micrograph of detrusor ultrastructure of female control patient



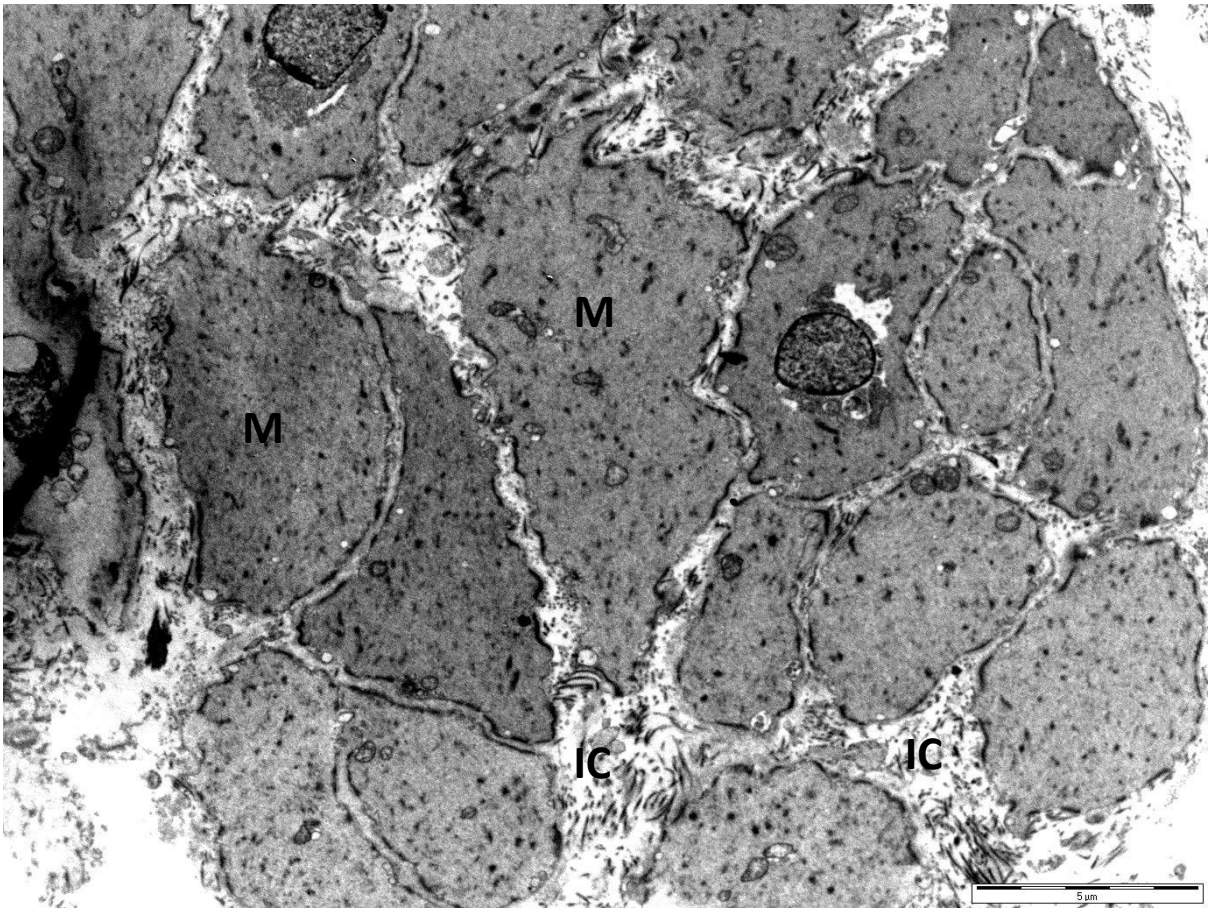
M = myocyte ; IC = intercellular collagen ; GJ = gap junction ‘ V = vacuolation.

Figure 4.3.50: Micrograph of detrusor ultrastructure of female control patient



M = myocyte ; IC = intercellular collagen ; E = elastin

Figure 4.3.51: Micrograph of detrusor ultrastructure of female control patient



M = myocyte ; IC = intercellular collagen.

Male bladder outlet obstruction patients

The detrusor ultrastructural features of males with bladder outlet obstruction are shown in the table below. There was a range of features identified.

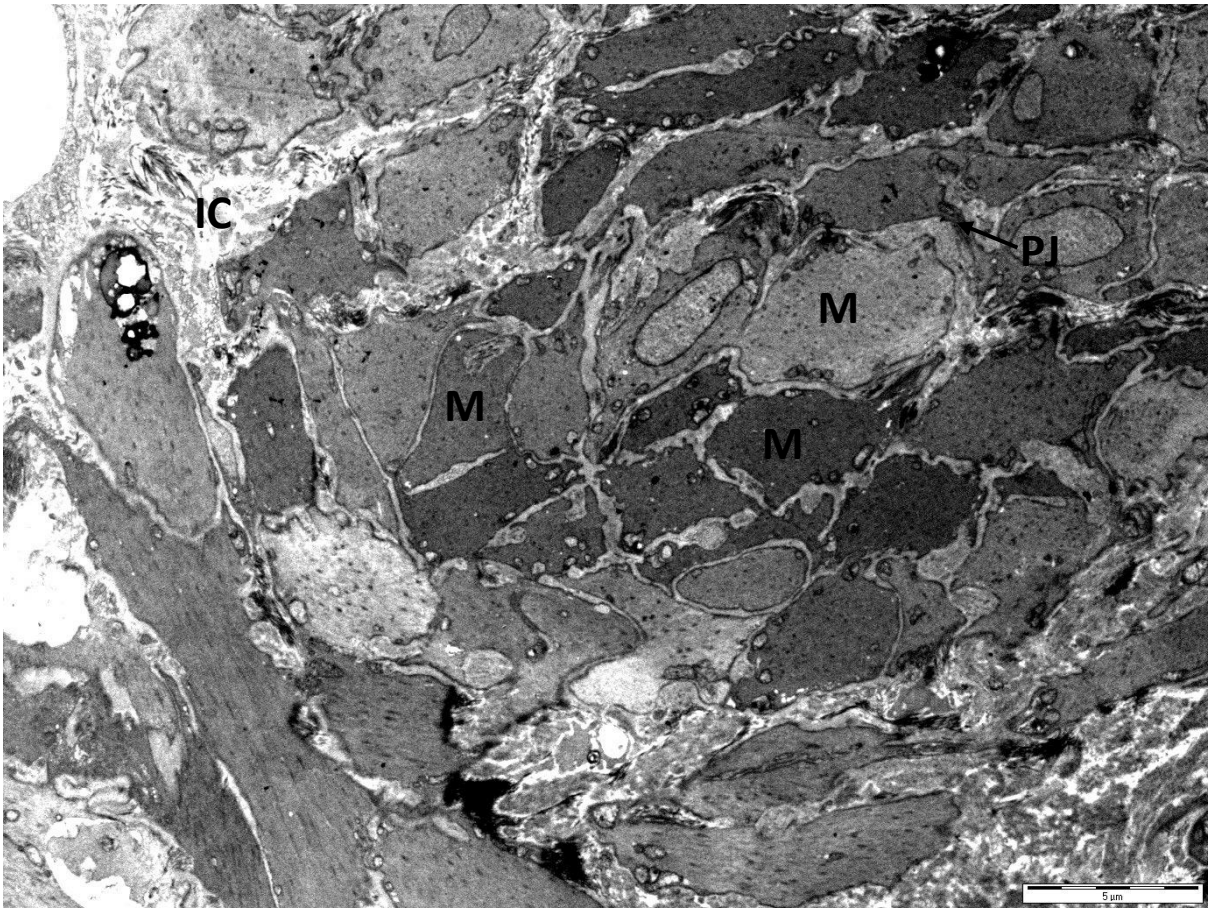
Table 4.5: Detrusor ultrastructural features of males with bladder outlet obstruction.

mBOO patient	Agg Global	Myoh Global	Cell	Sep	Coll	Degen	Var	Vac	Frag	Elas
1	2	2	1	2	2	2	1	2	1	1
2	1	1	1	1	1	2	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1
4	2	2	2	2	2	2	2	2	2	1
5	2	2	2	2	2	2	1	2	1	1
6	3	3	2	3	3	3	2	2	1	1

Agg Global = aggregate global impression score. Myoh Global = myohypertrophy category global impression score. Cell = cell size and irregularity. Sep = increased separation of cells. Coll = increase in intercellular collagen. Degen = Degeneration category global impression score. Var = electron density variation. Vac = vacuolation and dense bodies. Frag = fragmentation and exclusion. Elas = hyperelastosis.

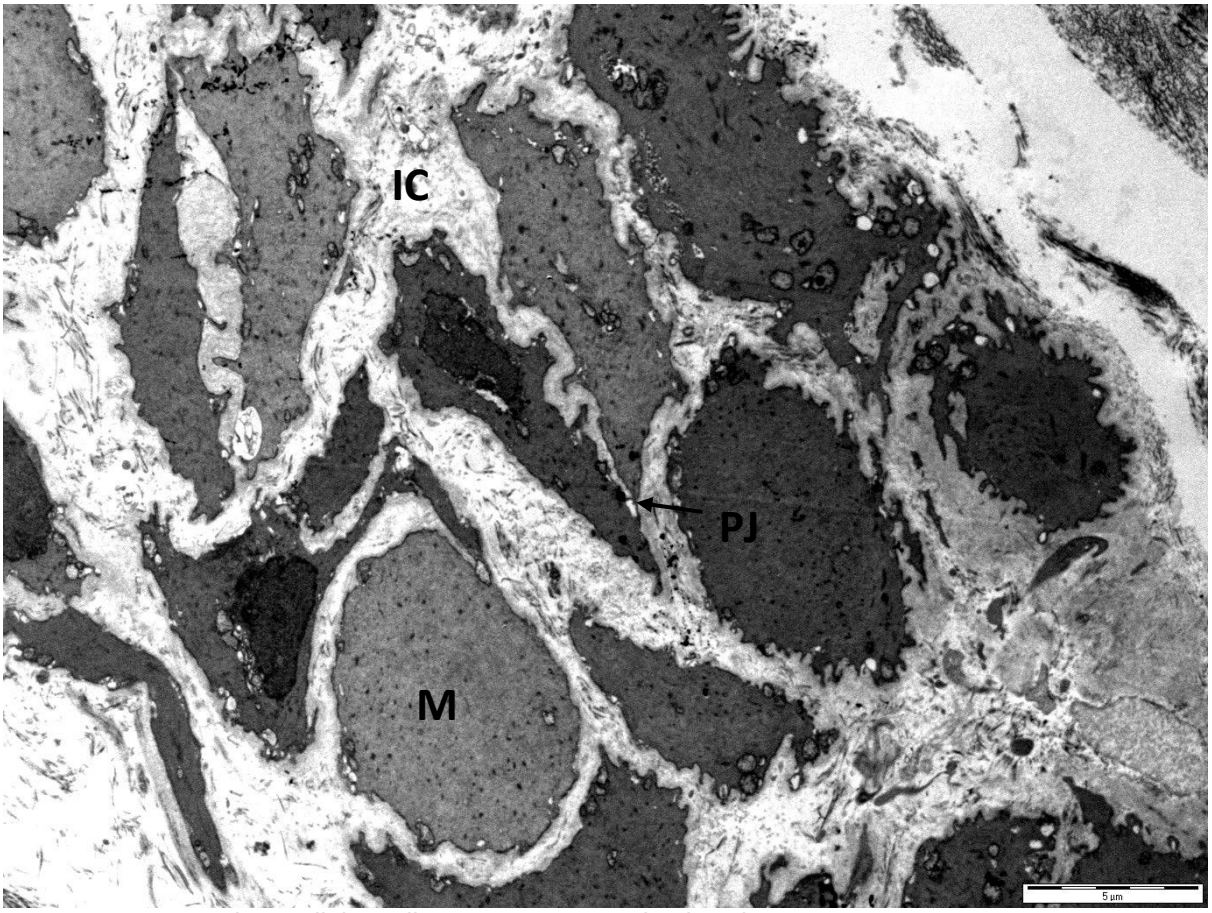
Figure 4.4.52: Micrograph of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6).

There is myohypertrophy pattern with muscle fascicle derangement, myocyte irregularity, cell separation, collagenosis, and abnormal junctions. There is also electron density variation.



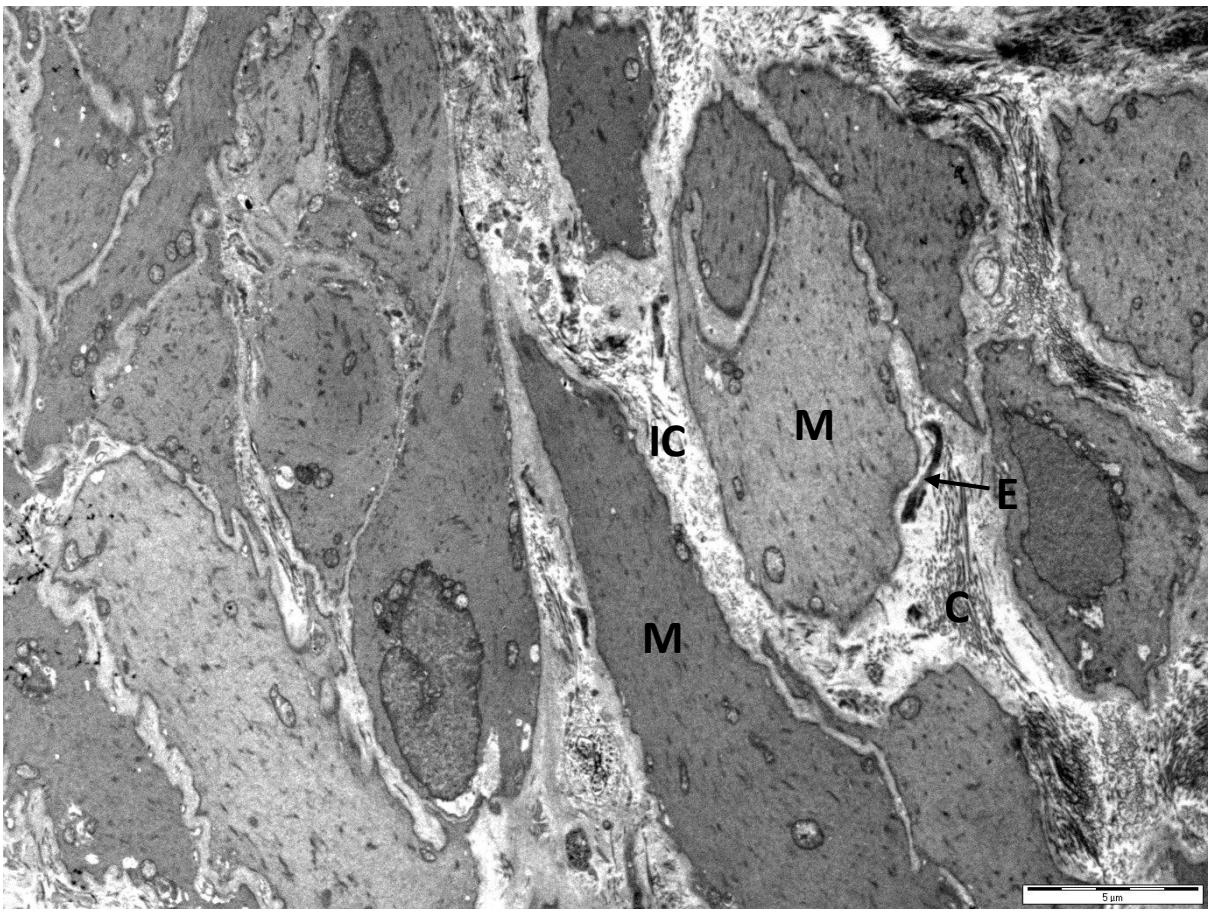
M = myocyte ; IC = intercellular collagen ; PJ = protrusion junction.

Figure 4.4.53: Micrograph of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6).



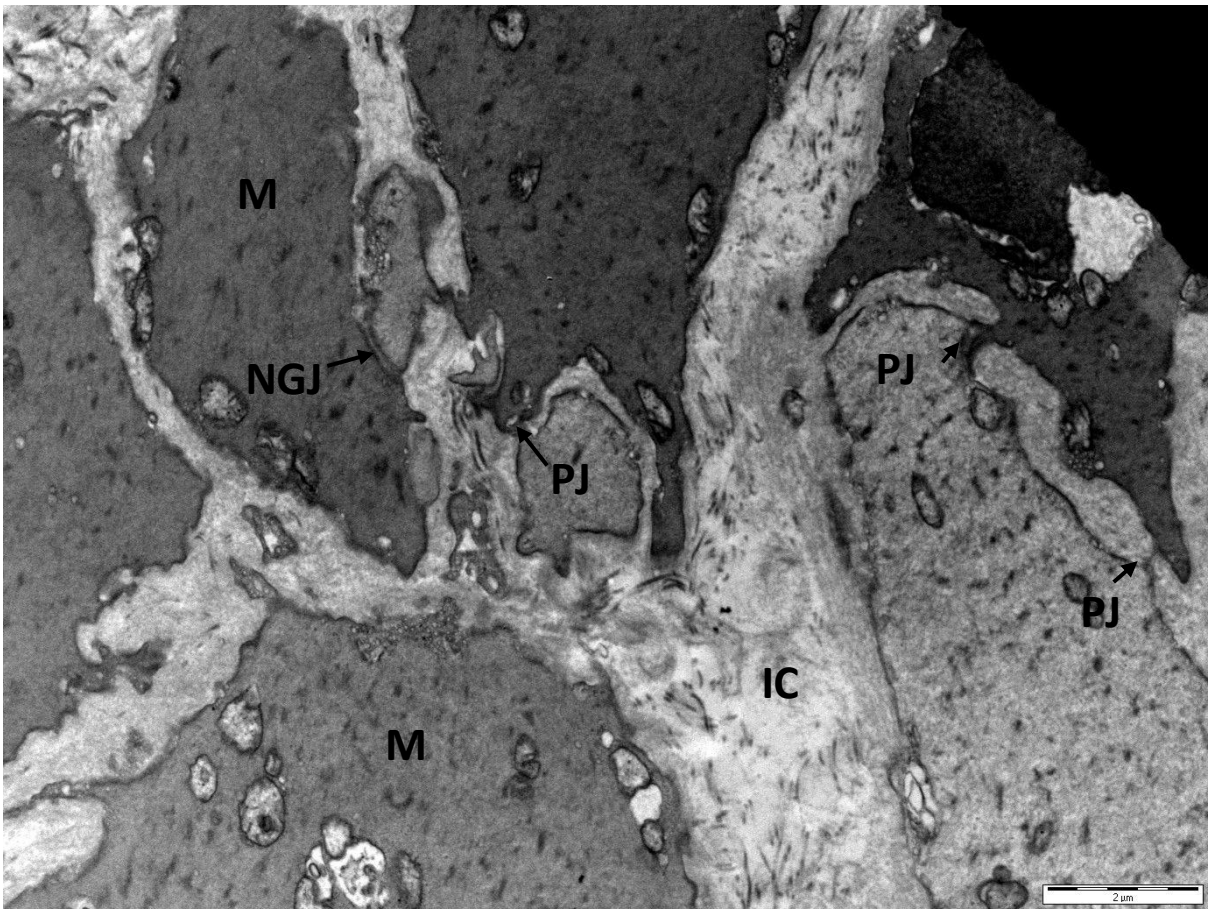
M = myocyte ; IC = intercellular collagen ; PJ = protrusion junction.

Figure 4.4.3: Micrograph of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6)



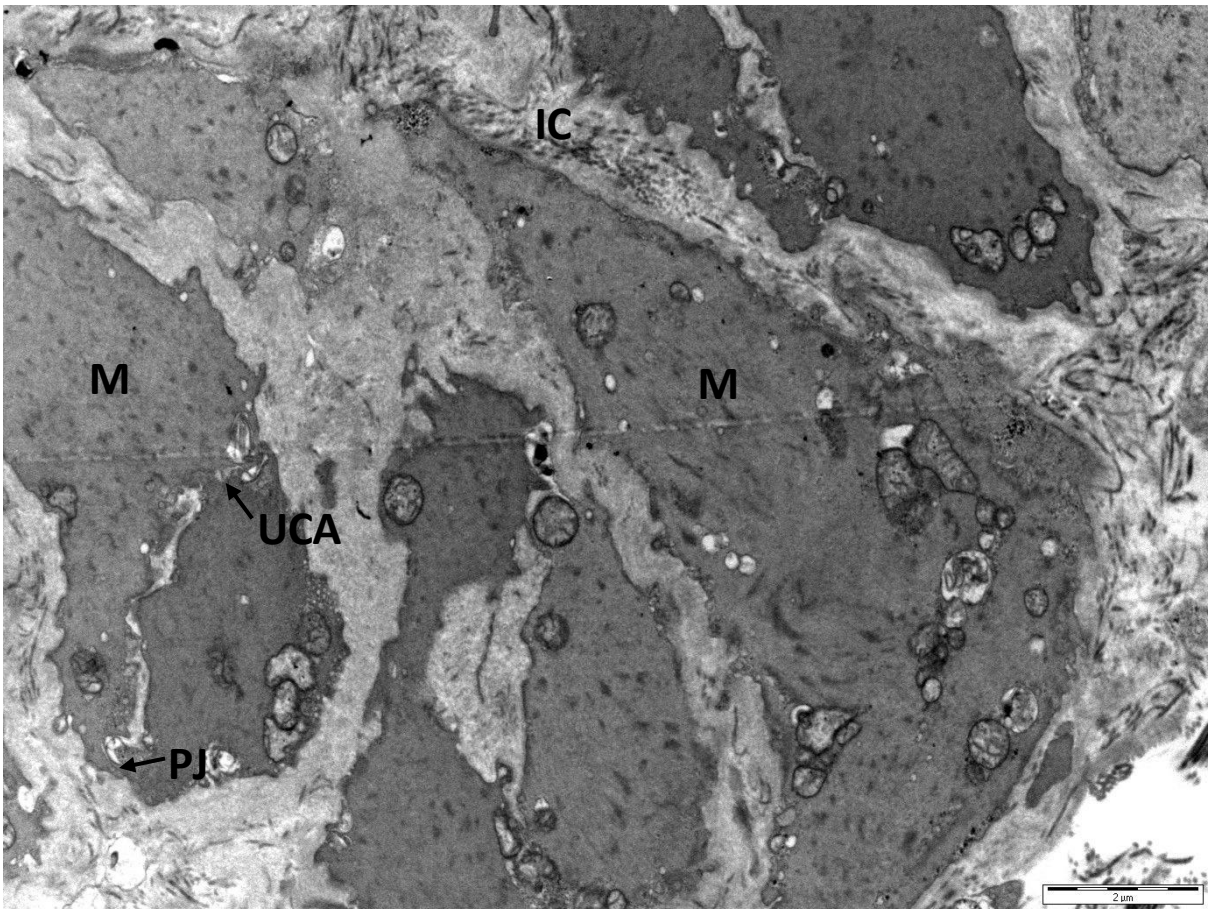
M = myocyte ; IC = intercellular collagen ; E = elastin ; C = collagen.

Figure 4.4.4: Micrograph of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6).



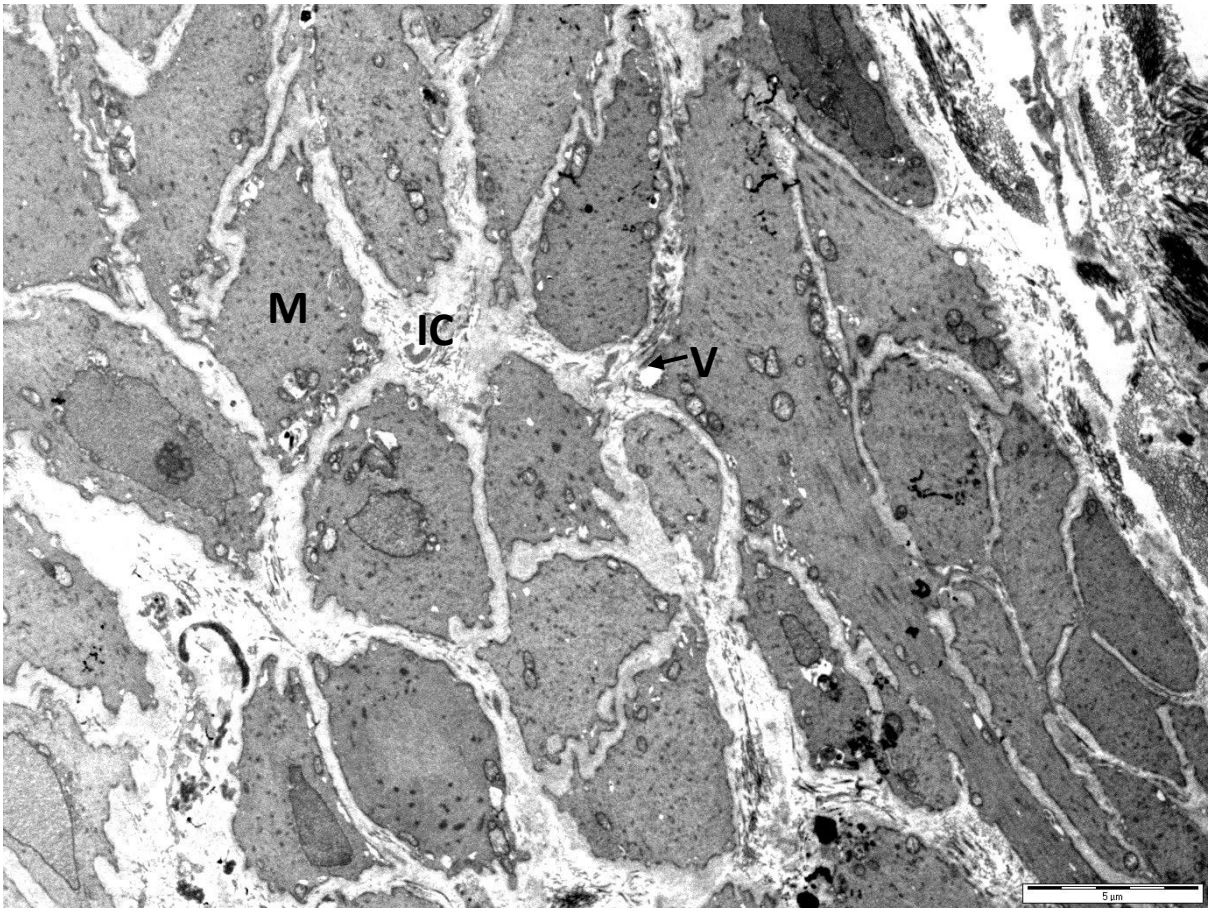
M = myocyte ; IC = intercellular collagen ; NGJ = normal gap junction ; PJ = protrusion junction.

Figure 4.4.5: Micrographs of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6).



M = myocyte ; IC = intercellular collagen ; PJ = protrusion junction ; UCA – ultraclose abutment.

Figure 4.4.6: Micrographs of detrusor ultrastructure of male patient with bladder outlet obstruction (mBOO patient 6).



M = myocyte ; IC = intercellular collagen ; V = vacuolation.

Female bladder outlet obstruction patients

The detrusor ultrastructural features of females with bladder outlet obstruction were analysed and are displayed in the following table.

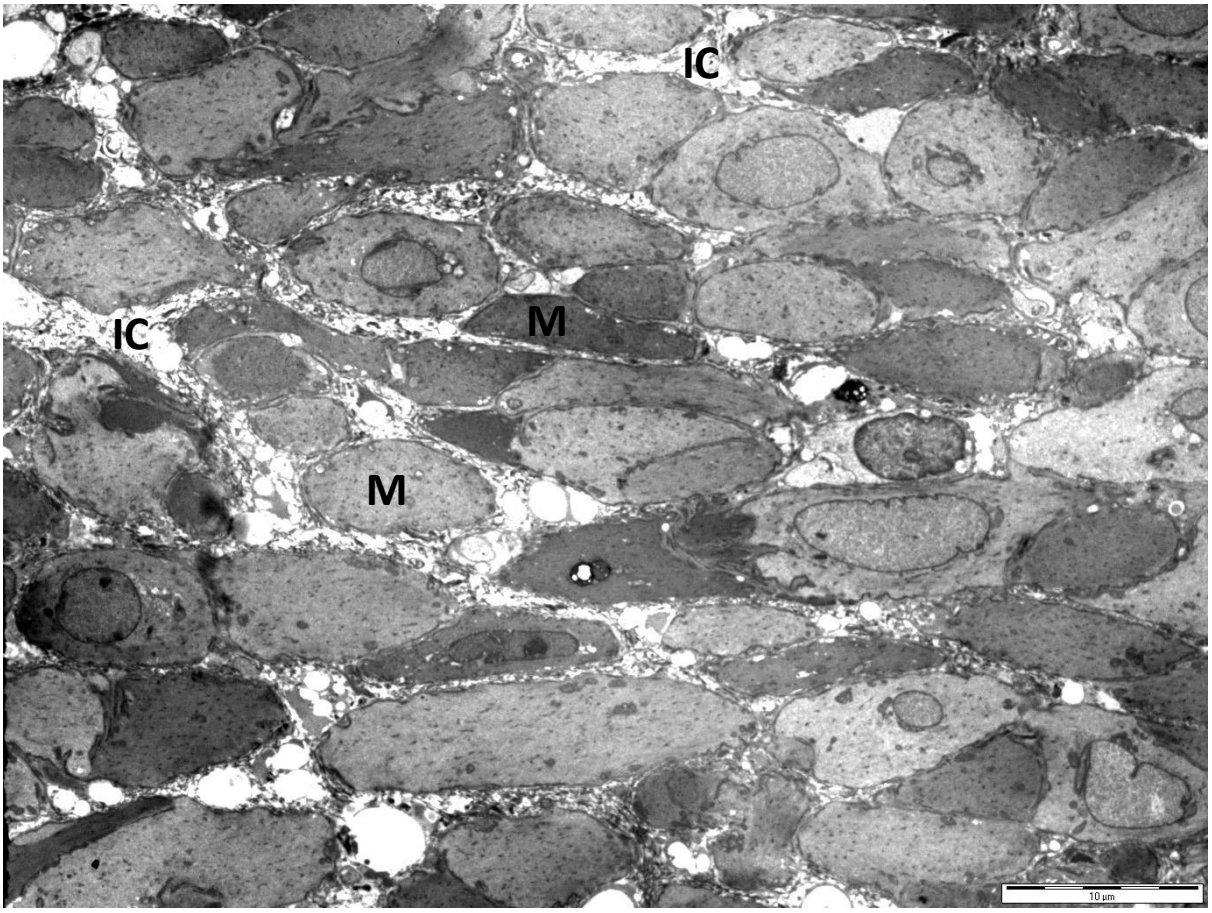
Table 4.6: Detrusor ultrastructural features in females with bladder outflow obstruction

fBOO patient	Agg Global	Myoh Global	Cell	Sep	Coll	Degen	Var	Vac	Frag	Elas
1	3	2	3	2	2	2	3	3	1	1
2	2	2	2	3	3	2	2	1	1	2
3	2	2	2	2	2	2	1	2	1	1
4	2	1	2	2	3	2	2	1	1	2
5	2	2	2	2	1	2	3	2	1	1
6	3	3	3	3	3	3	2	3	3	3
7	3	3	3	3	3	3	3	3	2	3
8	3	3	3	3	3	2	2	2	2	2

Agg Global = aggregate global impression score. Myoh Global = myohypertrophy category global impression score. Cell = cell size and irregularity. Sep = increased separation of cells. Coll = increase in intercellular collagen. Degen = Degeneration category global impression score. Var = electron density variation. Vac = vacuolation and dense bodies. Frag = fragmentation and exclusion. Elas = hyperelastosis.

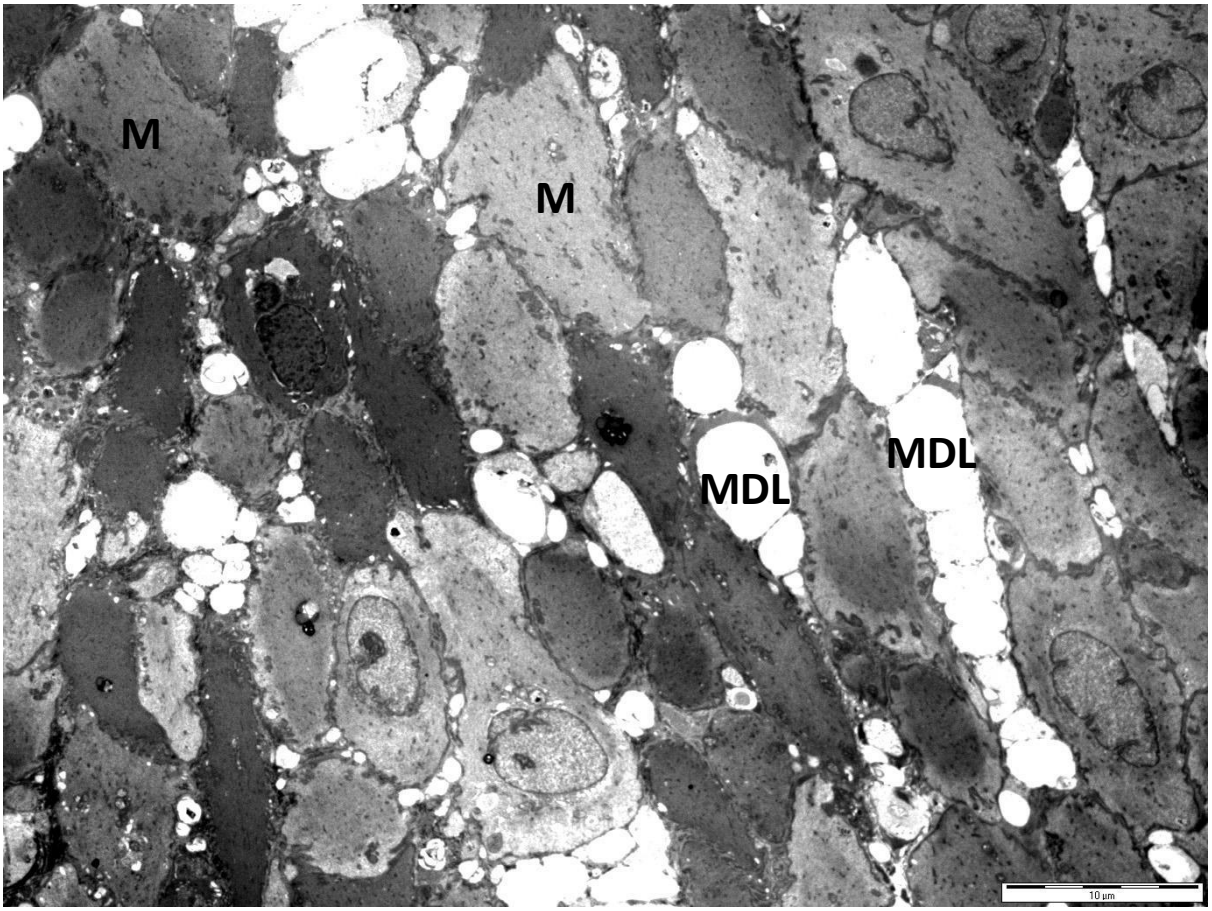
Figure 4.5.59: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction Female bladder outlet obstruction was in the context of history of synthetic mesh transvaginal pelvic organ prolapse repair and synthetic mesh transobturator midurethral sling. She subsequently presented with voiding lower urinary tract symptoms and recurrent urinary tract symptoms, leading to a 10 year history of absence of feeling of urgency to void, feeling of incomplete emptying, and eventually required an indwelling catheter for voiding dysfunction.

Her micrographs show myohypertrophy pattern with marked variation in myocyte size and shape, increased intercellular collagen, and electron density variation.



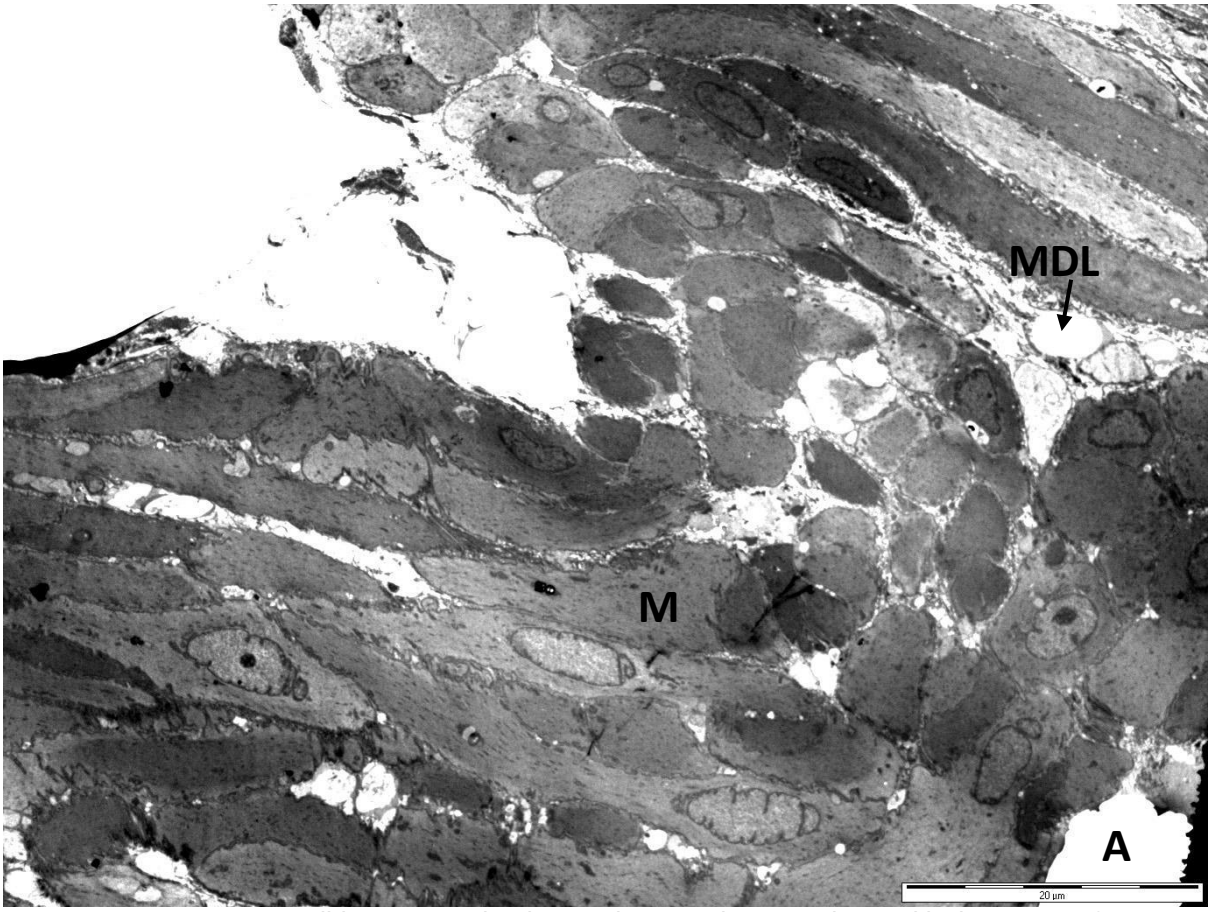
M = myocyte ; IC = intercellular collagen.

Figure 4.5.60: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction



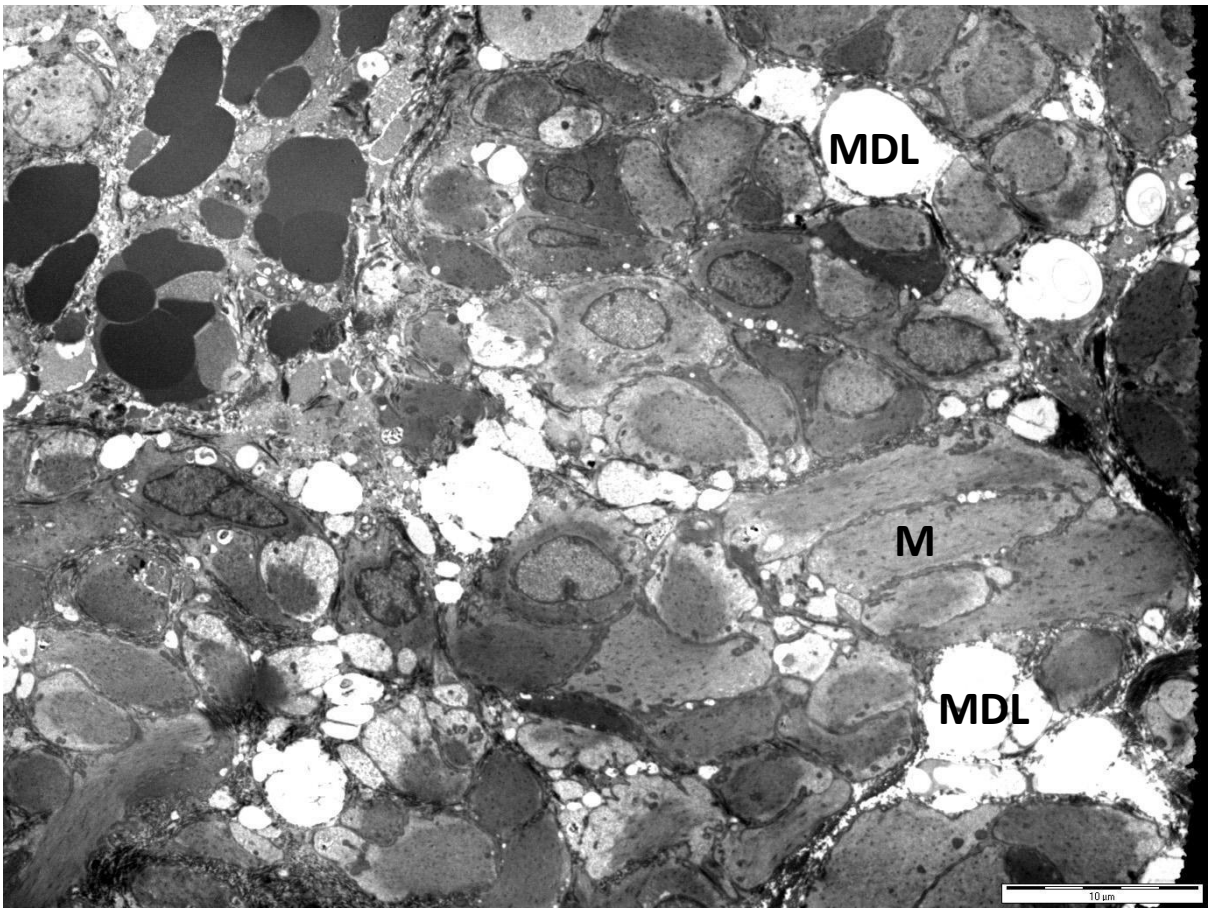
M = myocyte ; MDL = possible myocyte that has undergone degeneration and lysis.

Figure 4.5.61: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction



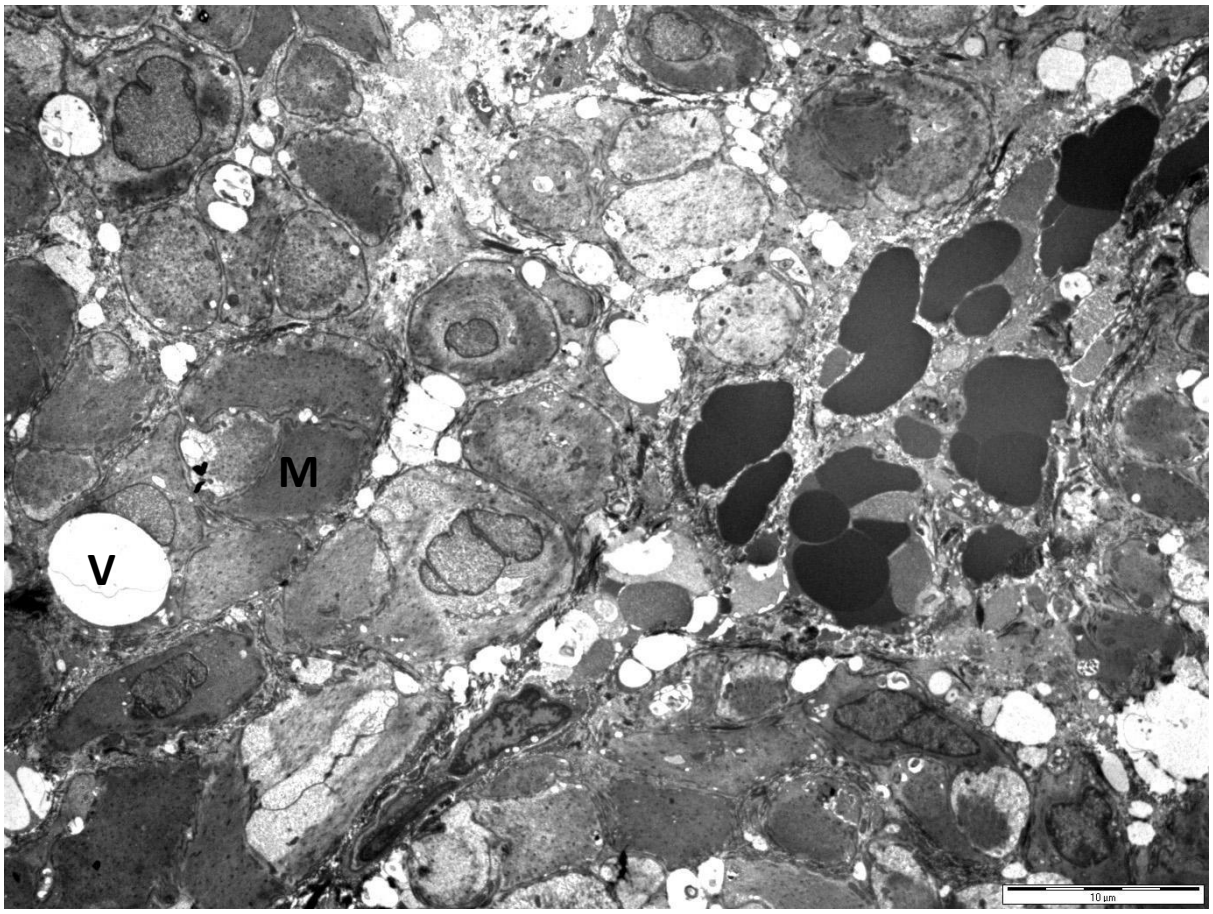
M = myocyte ; MDL = possible myocyte that has undergone degeneration and lysis ; A = artefact near edge of copper grid.

Figure 4.5.62: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction



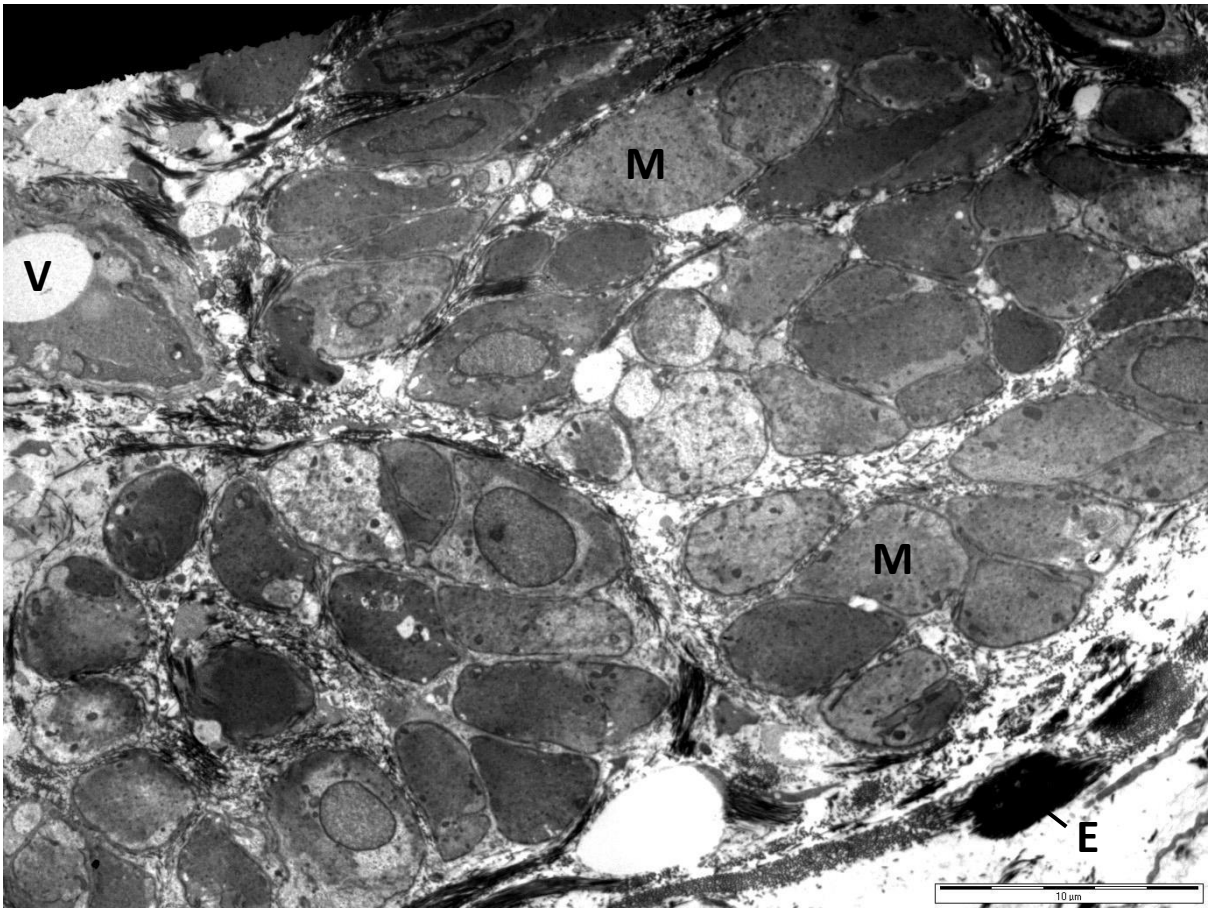
M = myocyte ; MDL = possible myocyte that has undergone degeneration and lysis.

Figure 4.5.63: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction



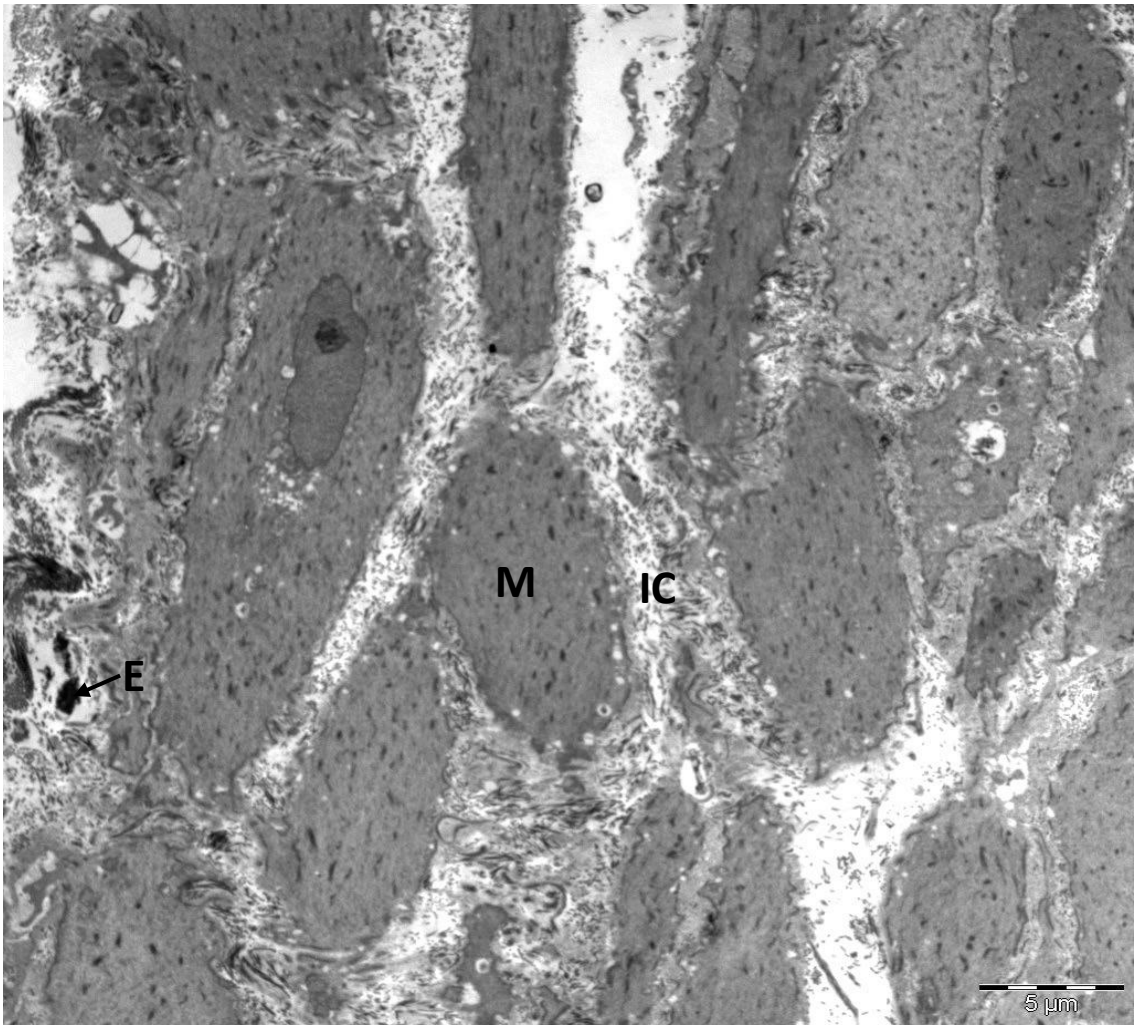
M = myocyte ; V = large vacuole within cell.

Figure 4.5.64: Micrograph of detrusor ultrastructure of female with bladder outlet obstruction



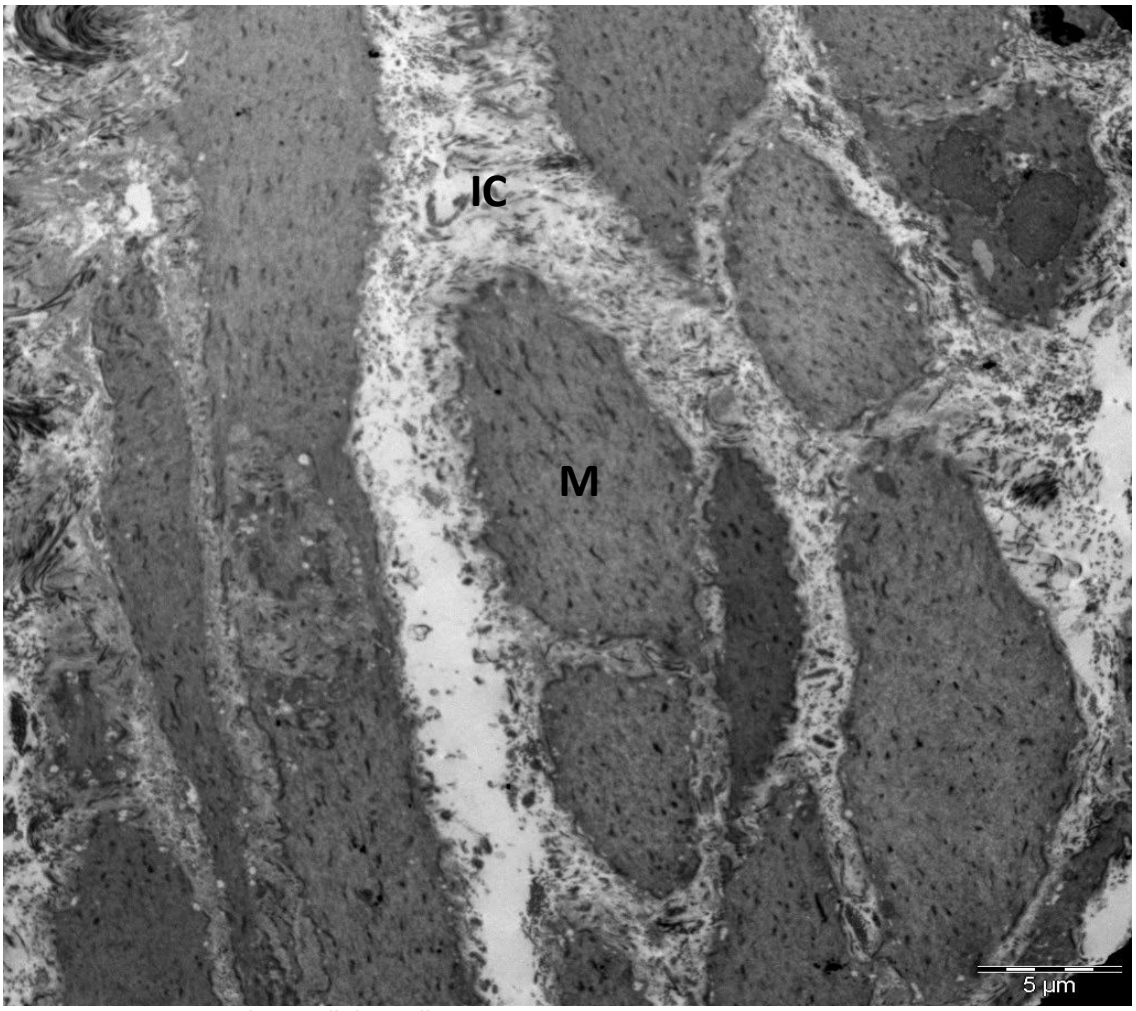
M = myocyte ; V = vacuolation ; E = elastin.

Figures 4.6.1: Micrograph of female bladder outlet obstruction patient 3. Female bladder outlet obstruction with aetiology due to sphincter active voiding.



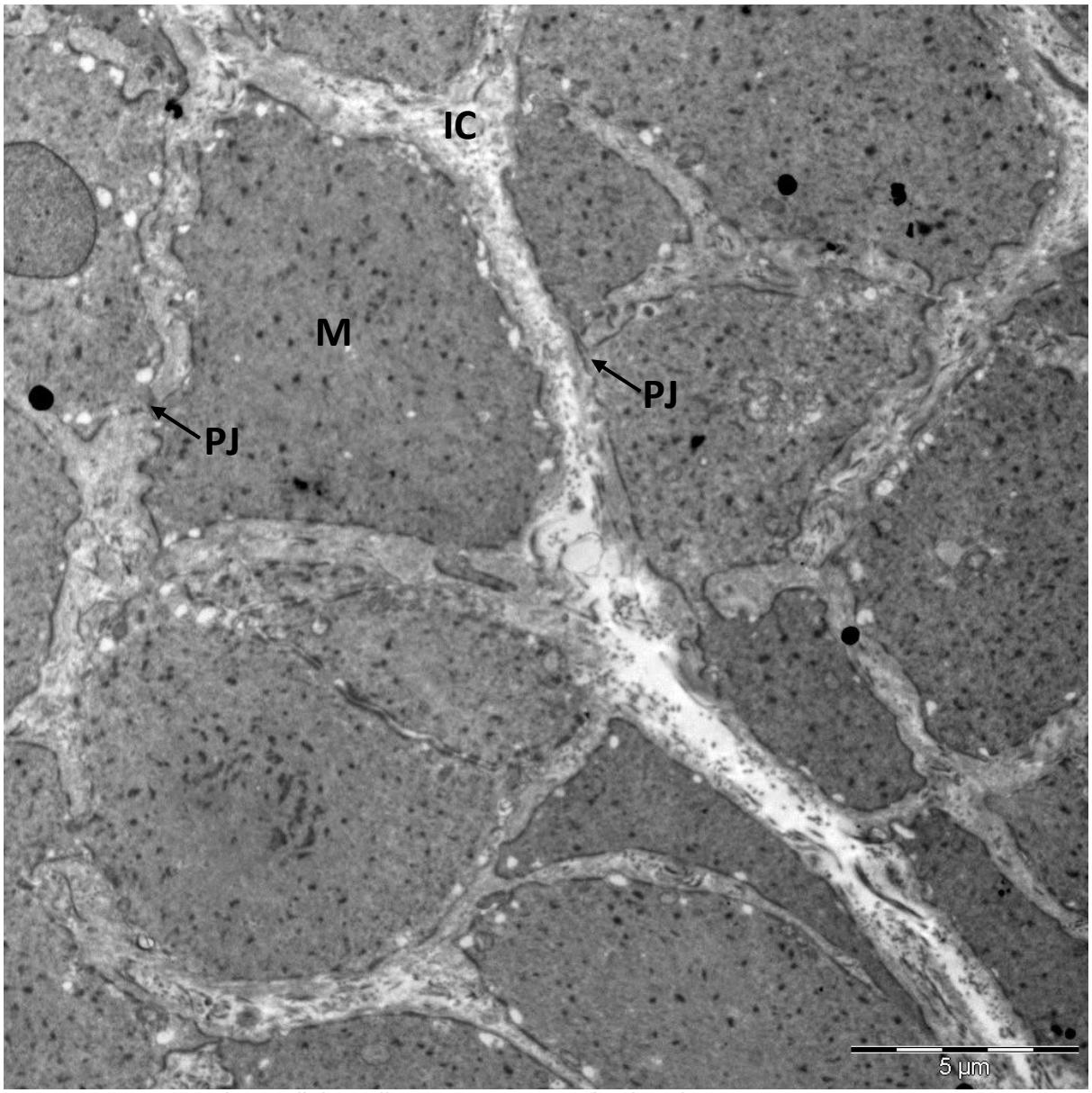
M = myocyte ; IC = intercellular collagen.

Figure 4.6.2: Micrograph of female bladder outlet obstruction patient 3.



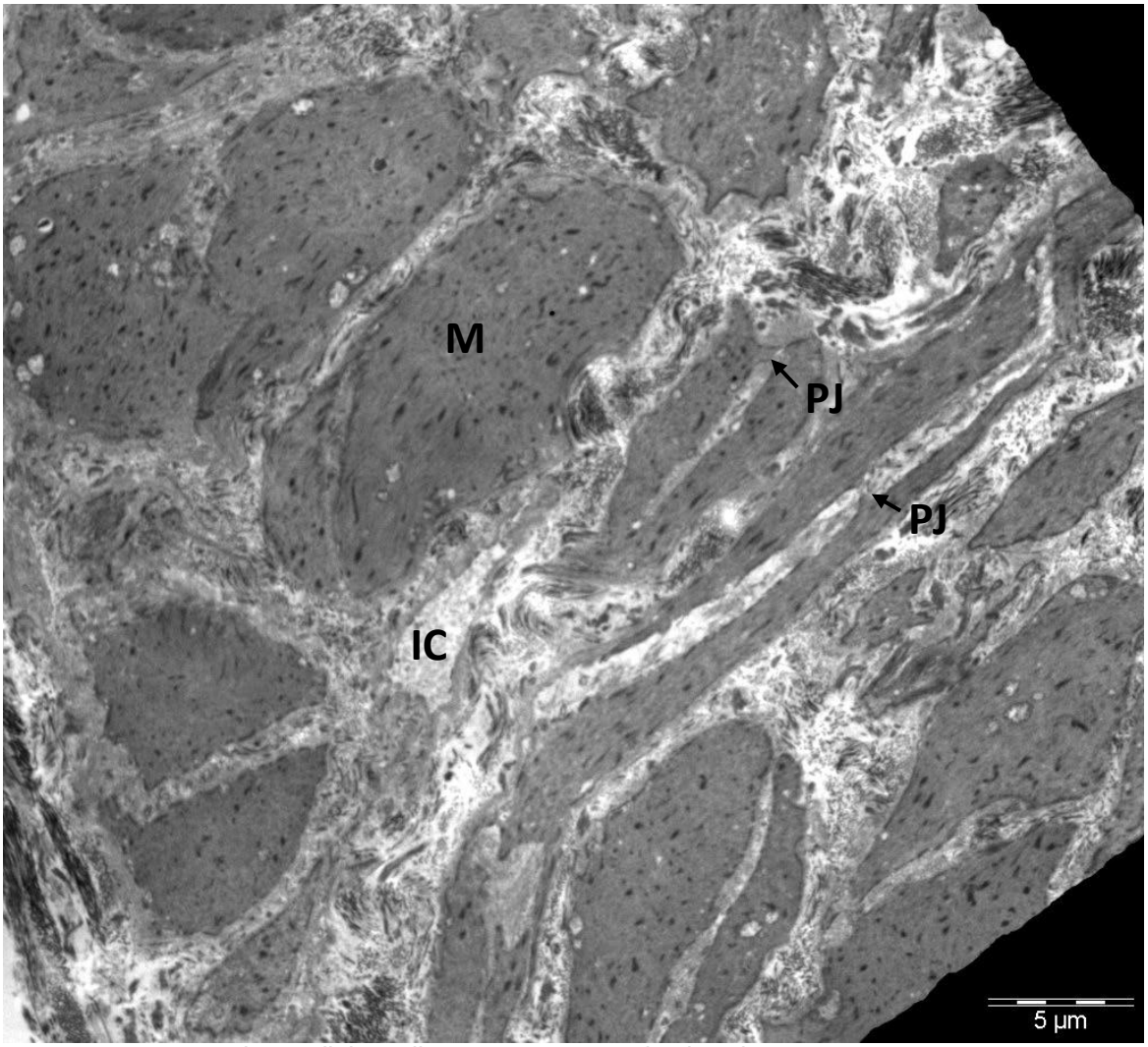
M = mycocyte ; IC = intercellular collagen.

Figure 4.6.67: Micrograph of female bladder outlet obstruction patient



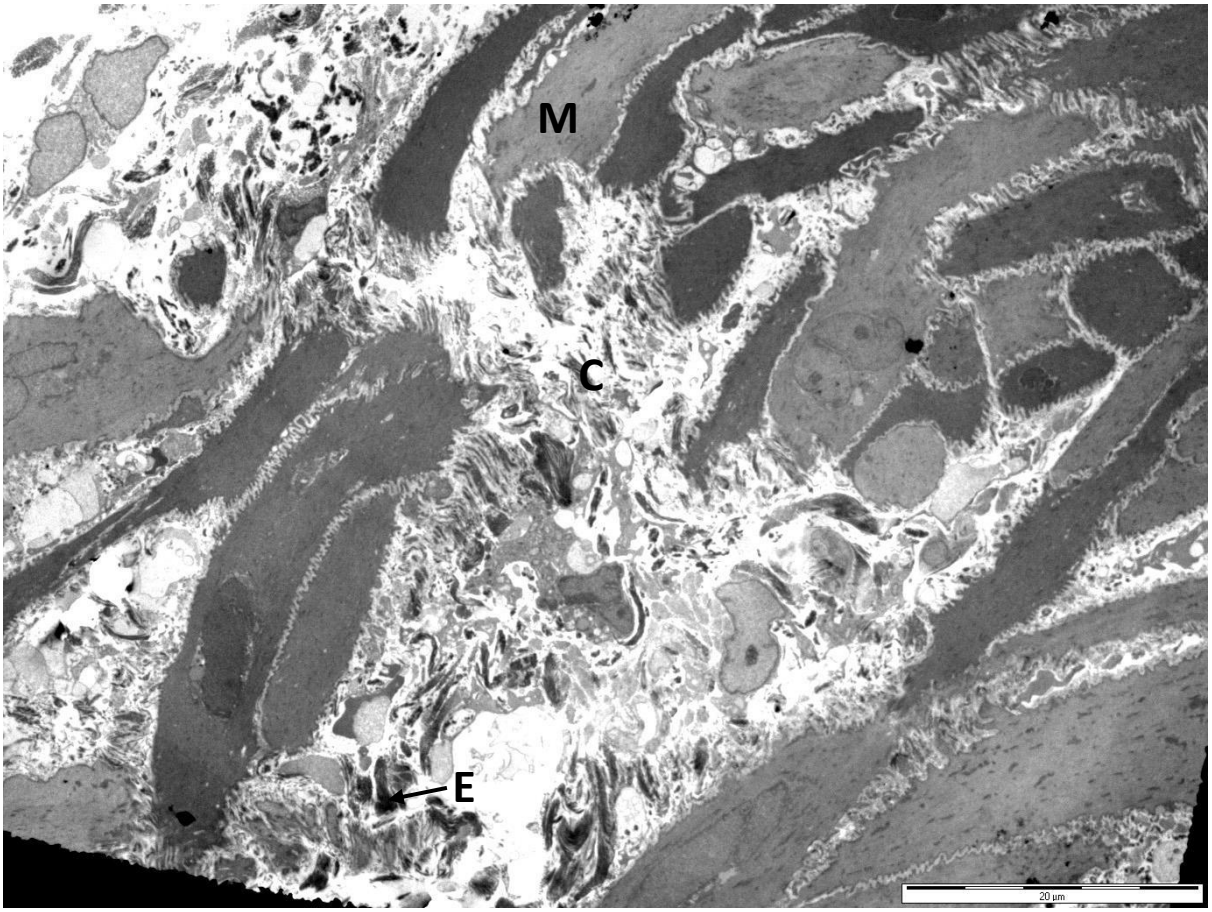
M = mycocyte ; IC = intercellular collagen ; PJ = protrusion junction.

Figure 4.6.68: Micrograph of female bladder outlet obstruction patient



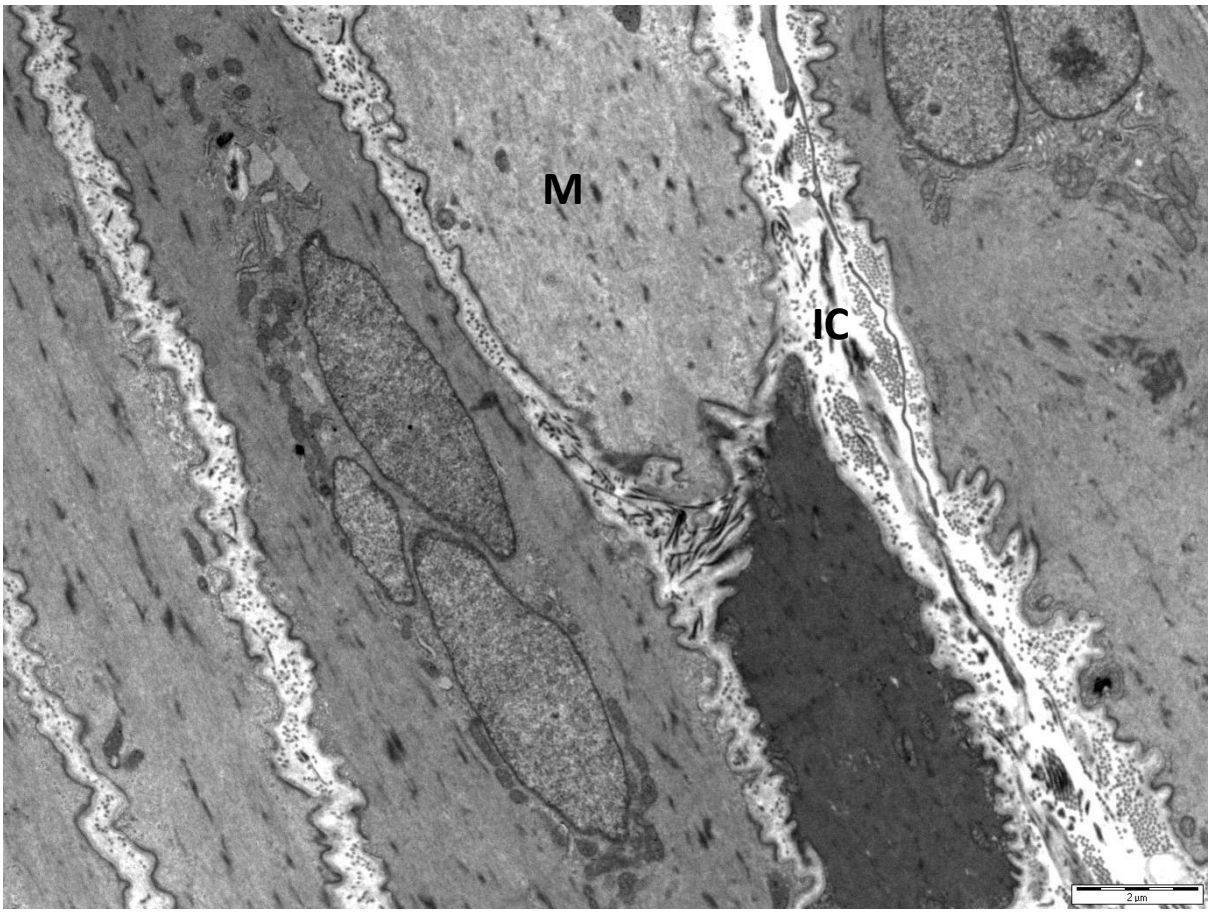
M = mycocyte ; IC = intercellular collagen ; PJ = protrusion junction.

Figure 4.7.1: Micrograph of female bladder outlet obstruction patient 7. This patient had female bladder outlet obstruction with aetiology due to urethral stricture.



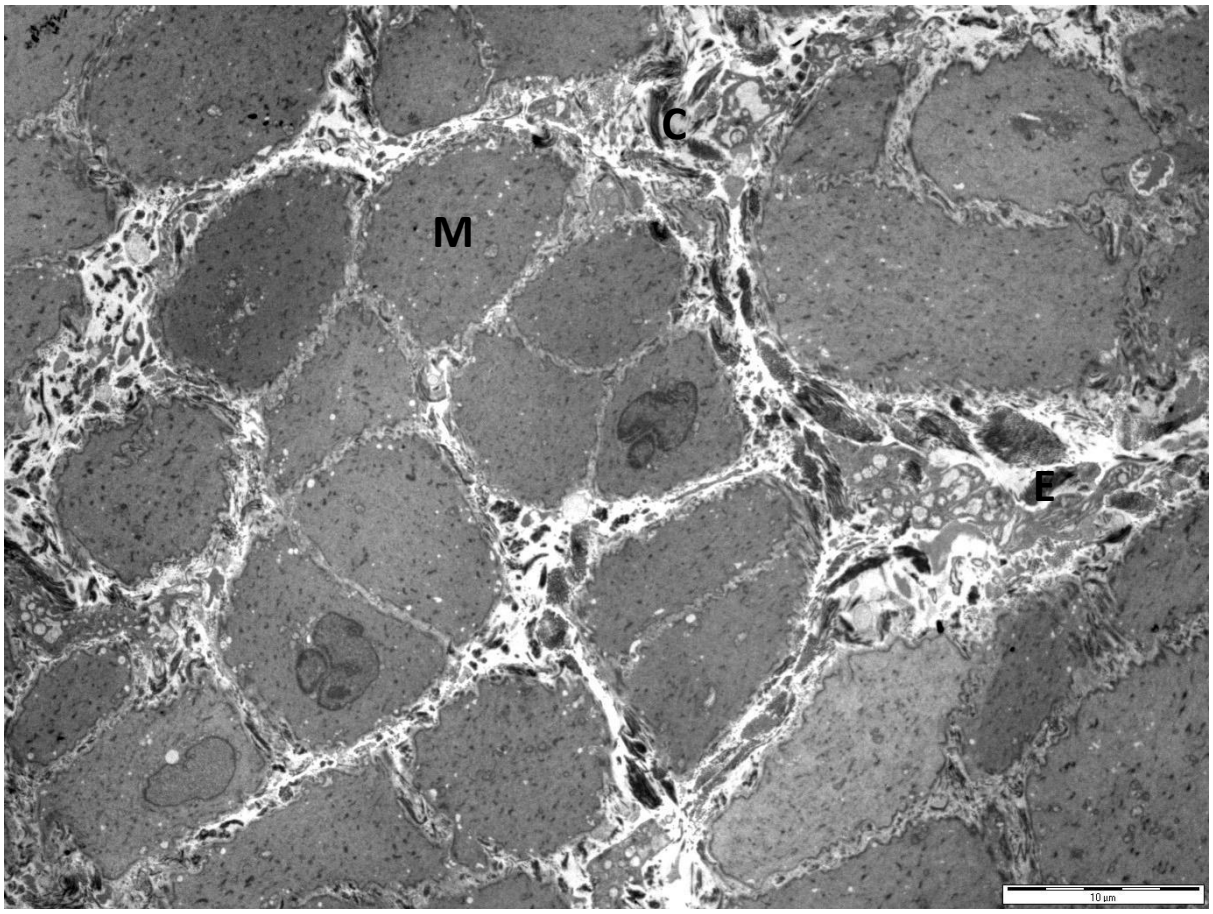
M = mycocyte ; C = collagen ; E = elastin.

Figure 4.7.70: Micrograph of female bladder outlet obstruction patient



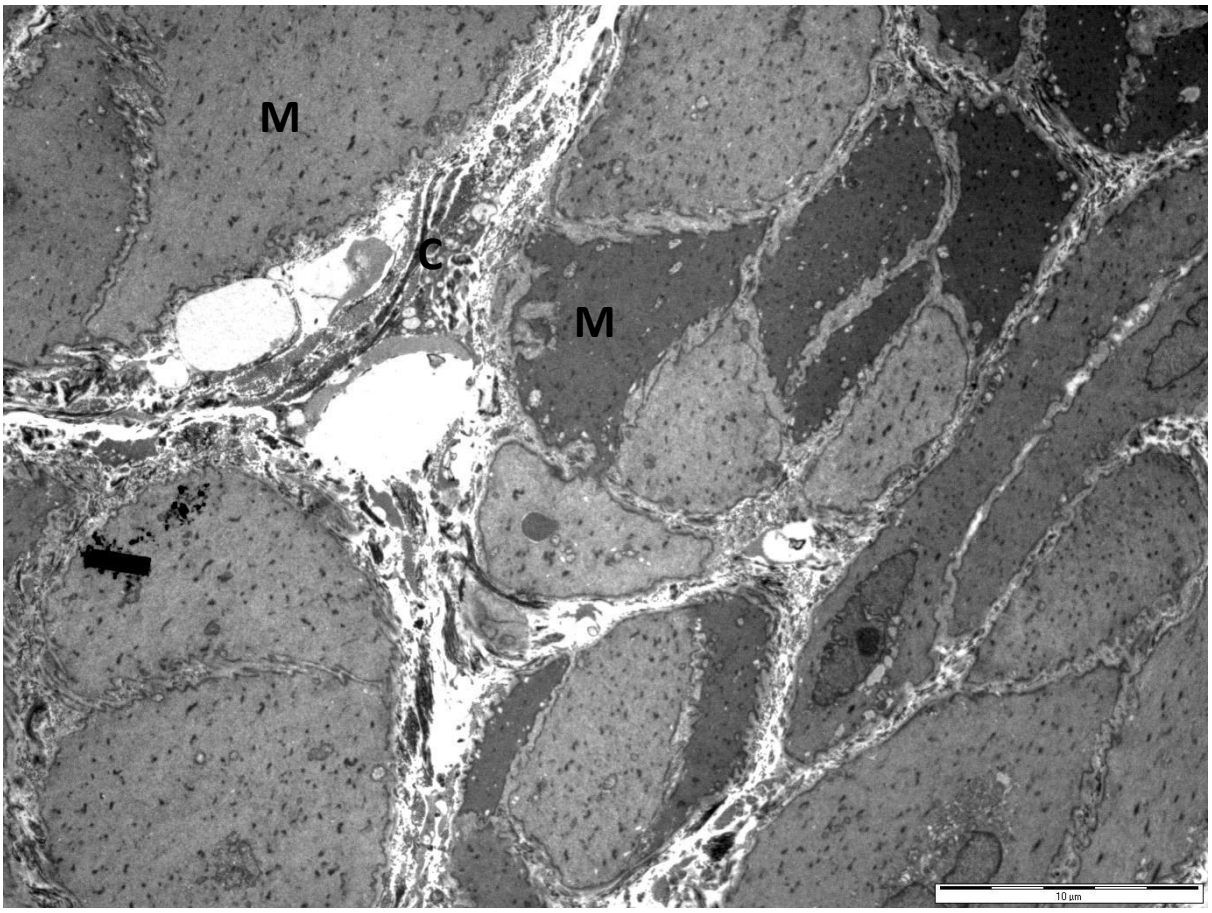
M = mycocyte ; IC = intercellular collagen.

Figure 4.7.71: Micrograph of female bladder outlet obstruction patient



M = mycocyte ; C = collagen ; E = elastin..

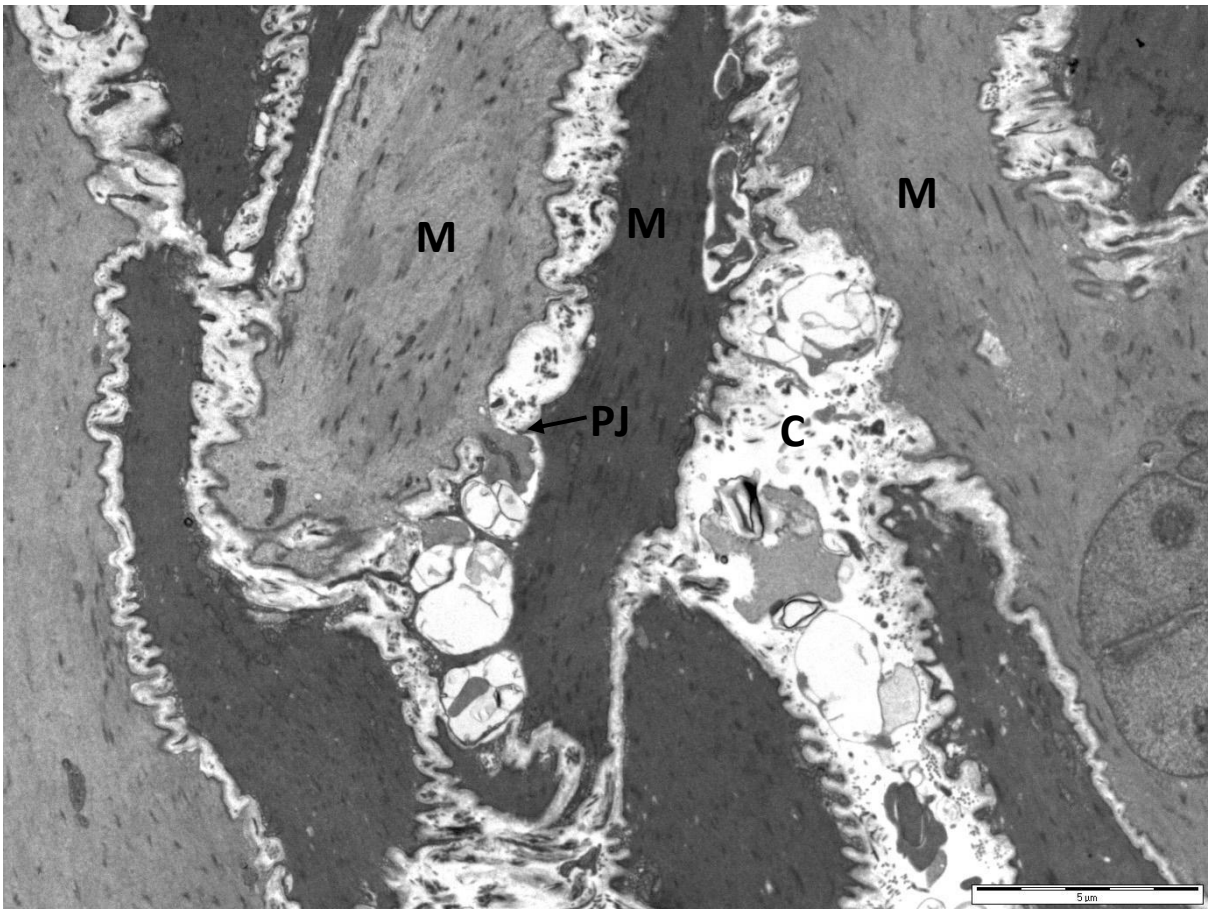
Figure 4.7.72: Micrograph of female bladder outlet obstruction patient



M = myocyte ; C = collagen.

Figure 4.7.73: Micrograph of female bladder outlet obstruction patient

Micrograph shows variation in electron density and protrusion junctions between myocytes. It is noted that the myocytes have very 'scalloped' edges for which the cause, relevance and significance is unknown. There are detrusor ultrastructure features which are yet to be explained.



M = myocyte ; PJ = protrusion junction ; C = collagen.

Comments regarding the results of ultrastructural analysis in correlation with clinical scenario

Myohypertrophy changes (muscle fascicle derangement, collagenosis, and variation in myocyte size/shape) were present in the detrusor specimens of all female and male patients with BOO, but absent in the detrusor specimens of the control patient.

Myohypertrophy features were as pronounced, if not more severe, in our cohort of females with BOO compared to males with BOO. It was interesting to note that myohypertrophy features were seen in the patient with only 4 months' history of an obstructive sling (fBOO patient 2), suggesting that structural changes in response to outlet obstruction can occur within a relatively short time frame. In fBOO patient 7 with sphincter active voiding, the detrusor ultrastructure features of myohypertrophy were less marked than in fBOO patient 3 (urethral stricture), which showed moderate severity features.

Features of degeneration were present in varying degrees in all patients, consistent with our previous study in males that suggested degeneration correlates with age rather than degree of obstruction.

Discussion

Currently, female bladder outlet obstruction is often diagnosed using clinical information, including symptomatology of voiding symptoms such as slow urinary flow, urinary hesitancy, sensation of incomplete emptying in combination with findings of poor urinary flow rates on uroflowmetry, and

elevated postvoid residual. However, clinical tests such as uroflowmetry and post void residual urine volume are unable to differentiate voiding dysfunction due to obstruction versus detrusor underactivity. Urodynamic studies are the “gold standard” way to diagnose lower urinary tract dysfunction from the functional aspect (as opposed to anatomical), but has its limitations and is invasive.(11) Whilst there is general consensus in the definition of male BOO using urodynamic pressure-flow parameters (the BOOI/ AGN), there is less agreement for the diagnosis of outlet obstruction in females, apart from the principles of high pressure, low flow voiding.

Several nomograms have been proposed to calculate female bladder outlet obstruction. Of these, the Solomon-Greenwell Number (SGN) calculated by Pdet at Qmax minus 2.2*Qmax has been reported to have excellent specificity when a cutoff of >18 is used (obstruction almost certain), but sensitivity is unsatisfactory, particularly for patients with primary bladder neck obstruction.(12) To improve sensitivity, BOOIf in this study was defined as BOOIf >5, based on the Solomon-Greenwell nomogram, whereby BOOIf>5 represents likely obstructed (>50%), and BOOIf>18 represents almost certainly obstructed (>90%). The BOOIf and SGN acknowledge high detrusor voiding pressure with low or no flow voiding.(13)

Another limitation of pressure-flow urodynamic parameters in the definition of obstruction is that this relies on the detection of elevated detrusor voiding pressures. If there is a degree of concomitant detrusor underactivity, the resulting lower voiding pressures and low flow voiding presents a diagnostic challenge in differentiating between obstruction and detrusor failure. Furthermore, the theory of detrusor dysfunction due to bladder outlet obstruction from initial compensatory hypertrophy later progressing to detrusor failure means that we may not capture patients in the mid-part of this trajectory of a failing detrusor (the detrusor voiding pressure is initially excessively high, then deteriorates toward normal before deteriorating further to sub-normal).(14) This is particularly important in the context of iatrogenic causes of BOO such as sling obstruction, as there may be a narrow window of opportunity during which obstruction can be relieved to prevent end-organ (bladder) failure.

The myohypertrophy features in this cohort of female patients with bladder outlet obstruction were similar if not more marked than in our male bladder outlet obstruction cases. This is not surprising, given that all the female cases had severe outlet obstruction based on their clinical presentation and many were obstructed for a number of years. In correlating the clinical scenarios with structural changes, the most severe changes were observed in the 2 females with chronic BOO due to recurrent urethral stricture disease, and one patient with high pressure, sphincter active voiding. The exception was the patient with obstruction following mid urethral sling who had moderate myohypertrophy changes after only 4 months. The presence of ultrastructural changes in the bladder after such a short duration of obstruction is certainly concerning, and has clinical implications regarding the importance of prompt diagnosis and treatment of obstruction following sling procedures to prevent injury to the detrusor over time. Similarly, the more severe ultrastructural changes in the male BOO patients (patients 4,5,6) were older men with high grade BOO on urodynamics and high residual urines.

It would be clinically useful if detrusor ultrastructure analysis, apart from quantifying structural derangement, could also help prognosticate a patient’s response to treatment and likelihood of return to normal voiding post surgery. It could be used to help differentiate those in retention due to bladder outlet obstruction versus those with features to suggest detrusor failure or detrusor underactivity. Extrapolating from Blatt’s work (9) at our institution whereby male bladder outlet obstruction patients had worse voiding outcomes after transurethral resection of the prostate when there were concomitant features of hypocontractile bladder, it is reasonable to hypothesise that it may be similar for female bladders which are obstructed–that those with concomitant hypocontractile features may have worse voiding outcomes despite sling division or, for example, other BOO surgery. Further studies are warranted to investigate this hypothesis.

Conclusions

There are similar ultrastructural features in detrusor biopsies of female patients with BOO compared to male BOO, using a standardized protocol. The myohypertrophy pattern (variation in myocyte shape and size, separation of cells, increased collagen deposition and degeneration changes) appears to correlate with the duration and severity of obstruction, although structural changes were observed in detrusor biopsy of one female who had obstruction from a mid urethral sling placed for 4 months.

We have shown that the use of detrusor biopsy for ultrastructural assessment using our established protocol for diagnosis of BOO in males is feasible and applicable for females with BOO. In the light of difficulties defining obstruction in females, there are possible clinical applications for ultrastructural studies in the management of females with suspected bladder outlet obstruction as a diagnostic tool, and potentially also providing prognostic information about voiding function (see Chapter 5).

References

1. Diokno AC, Brown MB, Goldstein N, Herzog AR. Epidemiology of bladder emptying symptoms in elderly men. J Urol. 1992;148(6):1817-21.
2. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. IV. Bladder outlet obstruction. J Urol. 1993;150(5 Pt 2):1681-95.
3. Hailemariam S, Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. V. Standardized protocols for routine ultrastructural study and diagnosis of endoscopic detrusor biopsies. J Urol. 1997;157(5):1783-801.
4. Elbadawi A, Hailemariam S, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. VI. Validation and update of diagnostic criteria in 71 detrusor biopsies. J Urol. 1997;157(5):1802-13.
5. Brierly RD, Hindley RG, McLarty E, Harding DM, Thomas PJ. A prospective evaluation of detrusor ultrastructural changes in bladder outlet obstruction. BJU Int. 2003;91(4):360-4.
6. Holm NR, Horn T, Smedts F, Nordling J, de la Rossette J. The detrusor muscle cell in bladder outlet obstruction--ultrastructural and morphometric findings. Scand J Urol Nephrol. 2003;37(4):309-15.
7. Yadav SS, Bhattar R, Sharma L, Banga G, Sadasukhi TC. Electron microscopic changes of detrusor in benign enlargement of prostate and its clinical correlation. Int Braz J Urol. 2017;43(6):1092-101.
8. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
9. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.
10. Lindsay J, Solomon E, Nadeem M, Pakzad M, Hamid R, Ockrim J, et al. Treatment validation of the Solomon-Greenwell nomogram for female bladder outlet obstruction. Neurourol Urodyn. 2020;39(5):1371-7.
11. VW. N. Pressure flow urodynamic studies: the gold standard for diagnosing bladder outlet obstruction. . Rev Urol 2005 ;7 Suppl 6(Suppl 6):S14-21. 2005.
12. Mai HC, Wu RC, Lin VC, Kuo WW, Wu CH. External validation of Solomon-Greenwell nomogram for female bladder outlet obstruction. Neurourol Urodyn. 2022;41(2):626-32.
13. Solomon E, Yasmin H, Duffy M, Rashid T, Akinluyi E, Greenwell TJ. Developing and validating a new nomogram for diagnosing bladder outlet obstruction in women. Neurourol Urodyn. 2018;37(1):368-78.
14. Fusco F, Creta M, De Nunzio C, Iacovelli V, Mangiapia F, Li Marzi V, et al. Progressive bladder remodeling due to bladder outlet obstruction: a systematic review of morphological and molecular evidences in humans. BMC Urol. 2018;18(1):15.

CHAPTER 5: DETRUSOR ULTRASTRUCTURAL FEATURES IN MALES WITH IMPAIRED DETRUSOR CONTRACTILITY

Introduction and objectives

In the detrusor ultrastructure literature on the underactive bladder, the numbers of patients in this cohort have been low. In this part of my work, I aim to provide the largest sample size reported on detrusor ultrastructure features in males with detrusor underactivity and correlate the structural findings with functional outcome data in the short and long term.

Voiding dysfunction and aging

Aging is associated with declining function in nearly every physiologic system.(1, 2) LUTS are more prevalent with increasing age, with the incidence of one or more voiding LUTS symptoms among men over the age of 60 years who have never had prostate surgery being 35%, and annual incidence rates being 16.4% and 16.1% over the next two years respectively.(3) Another study showed that among the population over 60 years of age, 11.8% of men and 10.8% of women had difficulty with bladder emptying.(4) The aging bladder has been described as being associated with urodynamic findings of detrusor overactivity, impaired contractility, or a combination of both overactive and underactive detrusor.(5)

Challenges in defining an underactive bladder

It is known that satisfactory voiding function requires both adequate detrusor contractility and an unobstructed outflow.(6) Underactive bladder (UAB) is a clinical syndrome characterised by a slow urinary stream, hesitancy, and straining to void, with or without a feeling of incomplete bladder emptying and sometimes associated with storage symptoms.(7) Detrusor underactivity (DU) is a diagnosis elicited on pressure-flow urodynamic studies and refers to low detrusor pressure or short detrusor contraction time, usually in combination with a low urine flow rate resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span. This is in comparison with the term “hypocontractile detrusor” or detrusor hypocontractility, which describes a detrusor contraction of reduced strength.(7) However, there are difficulties and controversies in solidifying the definition.

The LUTS are not diagnosis specific – voiding LUTS such as low flow rate and incomplete emptying may be due to BOO or DU, and the key symptoms of UAB have not been well defined. Additionally, in the diagnosis of DU, the magnitude of contraction and duration of contraction has not been standardised or accepted. In practice, it may be reasonable to consider DU a “relative” condition, whereby the contractility needs to be sufficient relative to the amount of outlet resistance, otherwise adequate emptying cannot occur. In essence, the contraction needs to be strong enough, and the resistance low enough, for the LUT system to work harmoniously. The contractility does not need to be as strong when the outlet resistance is low. Therefore, on a urodynamic study when a patient voids with low amplitude detrusor contraction but empties completely, it may not actually be detrusor hypercontractility, but rather a lower urinary tract system where the detrusor is not having to mount a significant strength of detrusor contraction in order to achieve emptying.

It is believed that some males with underactive bladder have de novo underactive bladder and some have underactive bladder secondary to bladder outlet obstruction (such as benign prostatic obstructrion). It is not always possible to know retrospectively whether a patient who is diagnosed at this time-point has de novo or secondary underactive bladder, although sometimes a thorough history of timeline of symptoms and symptom types can lead a clinician to understand it to be de novo or secondary, if there is absence of previous investigations (such as previous urodynamic studies) showing conclusively what is the case.

Urodynamic diagnosis of detrusor underactivity

There are clear limitations of urodynamic studies, which are the gold standard despite potential morbidity and limitations in being able to diagnose hypocontractile bladder. (8) Several calculations and nomograms have been proposed, including the calculation of a Bladder Contractility Index (BCI).(9) This index for bladder contractility can be calculated based on the Schaefer nomogram. The

bladder contractility index (BCI) is represented by the following formula: BCI = P_{det} Q_{max} + 5 Q_{max}. Using this formula, contractility can be divided into strong > 150, normal 100–150, and weak < 100.(9)

Management of underactive bladder

The management algorithm for an underactive bladder ranges from conservative lifestyle modifications to pharmacological treatments, and even includes surgical procedures, depending on patient and disease factors. How a patient transitions along the algorithm is based largely on nature

and severity of symptoms, as well as the type and presence of complications due to the underactive bladder condition, as guided by their clinician.

Initial lifestyle modification strategies include avoiding constipation, reviewing medications to remove medications that can cause urinary retention and constipation as side effects if possible, and improving overall wellbeing and mobilisation. Time from acute retention episodes can also help, allowing some spontaneous recovery from overdistension injury to the bladder. Timed or regular voiding can also help, with the theory being to avoid overdistension of the bladder, as it may be more difficult for an overdistended detrusor to contract fully to expel urine.(10)

Bethanechol (a cholinergic agonist) has been proposed for detrusor underactivity (DU), but has not been found to be effective, and may cause unwanted side effects.(11) Other UAB medications being studied include alpha 1-adrenoceptor antagonists, phosphodiesterase type 5 inhibitors, free radical scavengers, and beta 3-adrenoceptor agonists. It is postulated that chronic ischaemia has a significant role in LUTS in the older adult.(12)

Sacral neuromodulation is an established treatment for non-obstructive urinary retention. A systematic review found that the success rate ranged between 42.5-100% for the test phase, and 65.5-100% in the long term.(13) A more recent multicentre trial reported a success rate of 62% in women and 22% in men, with previous TURP or bladder neck incision being predictors of success in men.(14) Preoperative bladder contractility has also been reported as a predictor of SNM success, with 33% of patients with acontractile detrusor responding, and 57% of patients with BCI>0 responding.(15) Another form of neuromodulation via percutaneous tibial nerve stimulation (PTNS) has lower success rates of 50-60%. However, minimal complications were reported.(13)

The premise of alpha-blocker medication and cavitating prostate surgery (such as transurethral resection of the prostate and laser prostatectomy) in the setting of DU is to reduce outlet resistance. Results are variable, and improvement may be due to reduction of outlet resistance, or potentially due to presence of undiagnosed concomitant BOO in the presence of hypocontractile bladder.(16, 17) Patients ought to be counseled regarding poorer outcomes compared to patients without DU.

A recent study Investigating holmium laser enucleation of the prostate (HoLEP) outcomes in men (median age 71.5 years) with BPO and detrusor hypocontractility reported a good outcome, with all men voiding spontaneously without catheter, and improvement in their AUA-Symptom Scores at a median follow-up of 24 months. In men with BPO and acontractile detrusor at preoperative urodynamics (median age 75 years), 95% of men were able to void without catheter post-HoLEP, with 79% having return of detrusor contractility, and 21% voiding exclusively by Valsalva effort. Patient satisfaction was high for both groups.(18) Overall, there is reasonable consensus that in treating urinary retention and voiding dysfunction by attempting to reduce outflow resistance (such as by cavitating prostate surgery, urethroplasty, or sling excision), the presence of impaired detrusor contractility can reduce the success of such procedures. This has been demonstrated in the case of transurethral resection of the prostate (TURP). Our previous study on patients with chronic retention showed that ultrastructural features such as the ‘myohypertrophy pattern’ have been associated with poorer voiding outcomes after TURP.(19)

The role of reduction cystoplasty for impaired detrusor contractility is controversial and not generally recommended given the low level of evidence reported in the literature. In one small study, 7 of 8 men (mean age 60) had successful symptomatic outcomes (defined as PGII≤2). Of these patients, 3 had concurrent diverticulectomy, and 2 had concurrent open simple prostatectomy during reduction cystoplasty.(20)

Methododology and patient cohort

Patients who had urodynamic diagnosis of detrusor underactivity (as defined by BCI<100) and who were going to undergo cystoscopy as part of their clinical treatment were recruited into the study. Demographic, medical and surgical history, urodynamic data and voiding outcome data were recorded.

Detrusor biopsies were obtained at cystoscopy and processed for analysis by electron microscopy, as described in the methodology of Chapter 3. Detrusor ultrastructural features such as myohypertrophy pattern (cell size and irregularity), degenerative features, and hyperelastosis were scored from 0-3, with 0 being none and 3 being extensive changes. The electronic micrographs of detrusor ultrastructural features of the patients were scored by two independent observers (AC and VT), who were blinded to clinical and urodynamic data. Refer to the methodology of Chapter 3 for further details on cystoscopic, biopsy processing and ultrastructural analysis techniques.

Urodynamic data was analysed for calculations of BOOI, BCI and VE. Voiding outcomes were also recorded on presentation, and chart review conducted to record data on voiding outcomes in the intermediate term (3 months post-treatment) and long term (mean 16 years follow-up).

Ultrastructural features were correlated with urodynamic parameters. Furthermore, ultrastructural features were correlated with voiding outcomes. Statistical analyses were performed using SPSS® Version 28. Multivariate analyses were performed using binary logistic regression to test for ultrastructural and functional variables relating to clinical outcome. Non-parametric tests were used to analyse continuous variables. Chi-squared tests and Spearman's correlation were used for categorical variables. Fisher's Exact Test was used for univariate analysis between categorical variables when cell count was less than 5. $p<0.05$ was considered statistically significant.

Results

Twenty-three male patients (mean age 78 years, interquartile range 73-84 years) were recruited for this part of the study. All were identified to have detrusor underactivity (either hypocontractile or acontractile detrusor) on urodynamic study, with bladder contractility index (BCI) indicative of hypocontractile bladder whereby BCI<100 (BCI = pDet@Qmax + 5.Qmax). All patients had no evidence of bladder outlet obstruction as identified by urodynamic study and calculation of BOOI (with bladder outlet obstruction being defined as BOOI>40, whereby BOOI = Pdet@Qmax – 2.Qmax).

35% (8/23) of the cohort had concomitant urodynamic detrusor overactivity, poor compliance, or both.

Of the 23 patients, mean voided volume was 83ml (IQR 0-100), mean Qmax was 2.3ml/s (IQR 0-3.5), mean Pdet at Qmax was 17 cmH2O (IQR 0-36), and mean postvoid residual urine was 630ml (IQR 238-985). 30% (7/23) men were voiding spontaneously enough to not require catheterisation. 70% (16/23) men were catheter dependent on presentation to their urodynamic study.

As per eligibility criteria for this part of the study, all patients had BOOI<40. All patients had BCI<100 (that is, weak bladder contractility); mean BCI was 29 (IQR 0-45). Voiding efficiency was calculated; mean voiding efficiency was 19% (IQR 0-27).

Table 5.1: Urodynamic parameters of patients with underactive bladder.

Patient #	Age	Sex	DO / Poor Compliance	VV	Qmax	pDet@Qmax	PVR	BOOI	BCI	VE (%)
1	77	M	N	0	0	0	600	0	0	0
2	86	M	Y	200	8	20	400	4	60	33
3	86	M	Y	0	0	0	1200	0	0	0
4	78	M	N	0	0	0	1600	0	0	0
5	73	M	N	0	0	0	1200	0	0	0
6	75	M	N	100	6	50	400	38	80	20
7	71	M	N	0	0	40	1000	40	40	0
8	70	M	N	320	8	50	0	34	90	100
9	85	M	N	0	0	0	900	0	0	0
10	79	M	N	0	0	39	590	39	39	0
11	63	M	Y	0	0	0	100	0	0	0
12	90	M	Y	100	10	0	200	-20	50	33
13	84	M	N	0	0	0	970	0	0	0
14	100	M	Y	10	1	30	0	28	35	100
15	82	M	N	250	1	20	200	18	25	56
16	72	M	N	0	0	10	1050	10	10	0
17	81	M	Y	0	0	0	330	0	0	0
18	81	M	N	490	10	40	0	20	90	100
19	73	M	Y	30	1	0	350	0	5	8
20	84	M	Y	0	0	30	275	30	30	0
21	82	M	N	0	0	0	550	0	0	0
22	67	M	N	420	9	36	50	18	81	89
23	46	M	N	0	0	36	2064	36	36	0

In the intermediate term (within 3 months post-treatment), 9 patients (43%) were catheter-free, 12 patients (57%) required catheterisation in the form of urethral indwelling catheter, suprapubic catheter or clean intermittent self-catheterisation, and 2 men were lost to follow-up.

In the long term follow-up, 29% (6/21) patients were spontaneously voiding well enough to be catheter-free and 71% (15/21) were catheter-dependent. Of those that were catheter-dependent, 3 had deceased but were known to be catheter-dependent before end of life. Two men were lost to follow-up such that their catheter status was not known.

Table 5.2: Voiding outcomes, specifically catheter requirement, of patients with underactive bladder.

Patient #	Catheter present at time of urodynamic study? 0=no, 1=Yes	Catheter type. 0=catheter free, 1=urethral, 2=SPC, 3=CISC, u=unknown	Short term outcome 0=voiding catheter free, 1=eventually voided, 2=catheter dependent.	Long term outcome 0=voiding catheter free, 1=catheter dependent.	Long term Catheter type. 0=catheter free, 1=urethral, 2=SPC, 3=CISC, u=unknown.
1	1	2	2	1	2
2	0	0	0	1	2
3	1	1	2	1	1
4	1	u	2	1	1
5	1	1	2	1	1
6	0	0	0	1	2
7	1	1	2	1	1
8	0	0	1	1	1
9	1	u	2	1	1
10	1	1	2	1	1
11	1	1	1	0	0
12	1	1	2	1	2
13	1	1	2	1	1
14	1	1	u	u	u
15	0	0	0	0	0
16	0	1	2	1	2
17	1	1	1	0	0
18	0	u	0	0	0
19	1	1	u	u	u
20	1	1	1	0	0
21	1	1	2	1	2
22	0	u	0	0	0
23	1	3	2	1	3

There was a range of detrusor ultrastructural features identified in each of the domains. However, scoring 1-2 was generally more common in the cohort of patients with underactive bladder.

Table 5.3: Summary of detrusor ultrastructure features of patients with hypocontractile bladder.

Patient #	Myohypertrophy 0123 (global impression)	Cell size, irregularity 0123	Increased separation of cells 0123	Increase in intercellular collagen 0123	Degenerative features 0123 (global impression)	Electron density variation 0123	Vacuolation, dense bodies 0123	Fragmentation, exclusion 0123	Hyperelastosis 0123
1	2	2	2	2	2	3	3	2	3
2	2	2	2	2	2	2	3	2	2
3	2	2	1	2	3	2	2	1	1
4	1	1	2	2	1	1	2	1	2
5	3	3	3	3	3	3	3	3	3
6	2	2	2	2	2	2	1	1	2
7	2	2	2	2	2	1	2	1	1
8	1	1	1	1	1	1	1	0	1
9	1	1	2	1	1	1	1	1	1
10	3	3	3	3	2	2	2	2	2
11	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1
13	1	1	2	1	1	1	1	1	1
14	2	2	2	2	2	2	3	2	2
15	1	1	1	1	1	1	1	0	1
16	2	2	2	2	2	1	2	1	1
17	1	1	1	1	1	1	1	1	1
18	2	1	1	2	2	2	2	2	1
19	1	1	1	1	1	1	1	1	1
20	2	2	2	2	2	3	3	2	3
21	2	2	3	3	3	2	3	2	3
22	2	2	2	2	2	2	1	1	2
23	2	2	2	2	2	1	2	1	1

Figure 5.1.1: micrograph of detrusor ultrastructural features of male underactive bladder, showing severe degree of degeneration and hyperelastosis features (patient number 5).
This 73 year old male patient clinically was in urinary retention 1200ml PVR, and was unable to void with acontractile detrusor on urodynamic study. He was IDC dependent and never voided in the long term.

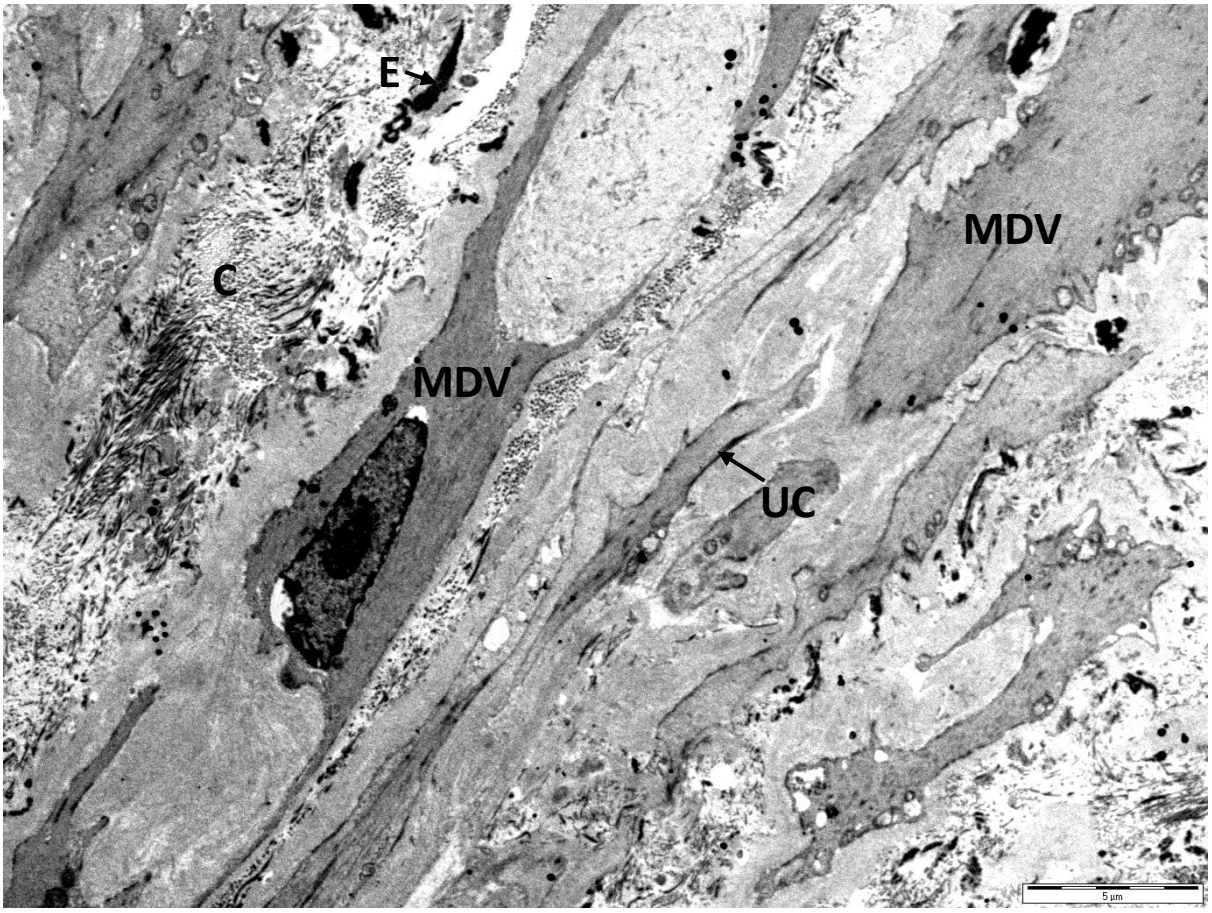
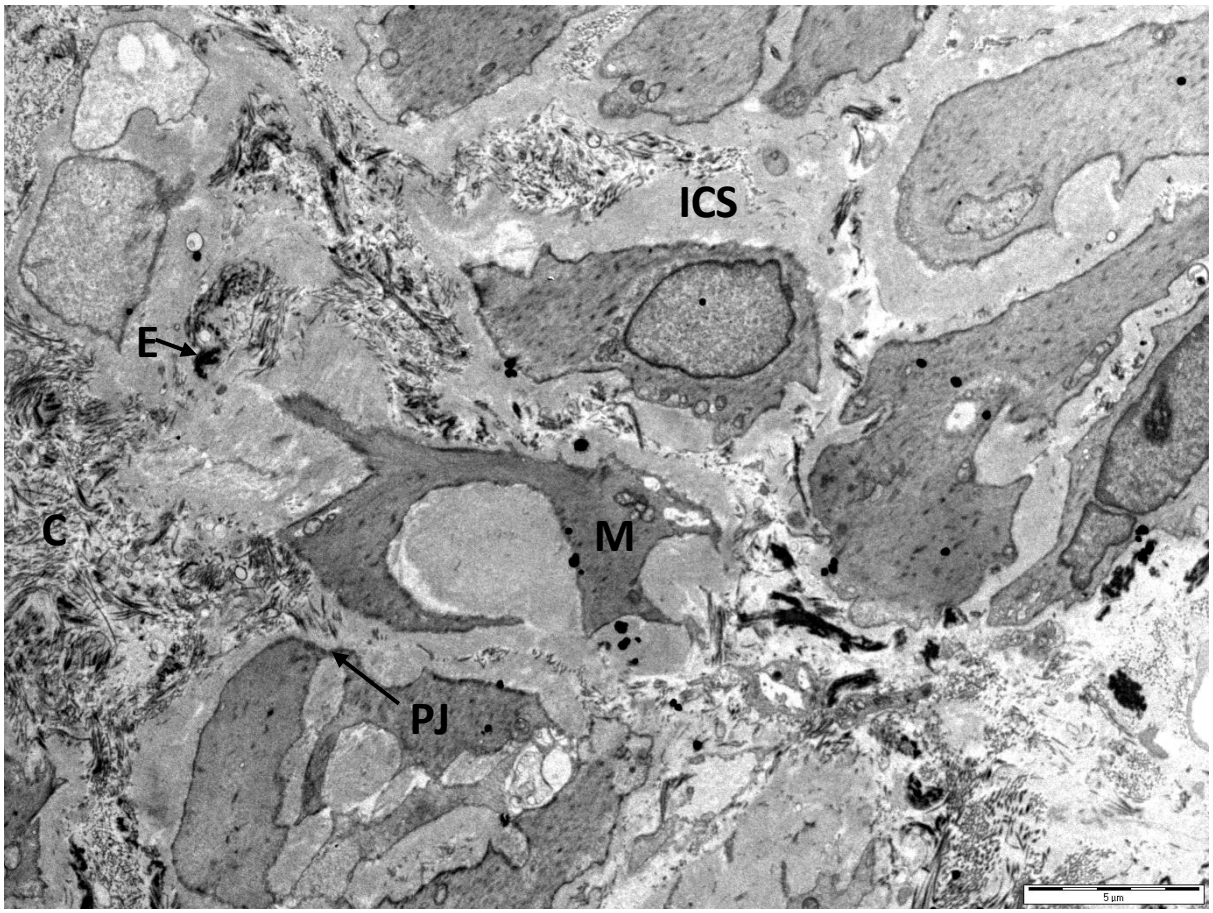
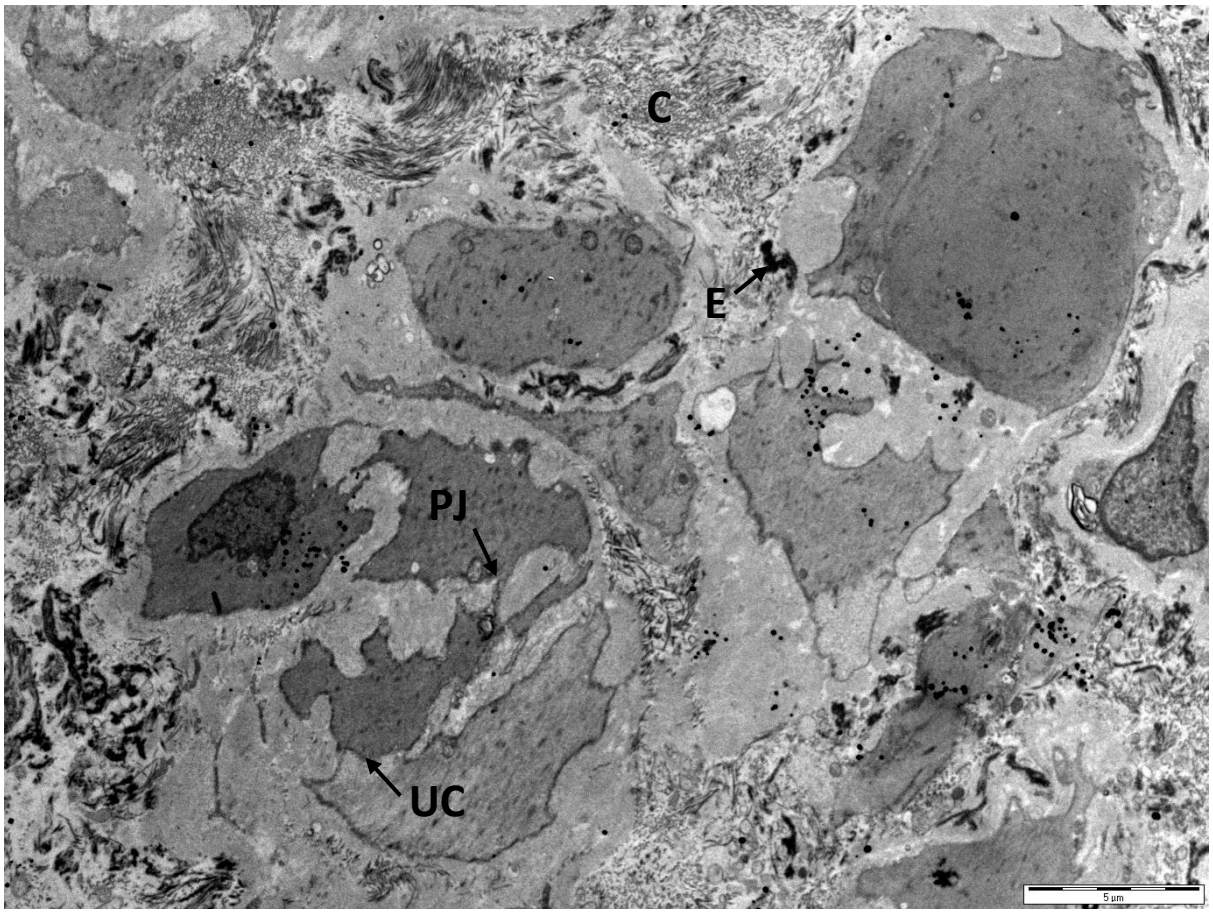


Figure 5.1.84: micrograph of detrusor ultrastructural features of male underactive bladder, showing severe degree of degeneration and hyperelastosis features (patient



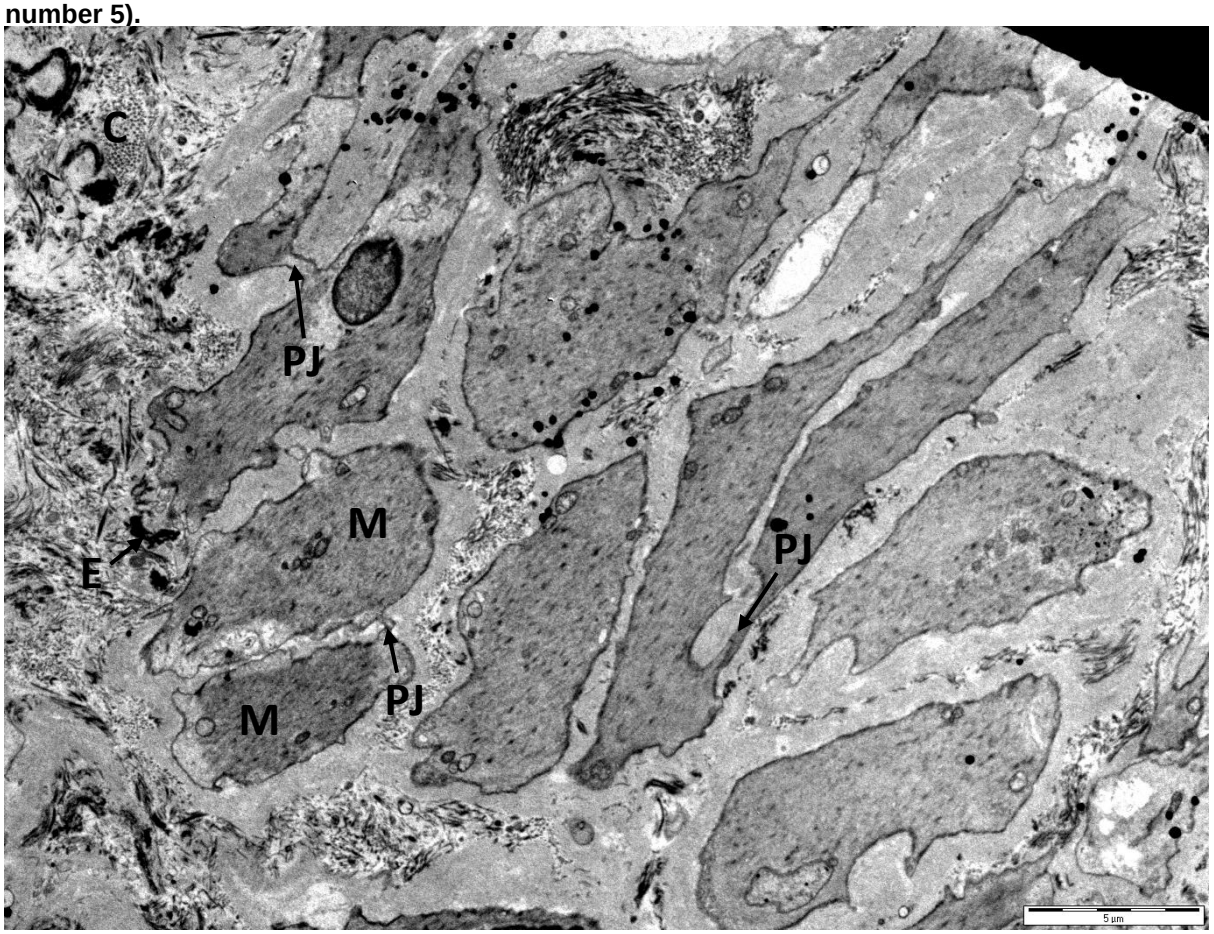
C = collagen, E = elastin, PJ = protrusion junction, M = abnormal shaped myocyte, ICS = wide intercellular space.

Figure 5.1.85: micrograph of detrusor ultrastructural features of male underactive bladder, showing severe degree of degeneration and hyperelastosis features (patient



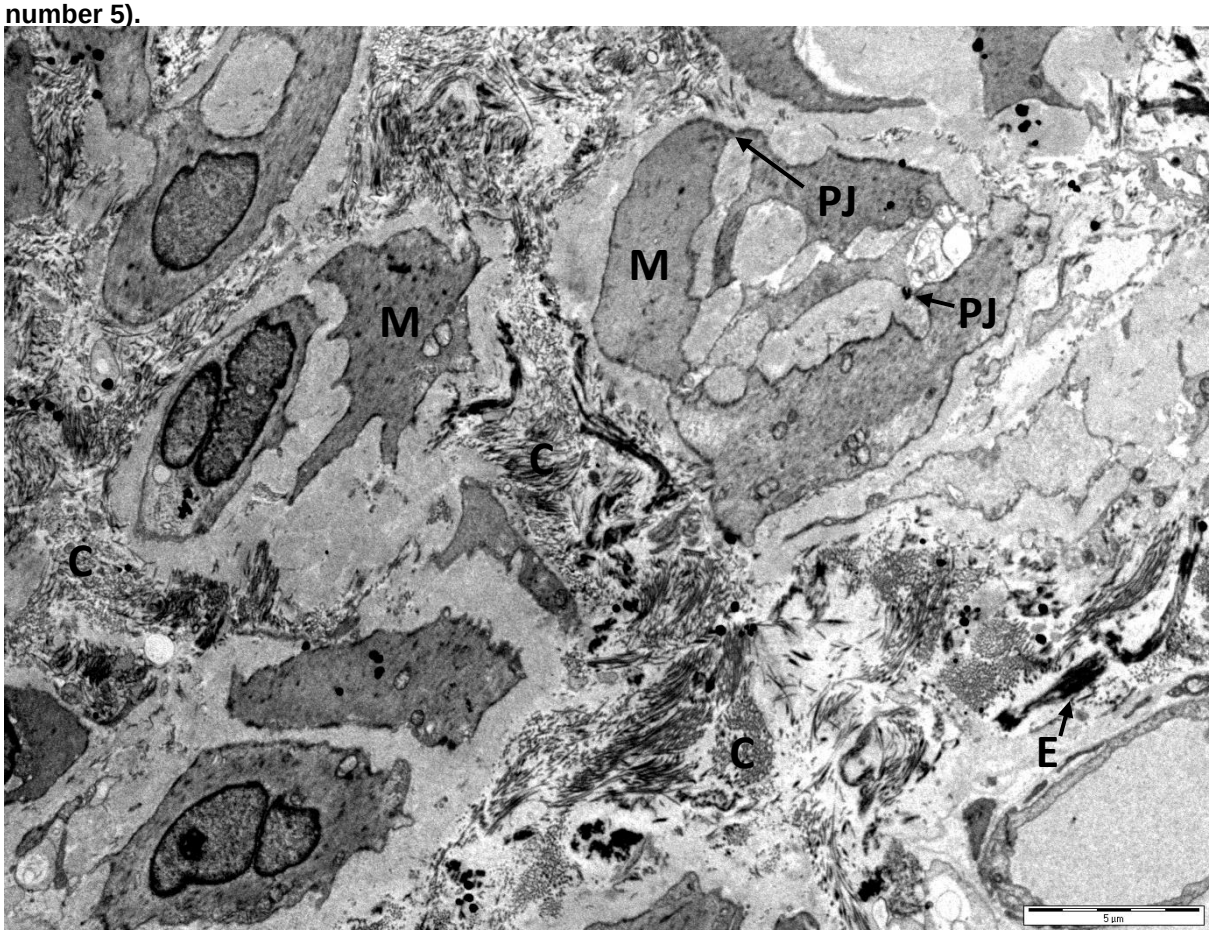
E = elastin, C = collagen, PJ = protrusion junction (appears as close contact over a point), UCA = ultraclose abutment (appears as close contact over a distance).

Figure 5.1.86: micrograph of detrusor ultrastructural features of male underactive bladder, showing severe degree of degeneration and hyperelastosis features (patient number 5).



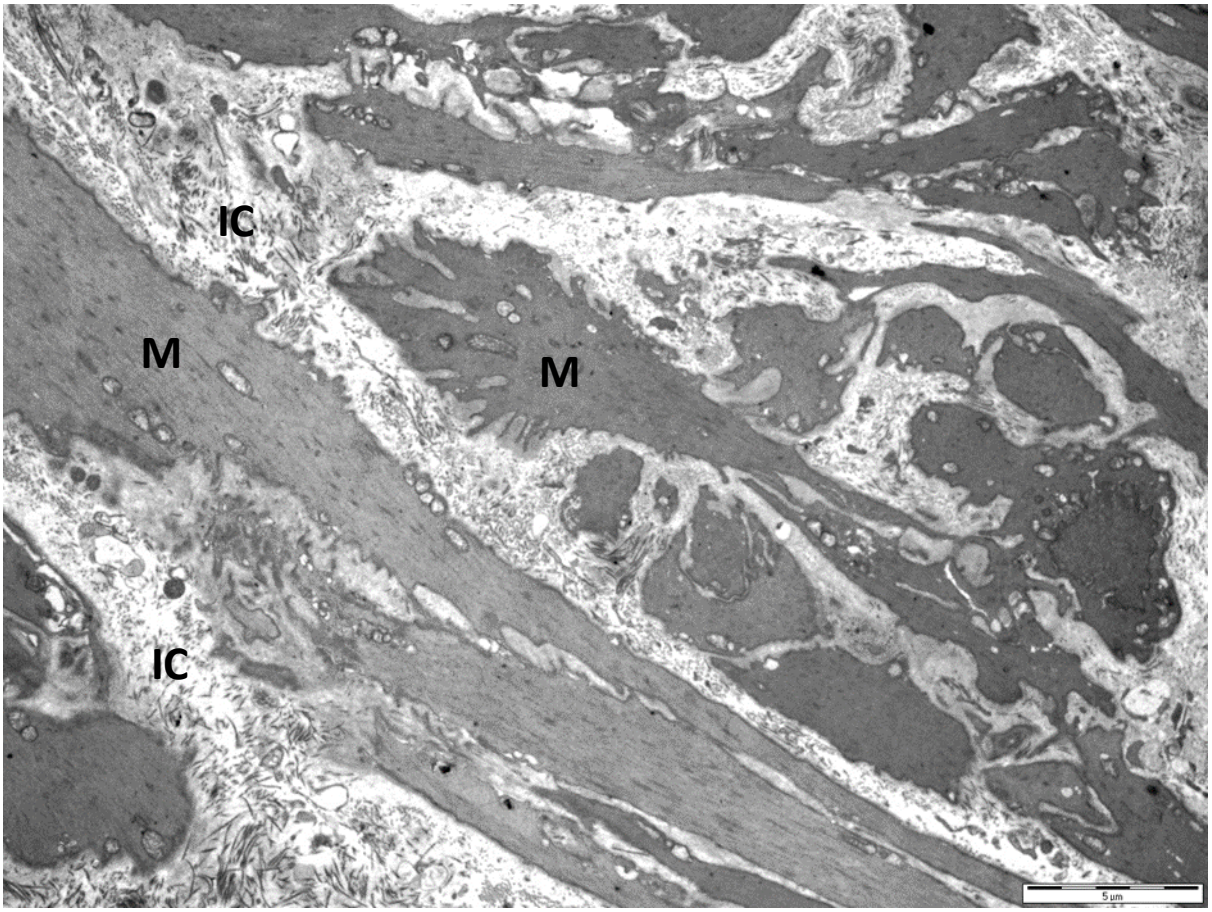
M = myocyte, E = elastin, C = collagen, PJ = protrusion junction

Figure 5.1.87: micrograph of detrusor ultrastructural features of male underactive bladder, showing severe degree of degeneration and hyperelastosis features (patient number 5).



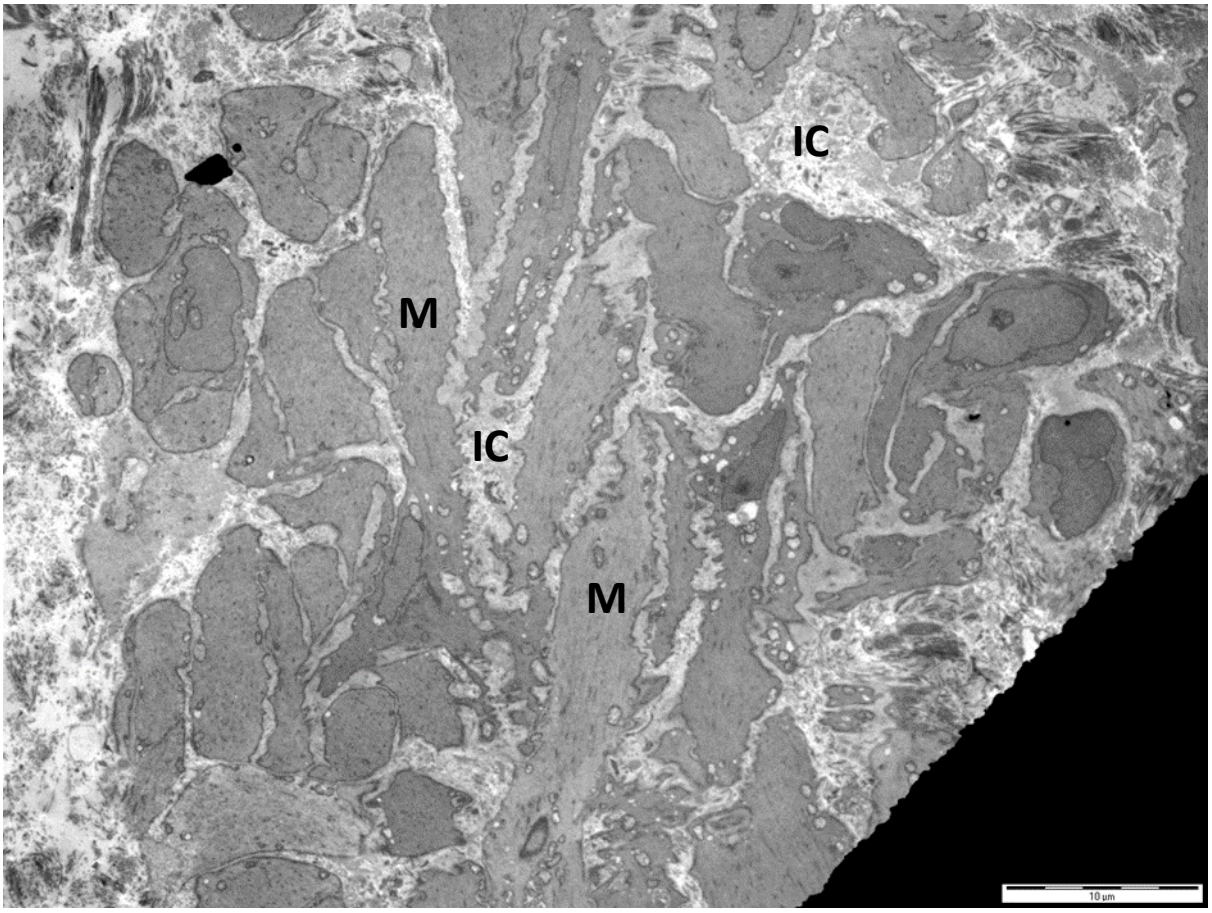
C = collagen, M = myocyte, E = elastin, PJ = protrusion junction.

Figure 5.2.1: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21). It is interesting that his detrusor shows patchy changes, highlighting the importance of examining a broad number of detrusor ultrastructure micrographs. Some of the micrographs show relatively severe changes, whilst other micrographs show only moderate changes. This 82-year-old patient was unable to void with urodynamic acontractile detrusor, and remained catheter-dependent with suprapubic catheter.



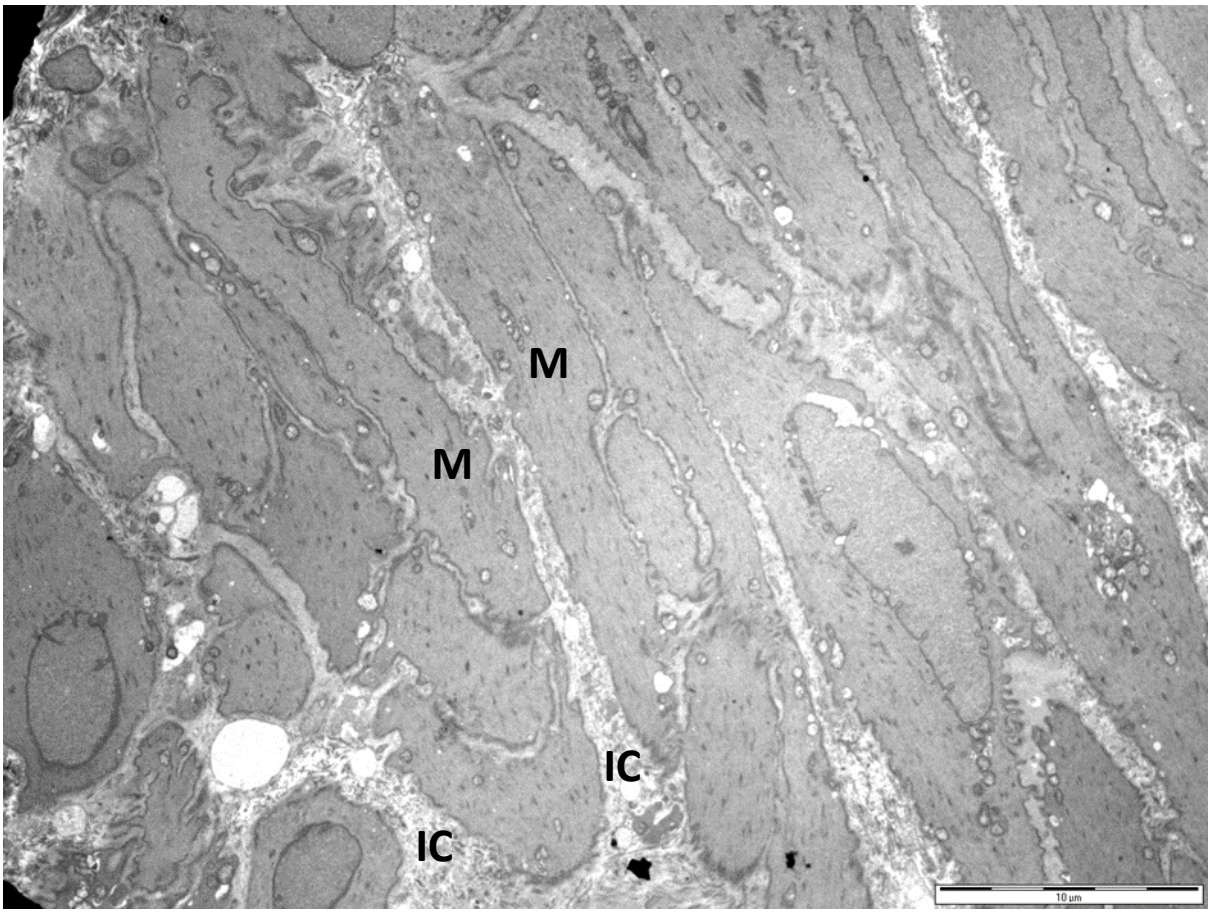
IC = intercellular collagen, M = myocyte.

Figure 5.2.89: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



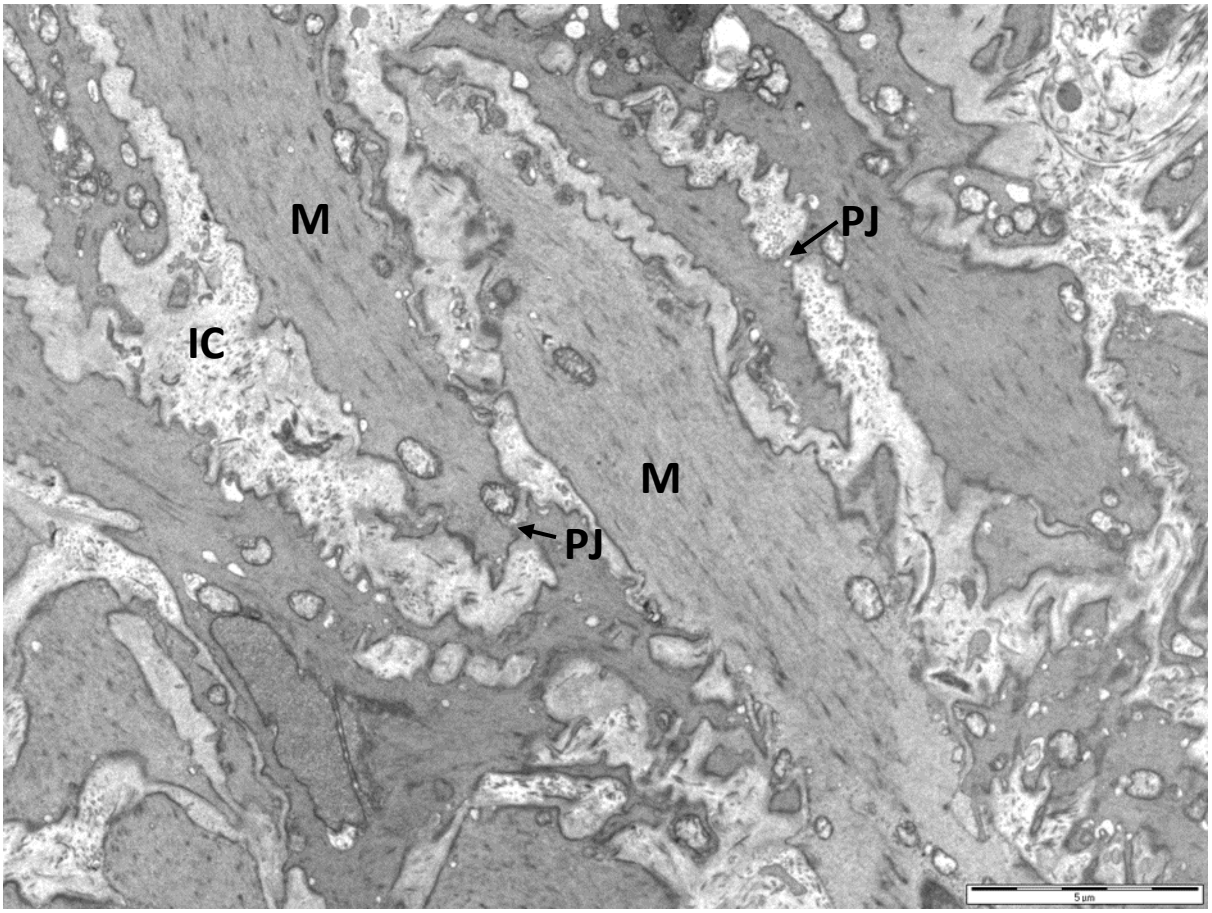
IC = intercellular collagen, M = myocyte.

Figure 5.2.90: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



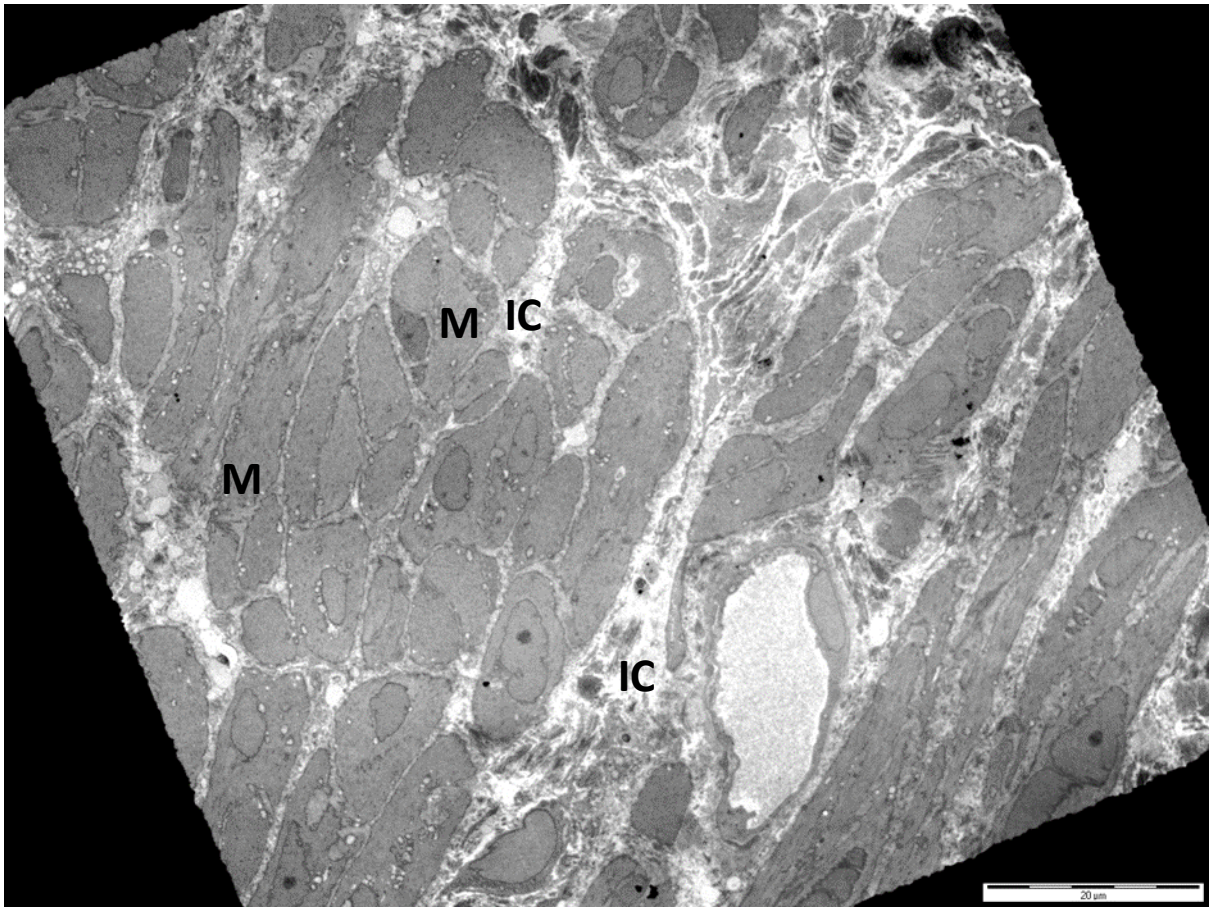
M = myocyte, IC = intercellular collagen.

Figure 5.2.91: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



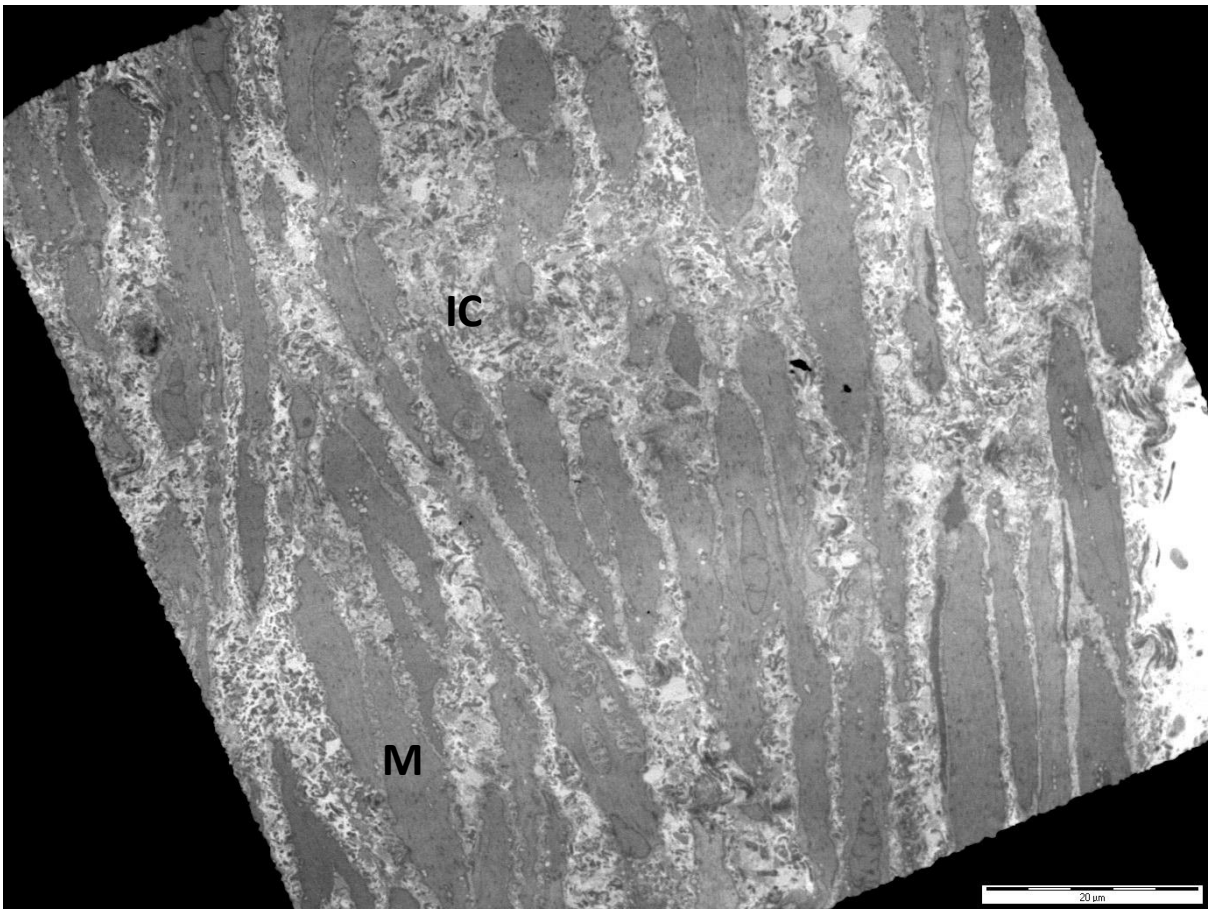
M = myocytes, IC = intercellular collagen, PJ = protrusion junction.

Figure 5.2.92: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



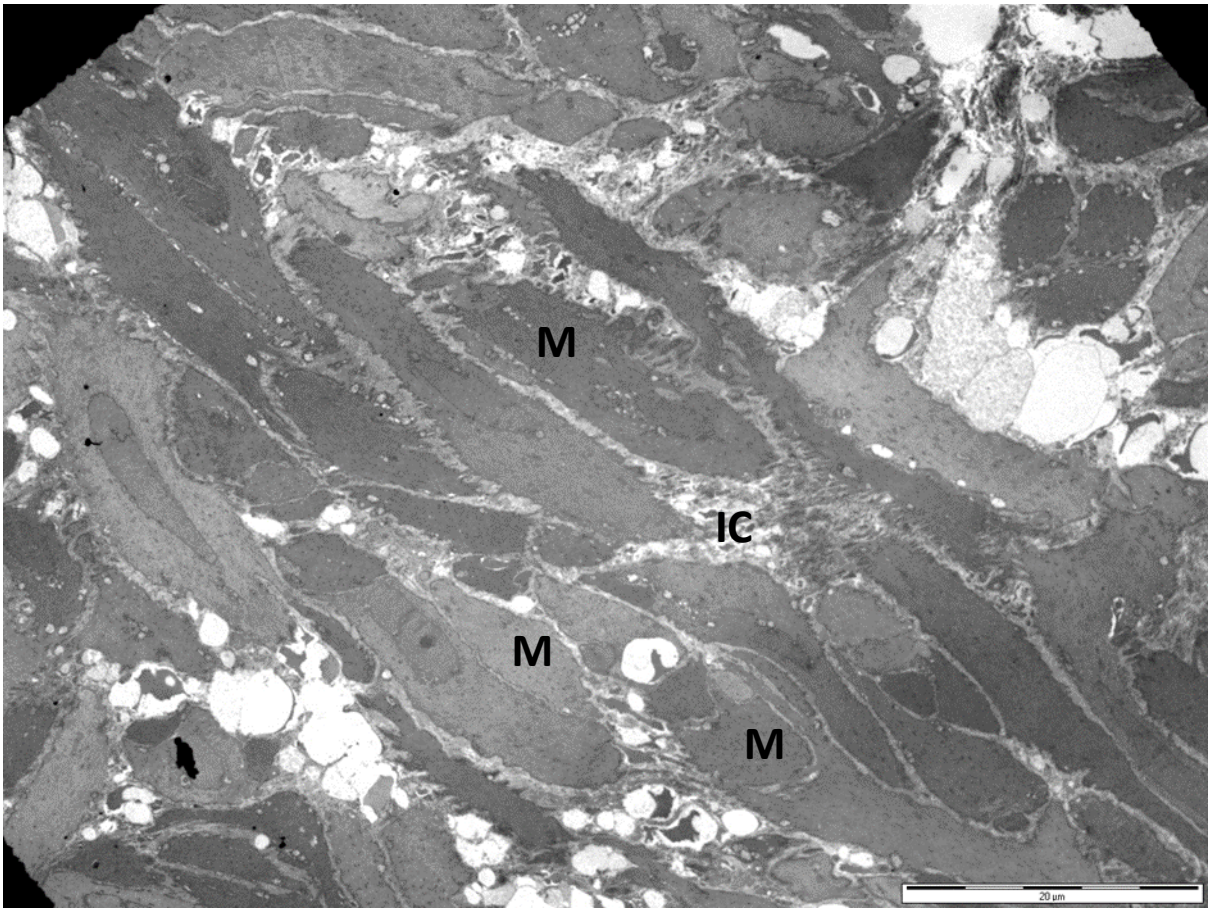
M = myocytes, IC = intercellular collagen.

Figure 5.2.93: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



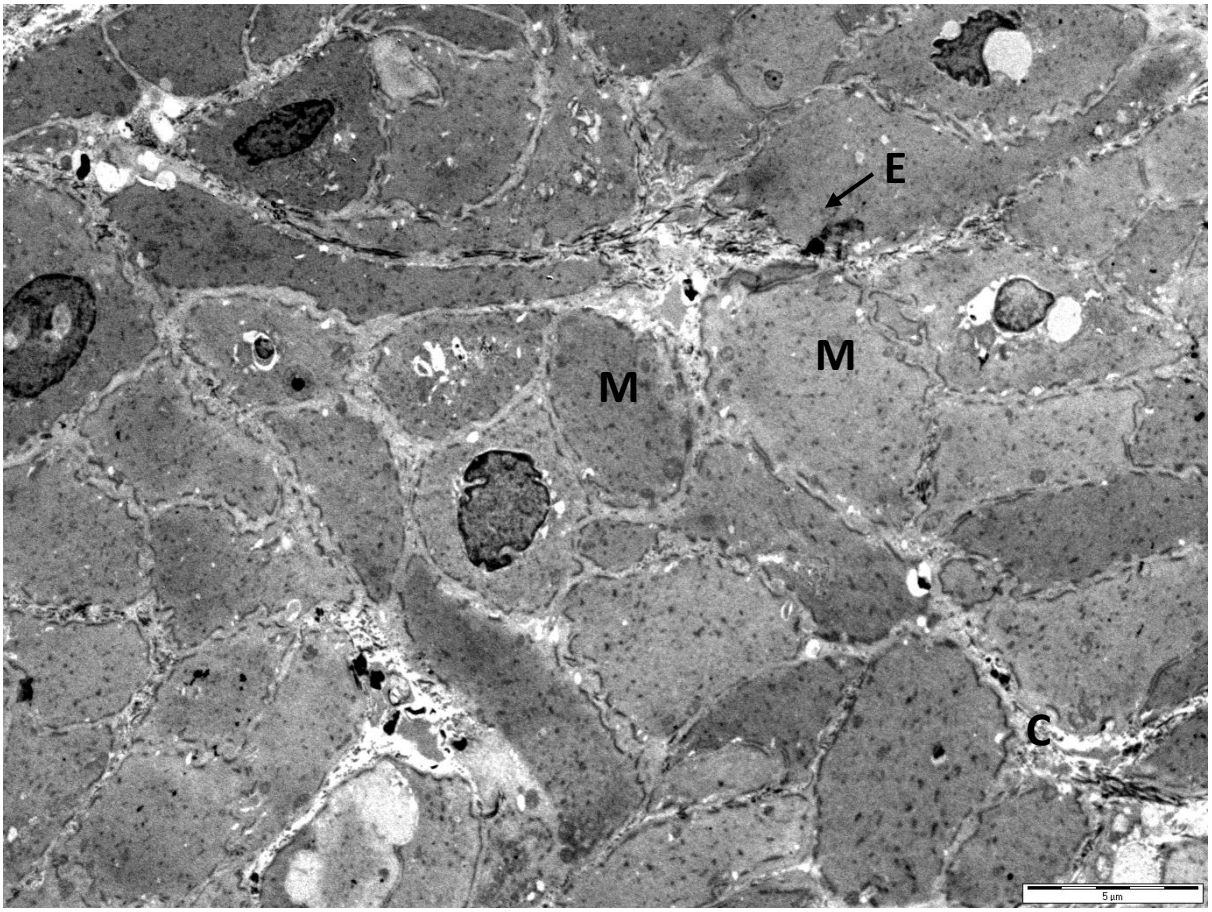
M = myocytes, IC = intercellular collagen.

Figure 5.2.94: micrograph of detrusor ultrastructural features of male underactive bladder showing moderate range myohypertrophy features and moderate to severe degenerative features and hyperelastosis (patient number 21).



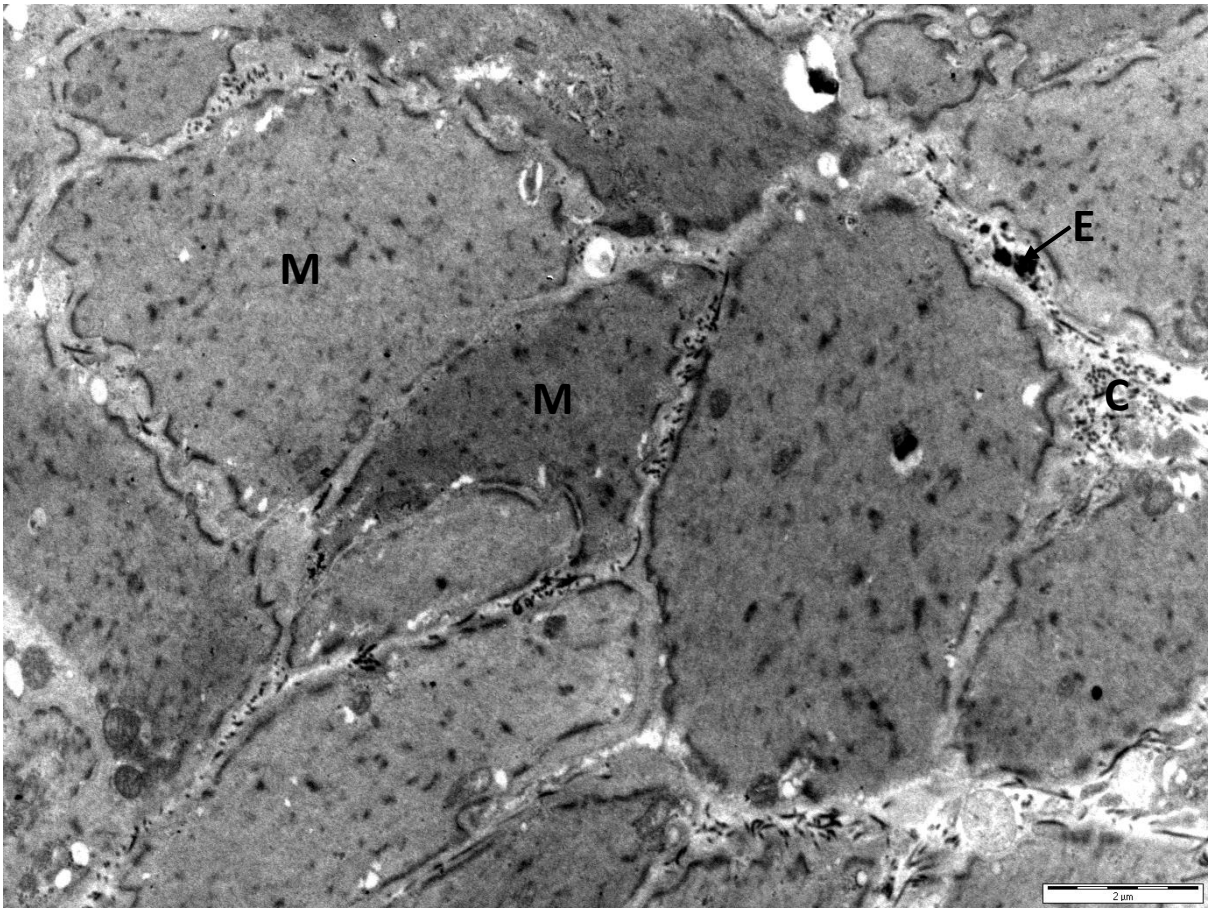
M = myocytes with electron density variation, IC = intercellular collagen.

Figure 5.3.1: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



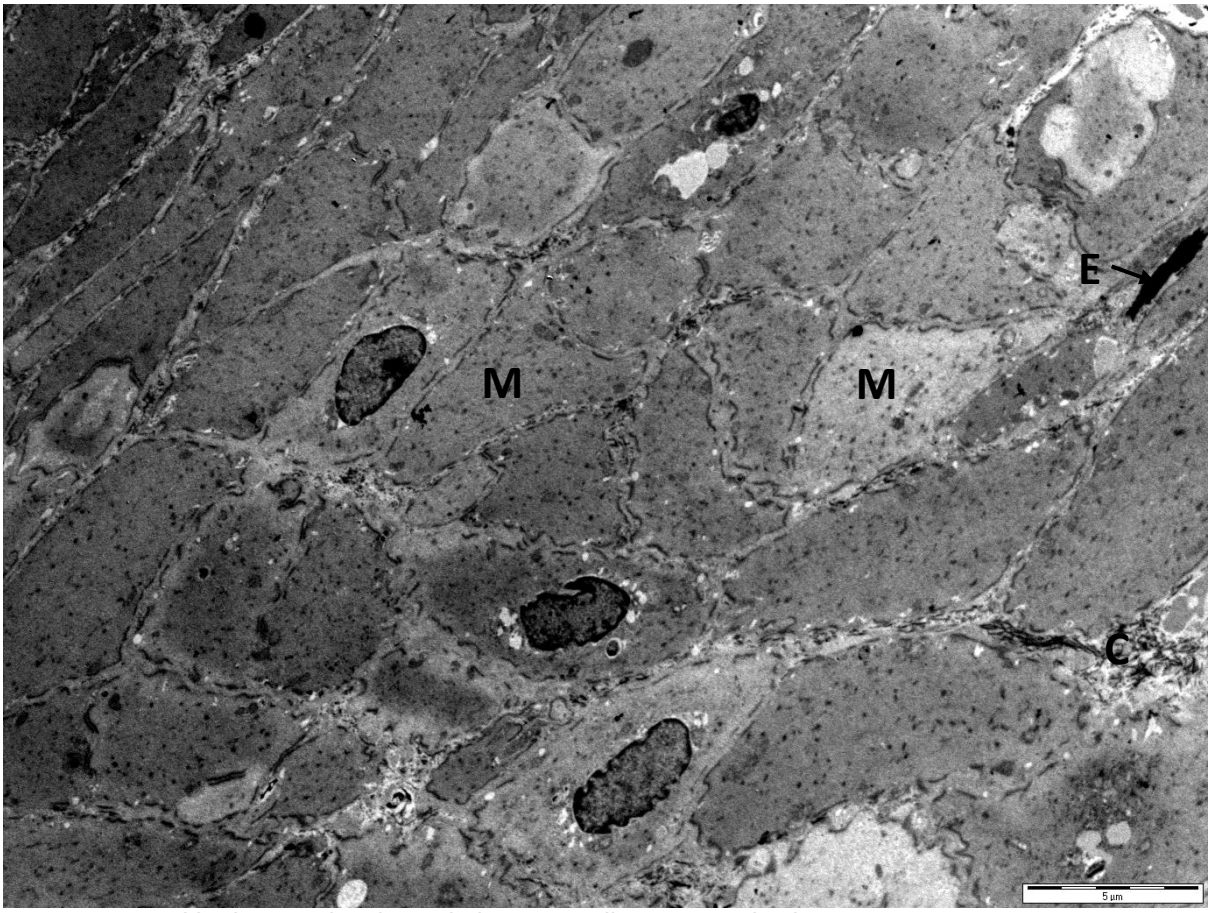
M = myocyte with electron density variation, C = collagen, E = elastin.

Figure 5.3.96: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



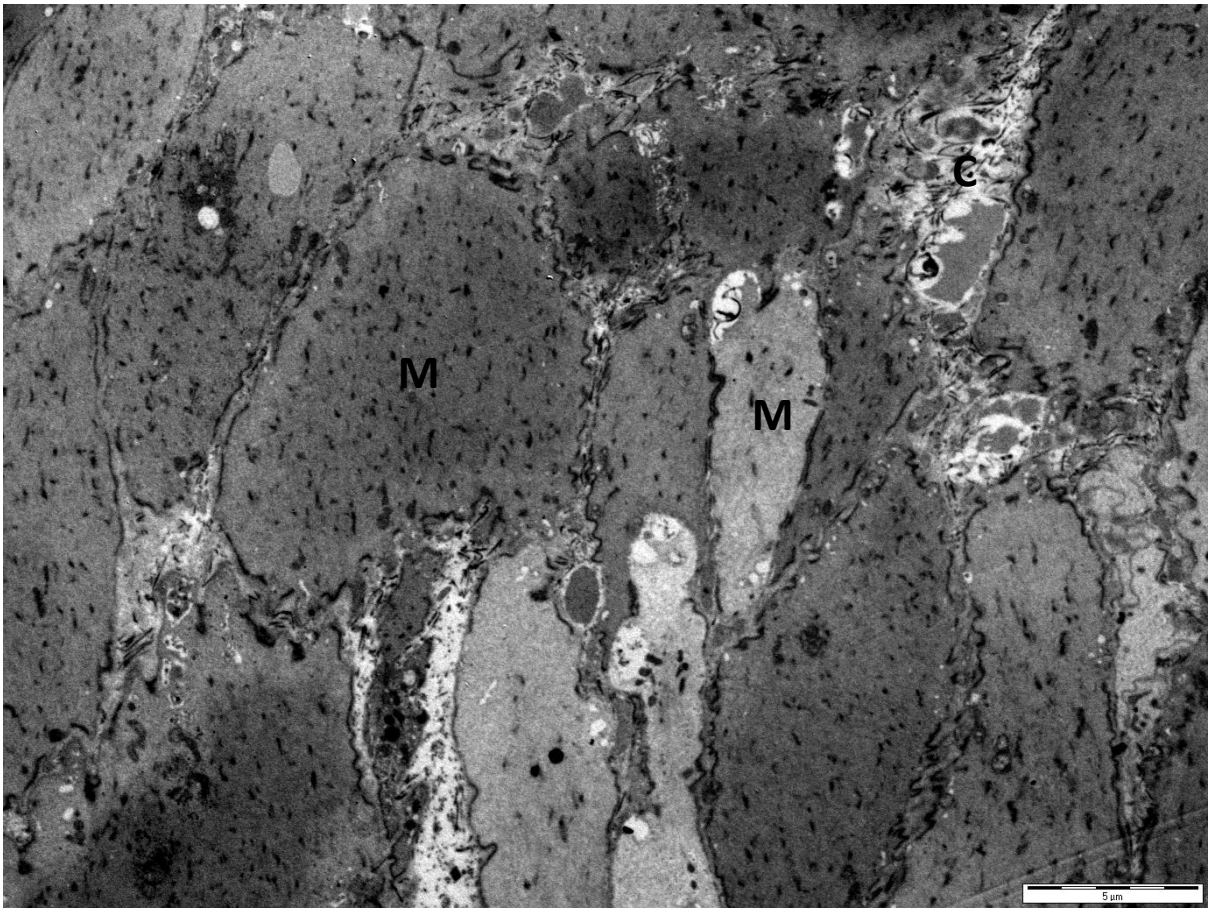
M = myocyte with electron density variation, C = collagen, E = elastin.

Figure 5.3.97: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



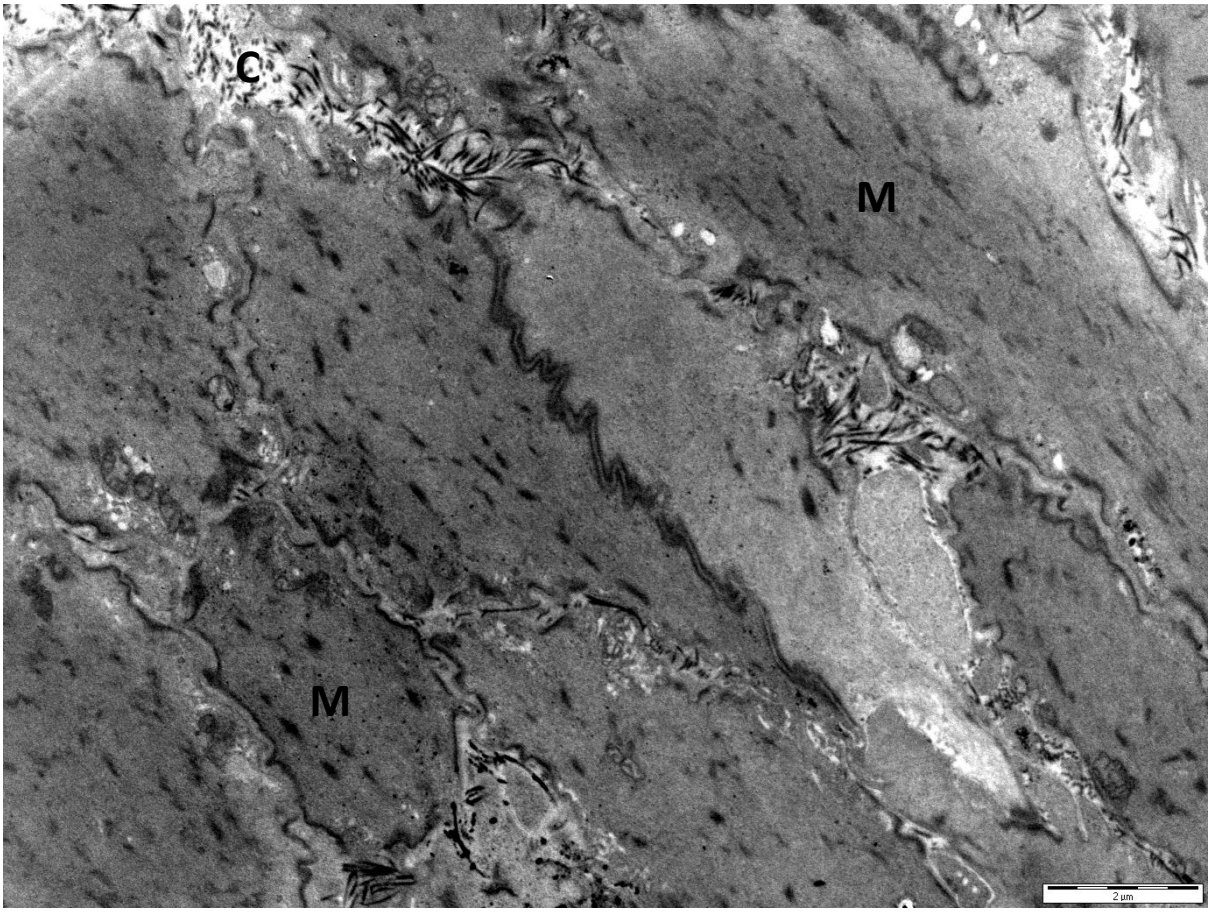
M = myocyte with electron density variation, C = collagen, E = elastin.

Figure 5.3.98: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



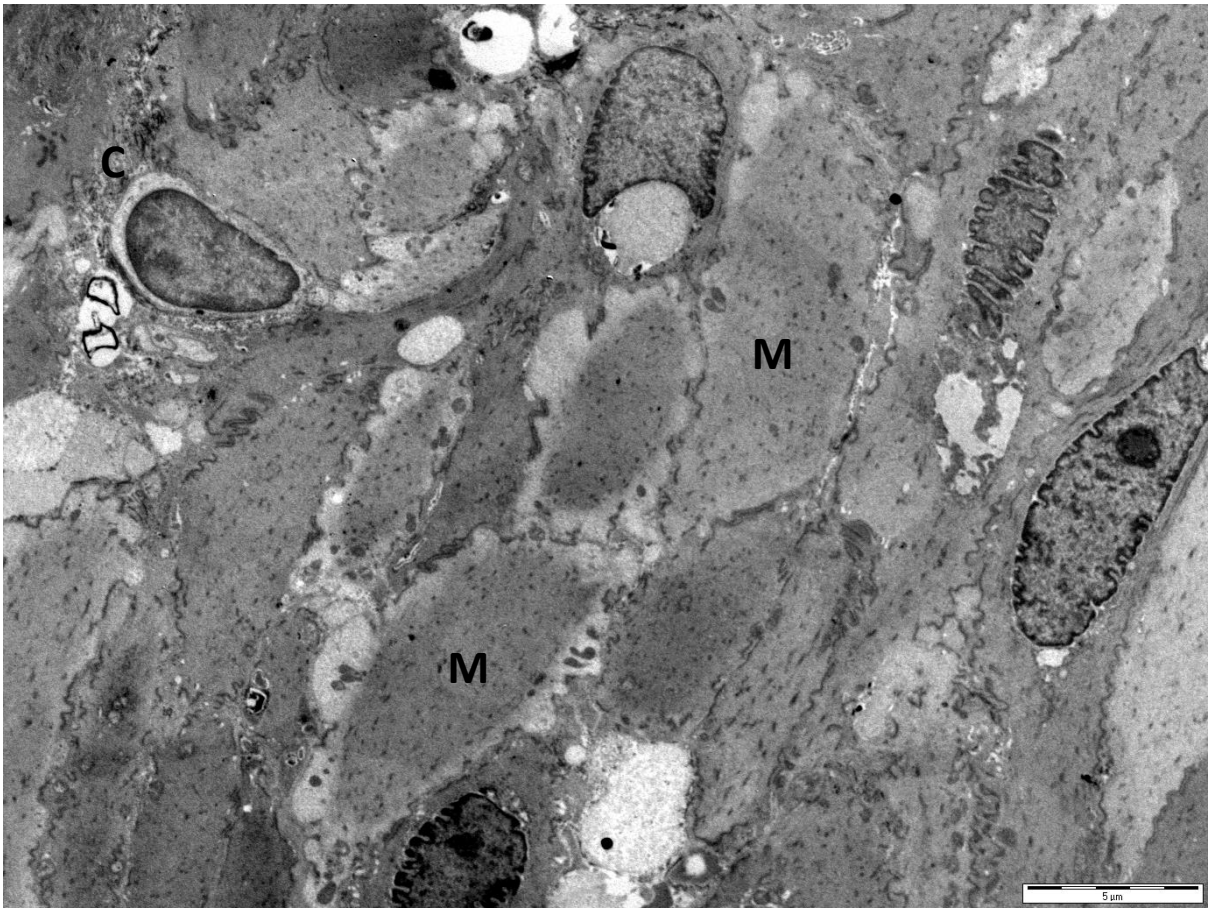
M = myocyte with electron density variation, C = collagen.

Figure 5.3.99: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



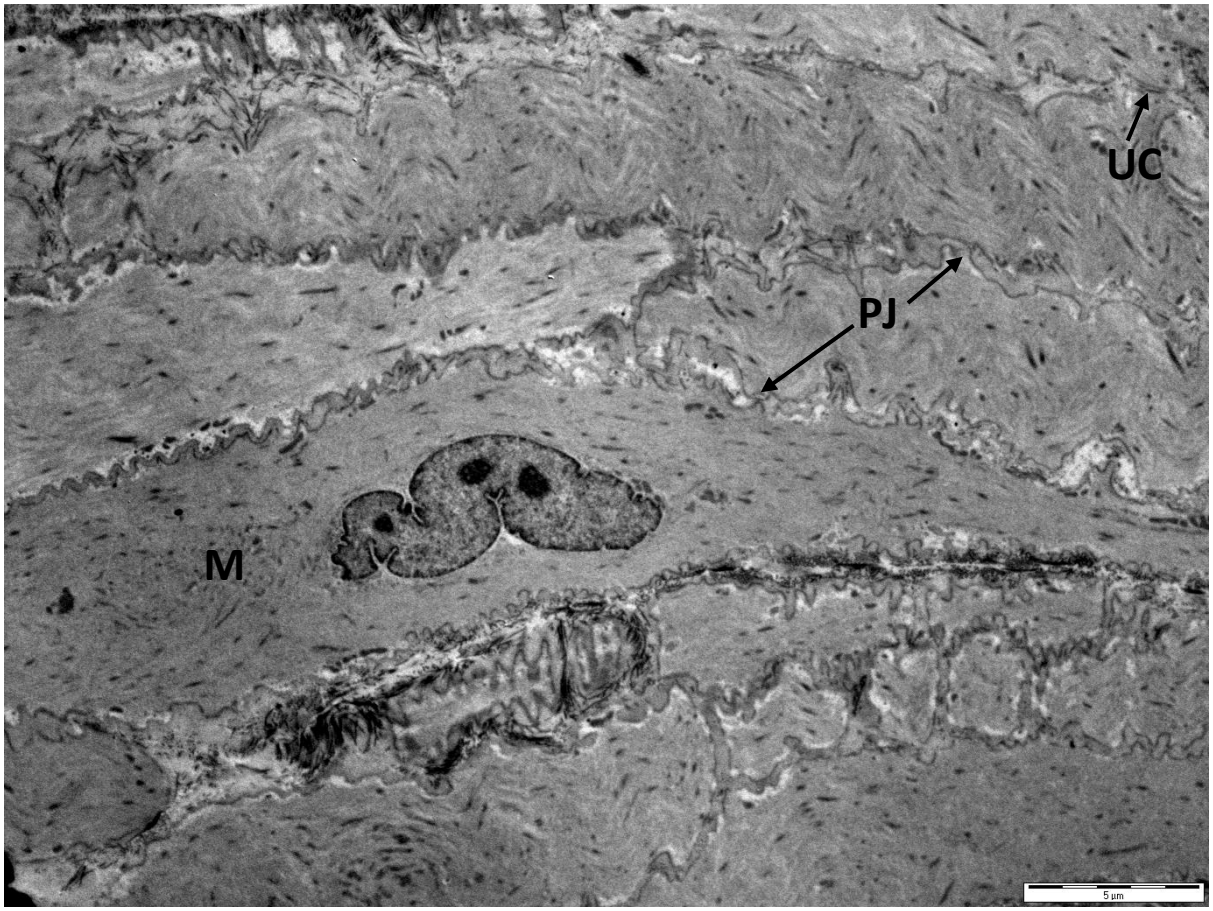
M = myocyte with electron density variation, C = collagen.

Figure 5.3.100: micrograph of moderate detrusor ultrastructural features of myohypertrophy and degeneration in male underactive bladder. This patient also had coexistent overactive bladder with underactive bladder.



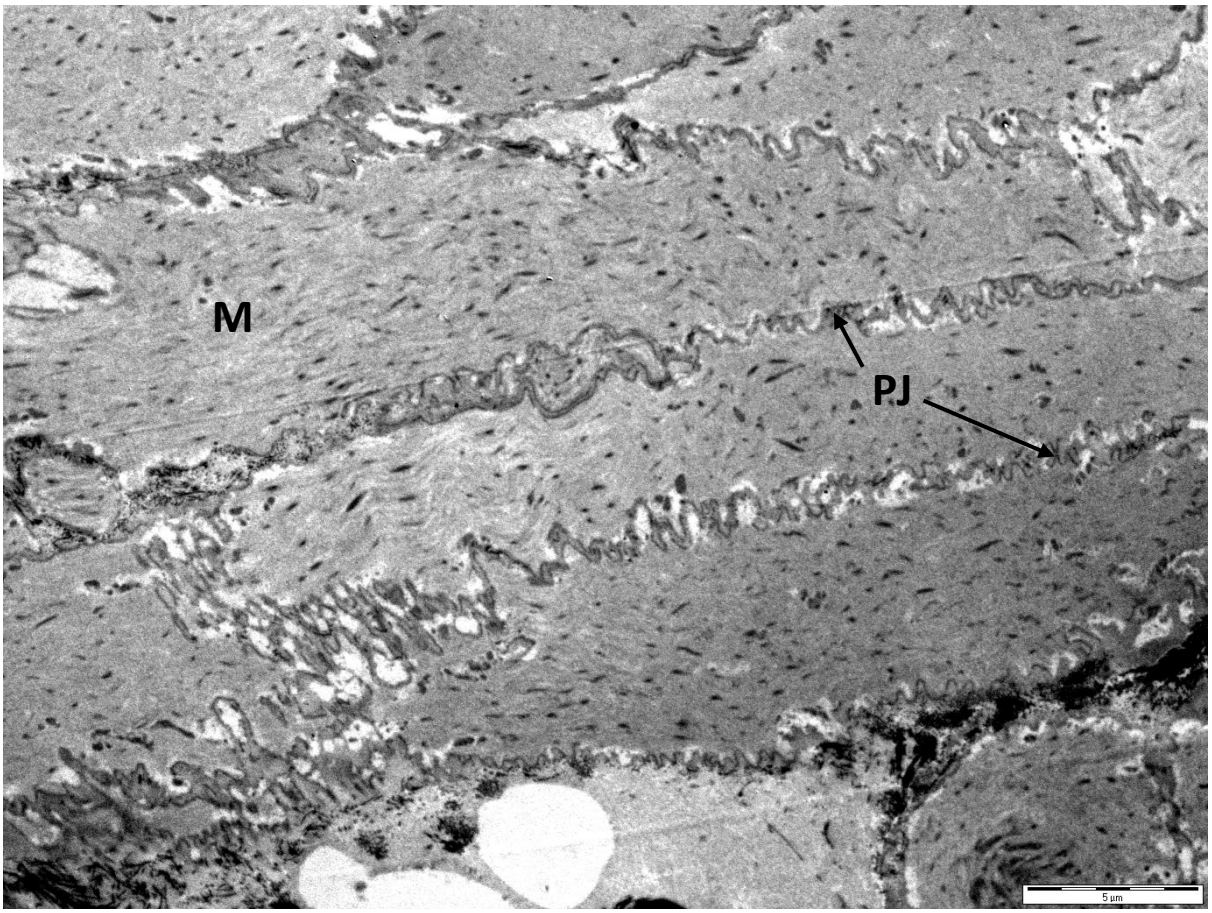
M = myocyte with electron density variation, C = collagen.

Figure 5.4.1: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17). It is interesting to note that his detrusor ultrastructure features show myocytes closely approximated with gap junctions and protrusions and close abutments, features associated with overactive bladder. This 81-year-old man had urodynamic overactive bladder as well as acontractile detrusor, with BCI 0 and VE 0%. Clinically, he initially presented with 330ml urinary retention, but had a subsequent successful trial of void and did not require further recatheterisation on long term follow-up.



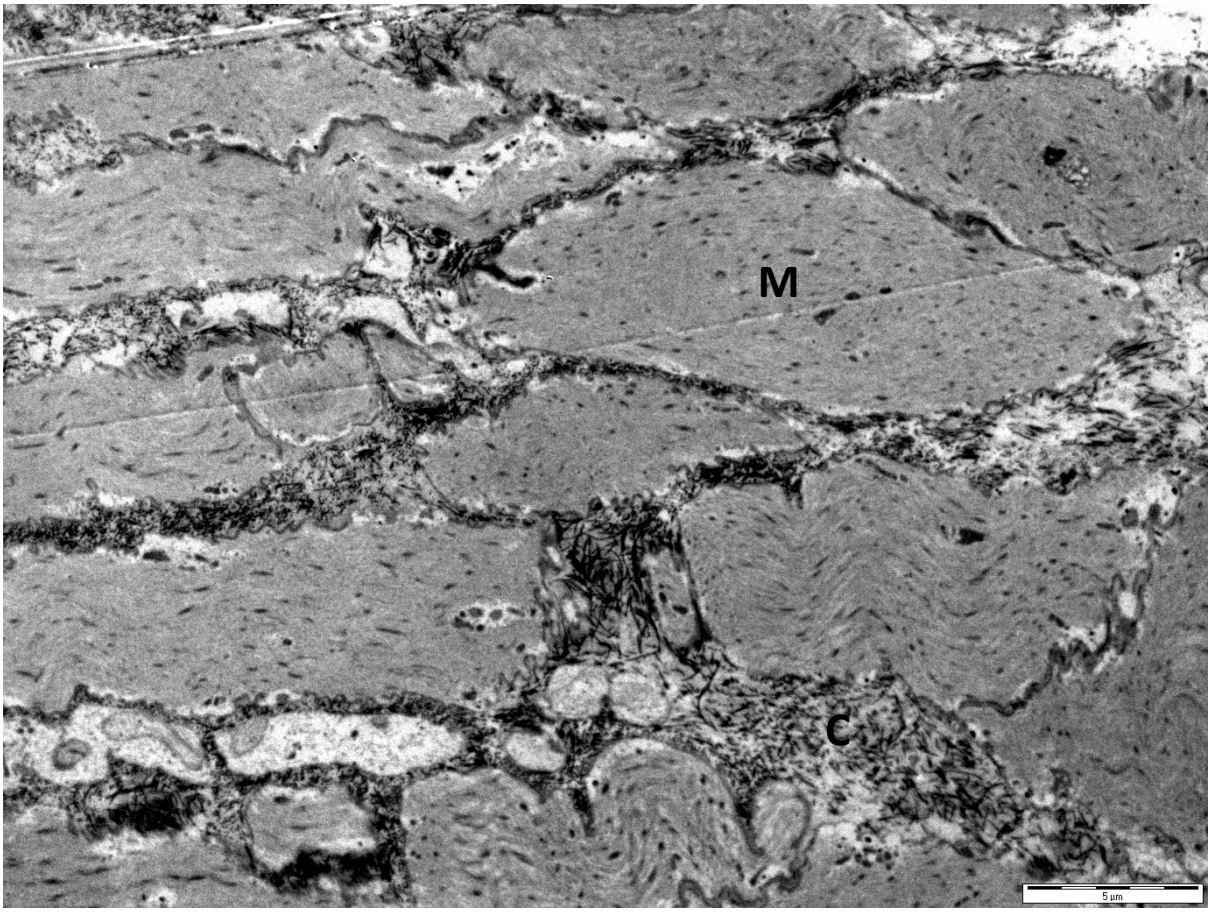
M = myocyte, PJ = multiple protrusion junctions, UC = ultraclose abutment.

Figure 5.4.102: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



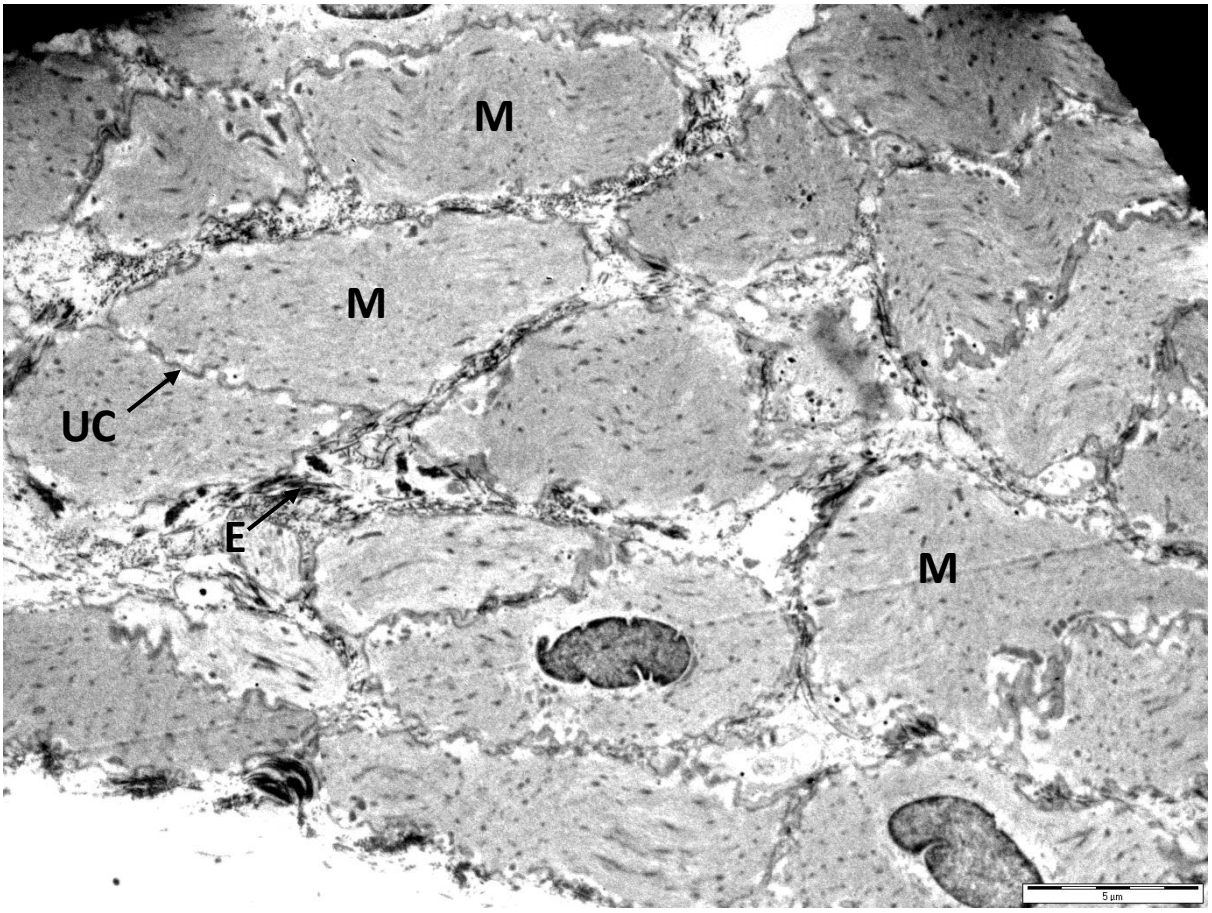
M = myocyte, PJ = multiple protrusion junctions.

Figure 5.4.103: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



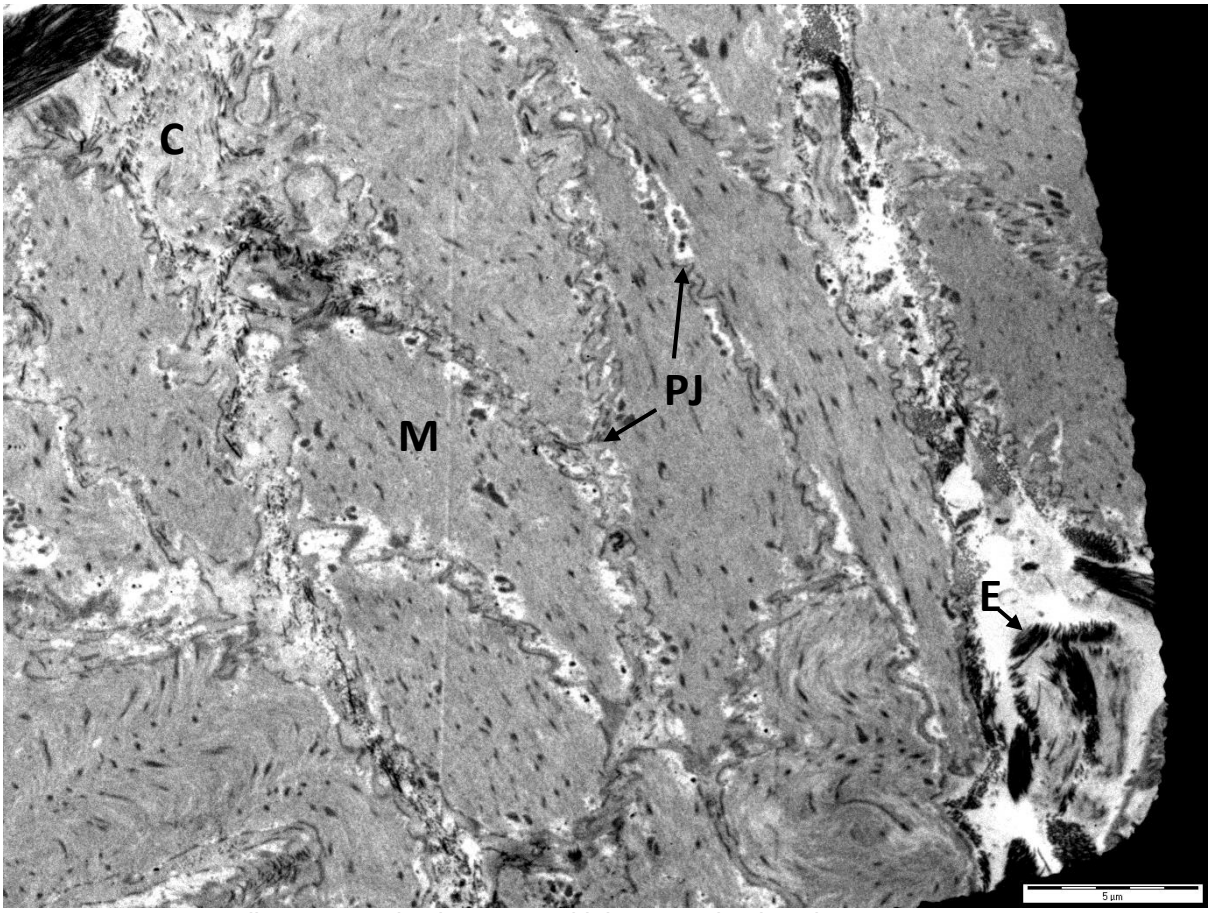
M = myocyte, C = collagen.

Figure 5.4.104: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



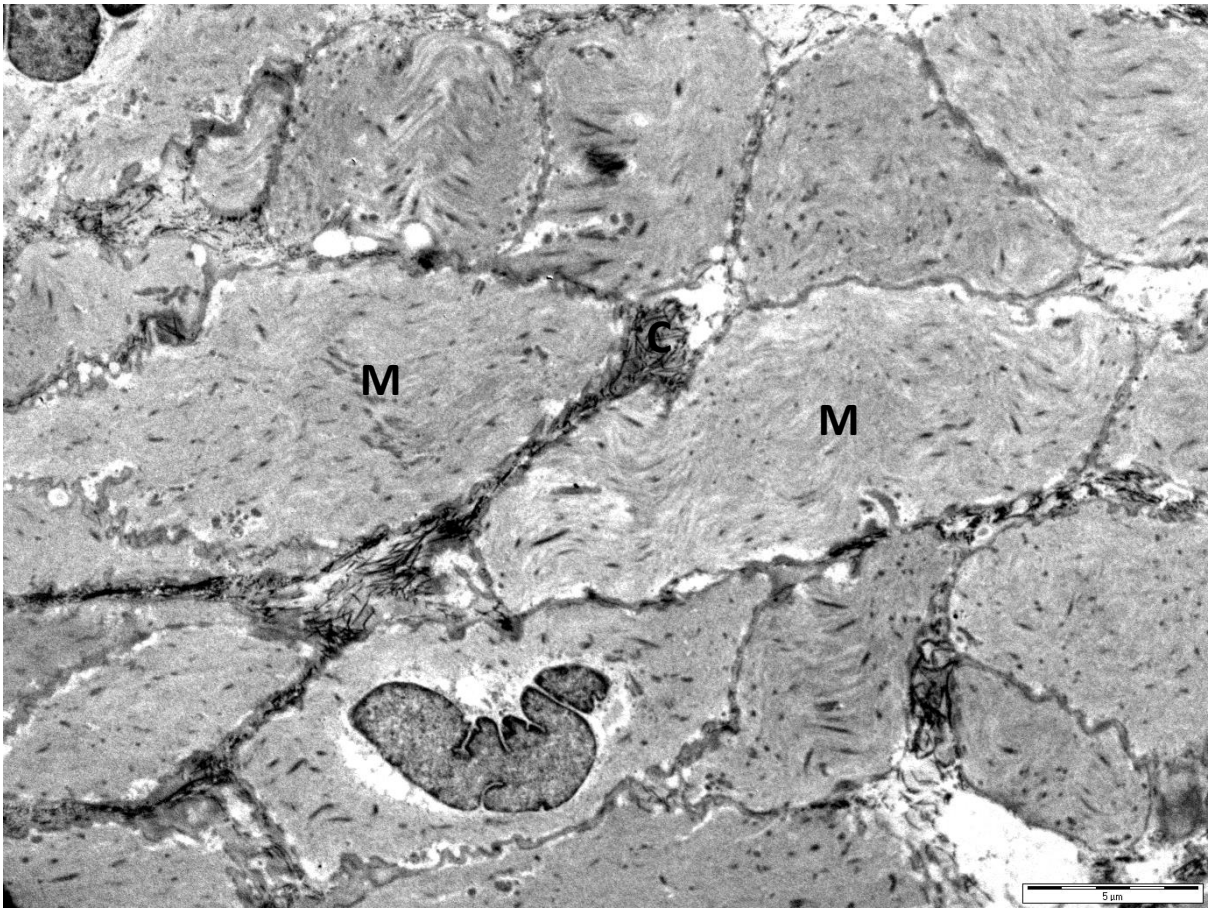
UC = massive ultraclose abutment, E = elastin, M = degenerating myocytes showing very light electron density, verging on vacuolation at the edges.

Figure 5.4.105: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



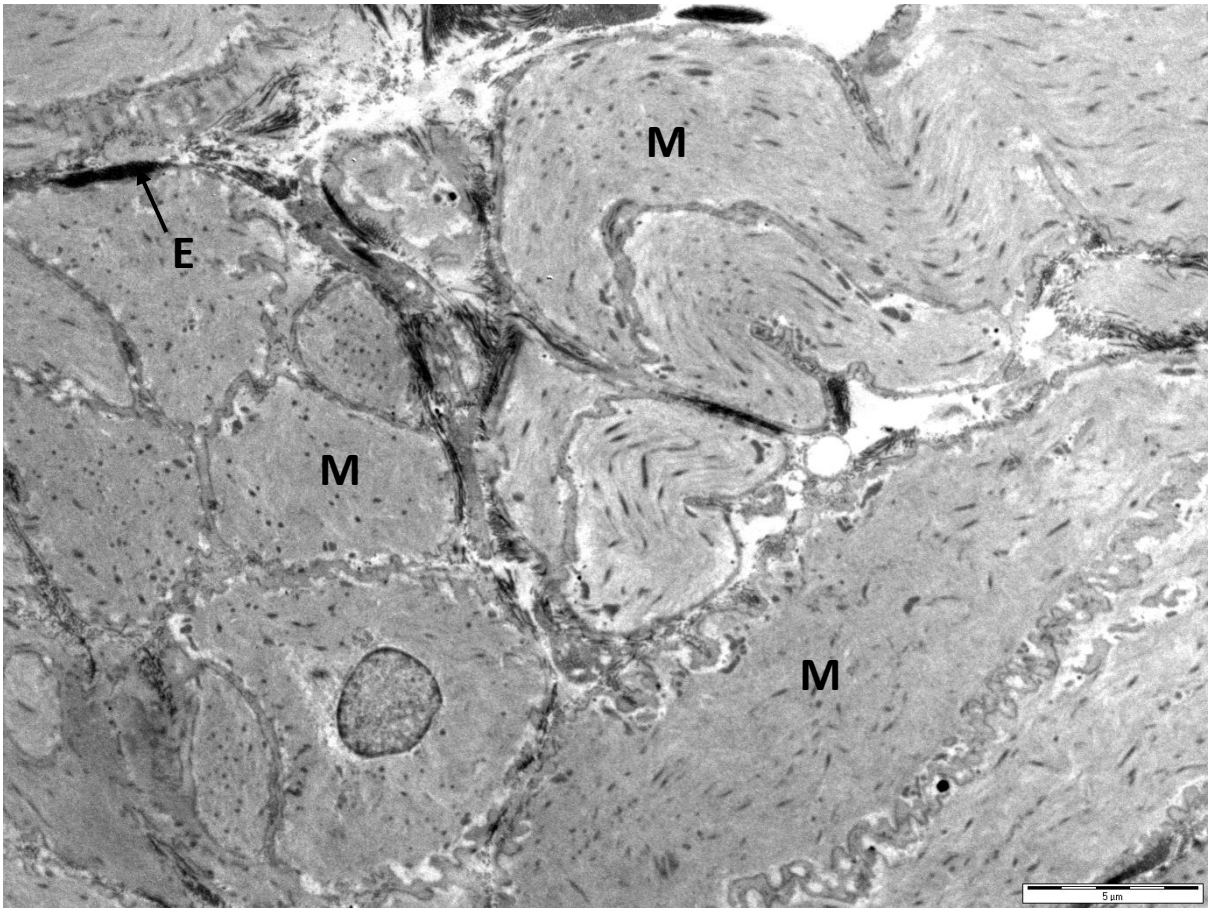
M = myocyte, C = collagen, E = elastin, PJ = multiple protrusion junctions.

Figure 5.4.106: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



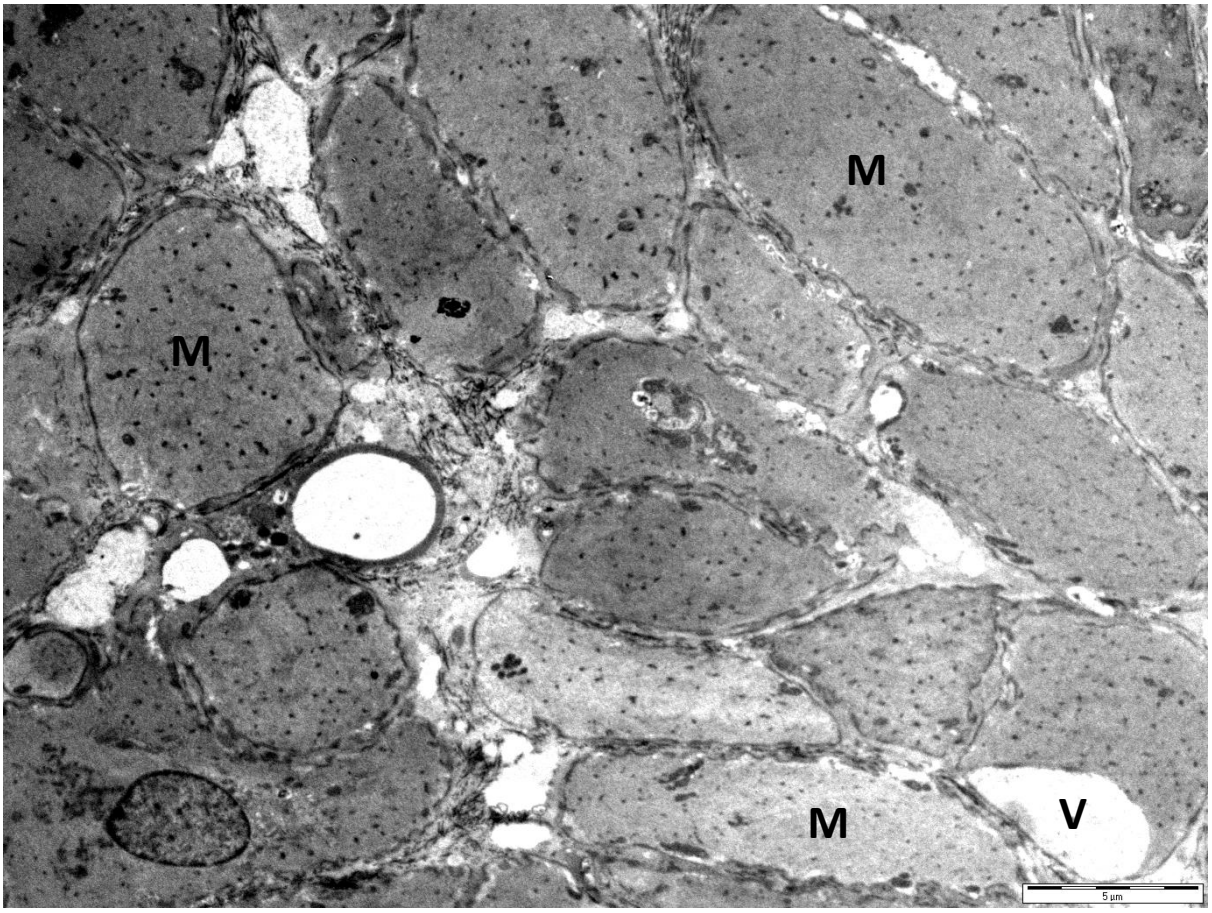
M = myocyte, C = collagen.

Figure 5.4.107: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



M = myocyte, E = elastin.

Figure 5.4.108: micrograph of detrusor ultrastructural features of male underactive bladder showing only mild features of degeneration, hyperelastosis and myohypertrophy on detrusor ultrastructure analysis (patient number 17).



M = myocyte, V = vacuolation.

Detrusor ultrastructural features were correlated with voiding parameters and both short term (within 3 months) and long term voiding outcome data. The results are displayed in the tables as follows.

Table 5.4: table showing postvoid residual measurement (sorted from smallest to largest) and corresponding short term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	PVR	Short term outcome
8	1	1	1	0	1
18	2	2	1	0	0
22	2	2	2	50	0
11	1	1	1	100	1
12	1	1	1	200	2
15	1	1	1	200	0
20	2	2	3	275	1
17	1	1	1	330	1
19	1	1	1	350	u
2	2	2	2	400	0
6	2	2	2	400	0
14	2	2	2	450	u
21	2	3	3	550	2
10	3	2	2	590	2
1	2	2	3	600	2
9	1	1	1	900	2
13	1	1	1	970	2
7	2	2	1	1000	2
16	2	2	1	1050	2
3	2	3	1	1200	2
5	3	3	3	1200	2
4	1	1	2	1600	2
23	2	2	1	2064	2

PVR= postvoid residual urine measurement (ml). Short term outcome 0 = voiding catheter-free, 1 = eventually voided during short term follow-up, 2 = never voided and catheter-dependent during short term follow-up, u = unknown.

Table 5.5: table showing BCI (sorted from highest to lowest) and corresponding short term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	BCI	Short term outcome
8	1	1	1	90	1
18	2	2	1	90	0
22	2	2	2	81	0
6	2	2	2	80	0
2	2	2	2	60	0
12	1	1	1	50	2
7	2	2	1	40	2
10	3	2	2	39	2
23	2	2	1	36	2
14	2	2	2	35	u
20	2	2	3	30	1
15	1	1	1	25	0
16	2	2	1	10	2
19	1	1	1	5	u
11	1	1	1	0	1
17	1	1	1	0	1
21	2	3	3	0	2
1	2	2	3	0	2
9	1	1	1	0	2
13	1	1	1	0	2
3	2	3	1	0	2
5	3	3	3	0	2
4	1	1	2	0	2

BCI = bladder contractility index. Short term outcome 0 = voiding catheter-free, 1 = eventually voided during short term follow-up, 2 = never voided and catheter-dependent during short term follow-up, u = unknown.

Table 5.6: table showing VE (sorted from highest to lowest) and corresponding short term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	VE	Short term outcome
8	1	1	1	100	1
18	2	2	1	100	0
22	2	2	2	89	0
15	1	1	1	56	0
2	2	2	2	33	0
12	1	1	1	33	2
6	2	2	2	20	0
19	1	1	1	8	u
14	2	2	2	2	u
7	2	2	1	0	2
10	3	2	2	0	2
23	2	2	1	0	2
20	2	2	3	0	1
16	2	2	1	0	2
11	1	1	1	0	1
17	1	1	1	0	1
21	2	3	3	0	2
1	2	2	3	0	2
9	1	1	1	0	2
13	1	1	1	0	2
3	2	3	1	0	2
5	3	3	3	0	2
4	1	1	2	0	2

VE = voiding efficiency (%). Short term outcome 0 = voiding catheter-free, 1 = eventually voided during short term follow-up, 2 = never voided and catheter-dependent during short term follow-up, u = unknown.

The following tables show detrusor ultrastructural features and select voiding outcomes (PVR, BCI, VE) on long term follow up.

Table 5.7 : table showing postvoid residual measurement (sorted from smallest to largest) and corresponding long term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	PVR	Long Term Outcome
8	1	1	1	0	1
18	2	2	1	0	0
22	2	2	2	50	0
11	1	1	1	100	0
15	1	1	1	200	0
12	1	1	1	200	2
20	2	2	3	275	0
17	1	1	1	330	0
19	1	1	1	350	u
2	2	2	2	400	2
6	2	2	2	400	2
14	2	2	2	450	.
21	2	3	3	550	2
10	3	2	2	590	1
1	2	2	3	600	2
9	1	1	1	900	1
13	1	1	1	970	1
7	2	2	1	1000	1
16	2	2	1	1050	2
3	2	3	1	1200	1
5	3	3	3	1200	1
4	1	1	2	1600	1
23	2	2	1	2064	3

PVR = postvoid residual urine measurement (ml). Long term outcome 0 = voiding catheter-free, 1 = eventually voided during long term follow-up, 2 = never voided and catheter-dependent during long term follow-up, u = unknown.

Table 5.8: table showing BCI (sorted from highest to lowest) and corresponding long term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	BCI	Long Term Outcome
8	1	1	1	90	1
18	2	2	1	90	0
22	2	2	2	81	0
6	2	2	2	80	2
2	2	2	2	60	2
12	1	1	1	50	2
7	2	2	1	40	1
10	3	2	2	39	1
23	2	2	1	36	3
14	2	2	2	35	u
20	2	2	3	30	0
15	1	1	1	25	0
16	2	2	1	10	2
19	1	1	1	5	u
11	1	1	1	0	0
17	1	1	1	0	0
21	2	3	3	0	2
1	2	2	3	0	2
9	1	1	1	0	1
13	1	1	1	0	1
3	2	3	1	0	1
5	3	3	3	0	1
4	1	1	2	0	1

BCI = bladder contractility index. Long term outcome 0 = voiding catheter-free, 1 = eventually voided during long term follow-up, 2 = never voided and catheter-dependent during long term follow-up, u = unknown.

Table 5.9: table showing VE (sorted from highest to lowest) and corresponding long term clinical outcomes and detrusor ultrastructure global impression scores.

Patient #	Myohypertrophy global impression score	Degenerative global impression score	Hyperelastosis score	VE	Long Term Outcome
8	1	1	1	100	1
18	2	2	1	100	0
22	2	2	2	89	0
15	1	1	1	56	0
2	2	2	2	33	2
12	1	1	1	33	2
6	2	2	2	20	2
19	1	1	1	8	u
14	2	2	2	2	u
7	2	2	1	0	1
10	3	2	2	0	1
23	2	2	1	0	3
20	2	2	3	0	0
16	2	2	1	0	2
11	1	1	1	0	0
17	1	1	1	0	0
21	2	3	3	0	2
1	2	2	3	0	2
9	1	1	1	0	1
13	1	1	1	0	1
3	2	3	1	0	1
5	3	3	3	0	1
4	1	1	2	0	1

VE = voiding efficiency (%). Long term outcome 0 = voiding catheter-free, 1 = eventually voided during long term follow-up, 2 = never voided and catheter-dependent during long term follow-up, u = unknown.

To further analyse the ultrastructural detrusor features with voiding outcomes, the data of four males with bladder outlet obstruction were used to compare with the data of the cohort of males with underactive bladder. The data of these four males with bladder outlet obstruction was reported in Chapter 4 (mBOO patients 2-5) and expanded here for ease of comparison. mBOO patients 2-5 were used for comparison with the data of males with underactive bladder, as those patients had mBOO with good detrusor function.

Table 5.10: table showing functional parameters of mBOO patients 2-5.

mBOO patient	Age	Sex	DO / Poor Compliance	VV	Qmax	pDet@Qmax	PVR	BOOI	BCI	VE
2	68	M	N	40	9	89	50	70	134	89
3	64	M	N	200	5	70	100	60	95	29
4	68	M	N	0	3	100	300	94	115	40
5	78	M	N	0	0	100	750	100	100	0

M = male. N = no. VV = voided volume (ml). Qmax = peak urinary flow rate (ml/s). pDet@Qmax in cmH2O. PVR = postvoid residual urine measurement (ml). BOOI = bladder outlet obstruction index. BCI = bladder contractility index. VE = voiding efficiency.

Table 5.11: table showing detrusor ultrastructural features data of males with bladder outlet obstruction (mBOO patient 2-5).

mBOO patient	Myohypertrophy 0123 (global impression)	Cell size, irregularity 0123	Increased separation of cells 0123	Increase in intercellular collagen 0123	Degenerative features 0123 (global impression)	Electron density variation 0123	Vacuolation, dense bodies 0123	Fragmentation, exclusion 0123	Hyperelastosis 0123	Myohypertrophy 0123 (global impression)
2	1	1	1	1	2	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1
4	2	2	2	2	2	2	2	2	1	2
5	2	2	2	2	2	1	2	1	1	2

Myoh Global = myohypertrophy category global impression score. Cell = cell size and irregularity. Sep = increased separation of cells. Coll = increase in intercellular collagen. Degen = Degeneration category global impression score. Var = electron density variation. Vac = vacuolation and dense bodies. Frag = fragmentation and exclusion. Elas = hyperelastosis.

On multivariate analysis (binomial regression), none of the ultrastructural features, global impression scores, or urodynamic parameters alone predicted voiding outcomes. This may be because the sample size was too small, with too few patients and data points, noting that some patients had some incomplete data points. Overall, the ultrastructual feature of increased cell separation appears to be the strongest single predictor of poorer voiding outcome, but this did not reach statistical significance.

For further statistical analysis, using a combination of four key ultrastructural features of ‘myohypertrophy pattern’ - myocyte size variation, increased separation of cells, increased intercellular collagen, and degeneration as an aggregate score (that is, for the four features, scoring at least 2 in 3 of the 4 criteria, with a cut-off point of 7 or more), it was shown that these features did correlate with poorer voiding outcomes on long term follow up (p=0.029, with OR of catheter-dependence being 5.5). These combined features did not correlate with short term voiding outcomes, likely as more patients voided early but then eventually developed complete detrusor failure and became catheter-dependent over time. Therefore, detrusor ultrastructural features of myocyte size variation, increased separation of cells, increased intercellular collagen, and degeneration were predictive of poor voiding outcomes in the long term follow up.

On univariate chi-squared analysis, increased cell separation did correlate with short term (p=0.047) and long term (p=0.034) voiding outcomes (see Tables 5.12 and 5.13). Age did not appear to correlate to myohypertrophy, degeneration, or voiding parameters (PVR, BCI, VE).

Univariate analyses were also conducted to assess for correlations between ultrastructural features and functional parameters. This showed that the ultrastructural features of myohypertrophy (p=0.025), degeneration (p=0.017) and an aggregate score (p=0.002) (reflecting myocyte size variation, increased separation of cells, increased intercellular collagen, and degeneration) all correlated with postvoid residual urine measurement, but not with bladder contractility index or patient age (see Table 5.14).

Table 5.12: Chi-squared test statistical analysis showing relationship between increased cellular separation on ultrastructure and short term voiding outcome.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.185[a]	1	0.023		
Continuity Correction[b]	3.546	1	0.060		
Likelihood Ratio	5.495	1	0.019		
Fisher's Exact Test				0.047	0.028
Linear-by-Linear Association	4.993	1	0.025		
N of Valid Cases	27				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.89.

b. Computed only for a 2x2 table

Table 5.13: Chi-square test statistical analysis showing relationship between increased cellular separation on ultrastructure and long term voiding outcome.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.250[a]	1	0.012		
Continuity Correction[b]	4.340	1	0.037		
Likelihood Ratio	6.421	1	0.011		
Fisher's Exact Test				0.034	0.018
Linear-by-Linear Association	6.000	1	0.014		
N of Valid Cases	25				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.00.

b. Computed only for a 2x2 table

Table 5.14: Spearman's rho statistical analysis of correlations between aggregate score (consisting of cell size and cell irregularity, increased separation between cells, and intercellular collagen) versus age, PVR, BCI and VE.

Correlations

			Age	PVR	BCI	VE (%)	AggregateScore
Spearman's rho	Age	Correlation Coefficient	1.000	-0.118	-0.142	0.049	-0.105
		Sig. (1-tailed)	.	0.287	0.260	0.411	0.305
		N	26	25	23	23	26
	PVR	Correlation Coefficient	-0.118	1.000	-0.504**	-0.726**	0.544**
		Sig. (1-tailed)	0.287	.	0.007	<0.001	0.002
		N	25	25	23	23	25
	BCI	Correlation Coefficient	-0.142	-0.504**	1.000	0.757**	0.047
		Sig. (1-tailed)	0.260	0.007	.	<0.001	0.416
		N	23	23	23	23	23
	VE (%)	Correlation Coefficient	0.049	-0.726**	0.757**	1.000	-0.344
		Sig. (1-tailed)	0.411	<0.001	<0.001	.	0.054
		N	23	23	23	23	23
	AggregateScore	Correlation Coefficient	-0.105	0.544**	0.047	-0.344	1.000
		Sig. (1-tailed)	0.305	0.002	0.416	0.054	.
		N	26	25	23	23	27

**. Correlation is significant at the 0.01 level (1-tailed).

Discussion

This study comprises of a large cohort of patients when compared with other studies published in the literature and therefore provides significant addition to the body of knowledge of detrusor ultrastructure in underactive bladders. It is not surprising that on multivariate analysis, no single ultrastructural feature or urodynamic parameter alone was able to predict voiding outcomes. There are limitations regarding the relatively low numbers of patients (despite this being the largest reported study in the world thus far). The scoring system was categorical rather than continuous for practical reasons.

The experience of the Concord ultrastructure research group over the past 20 years in analysing detrusor ultrastructure has shown that abnormal changes can be patchy across biopsy specimens, and it is important to examine all areas of the biopsy before imaging representative fields.

Nonetheless, when considering the key detrusor ultrastructural features of the 'myohypertrophy pattern' (myocyte size variation, increased cell separation, increased intercellular collagen, and degeneration) together as an aggregate score, there was a clear correlation with poorer long term voiding outcomes in our cohort with over 15 years follow-up (odds ratio of long term catheter-dependence being 5.5).

It is interesting to note that the aggregate scores of detrusor ultrastructural features of myocyte size variation, increased cell separation, increased intercellular collagen and degeneration did not correlate with short term outcomes, although they did correlate with long term voiding outcomes. This may be due to a number of patients voiding early but then subsequently failing to void and becoming catheter dependent in the long term. As such, we consider these severe 'myohypertrophy' pattern changes to be representative of the failing detrusor and high likelihood of long term catheter dependence.

Age had no significant correlation with voiding outcomes, and this aligns with the thought that myohypertrophy and degeneration patterns correlate with lower urinary tract function, rather than an aging phenomenon per se.(21)

Overall, this part of the PhD shows that, consistent with our previous studies, detrusor structural changes do seem to predict poor voiding outcomes in men with hypocontractile detrusors, even with extended follow-up and a larger cohort of patients.

As referenced in the introductory chapter, accurate diagnosis and prognosis of human lower urinary tract dysfunction can be elusive using current established testing, such as video-urodynamic studies. In light of the limitations of urodynamic studies in diagnosis of DU, it would be useful if detrusor ultrastructural studies could assist as a “piece of the puzzle” in determining a patient’s detrusor’s functional qualities, whether the degree of detrusor contractility or failure would allow a patient to be able to void spontaneously without need for catheterisation if their outlet resistance is reduced. That is, prognostication of clinical outcome after BOO surgery and treatments.
We have shown that detrusor biopsy with ultrastructural analysis may be a useful diagnostic and prognostic tool in men with hypocontractile bladder and urinary retention considering treatment options.(19)

Conclusions

Detrusor ultrastructural features of myocyte size variation, increased cell separation, increased intercellular collagen, and degeneration are associated with poor long term voiding outcomes and increased likelihood of catheter-dependence. This information may help clinicians counsel patients with diagnosis as well as prognosis, since delineating the diagnosis and prognosis is key to effective and appropriate management, whilst avoiding unnecessary harm associated with inappropriate therapy. Detrusor ultrastructural analysis has potential as a diagnostic and prognostic tool in lower urinary tract dysfunction assessment and management.

References

1. Rossi A, Ganassini A, Tantucci C, Grassi V. Aging and the respiratory system. Aging (Milano). 1996;8(3):143-61.
2. Geokas MC, Conteas CN, Majumdar AP. The aging gastrointestinal tract, liver, and pancreas. Clin Geriatr Med. 1985;1(1):177-205.
3. Diokno AC, Brown MB, Goldstein N, Herzog AR. Epidemiology of bladder emptying symptoms in elderly men. J Urol. 1992;148(6):1817-21.
4. Diokno AC, Brock BM, Brown MB, Herzog AR. Prevalence of urinary incontinence and other urological symptoms in the noninstitutionalized elderly. J Urol. 1986;136(5):1022-5.
5. Resnick NM, Yalla SV. Detrusor hyperactivity with impaired contractile function. An unrecognized but common cause of incontinence in elderly patients. JAMA. 1987;257(22):3076-81.
6. Chapple CR, Osman NI, Birder L, van Koeveringe GA, Oelke M, Nitti VW, et al. The underactive bladder: a new clinical concept? Eur Urol. 2015;68(3):351-3.
7. Chapple CR, Osman NI, Birder L, Dmochowski R, Drake MJ, van Koeveringe G, et al. Terminology report from the International Continence Society (ICS) Working Group on Underactive Bladder (UAB). Neurourol Urodyn. 2018;37(8):2928-31.
8. Kortmann BB, Sonke GS, Wijkstra H, Nordling J, Kallestrup E, Holm NR, et al. Intra- and inter-investigator variation in the analysis of pressure-flow studies in men with lower urinary tract symptoms. Neurourol Urodyn. 2000;19(3):221-32.
9. Abrams P. Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: three simple indices to define bladder voiding function. BJU Int. 1999;84(1):14-5.
10. Chung A, Noguchi N, Chan L, Tse V. Voiding dysfunction in older men. Curr Opin Urol. 2016;26(2):177-83.
11. Barendrecht MM, Oelke M, Laguna MP, Michel MC. Is the use of parasympathomimetics for treating an underactive urinary bladder evidence-based? BJU Int. 2007;99(4):749-52.
12. Nomiya M, Andersson KE, Yamaguchi O. Chronic bladder ischemia and oxidative stress: new pharmacotherapeutic targets for lower urinary tract symptoms. Int J Urol. 2015;22(1):40-6.
13. Ho FCS, He C, Yao HH, O'Connell HE, Gani J. Efficacy of sacral neuromodulation and percutaneous tibial nerve stimulation in the treatment of chronic nonobstructive urinary retention: A systematic review. Neurourol Urodyn. 2021;40(5):1078-88.
14. Coolen RL, Groen J, Stillebroer AB, Scheepe JR, Witte LPW, Blok BFM. Two-Staged Sacral Neuromodulation for the Treatment of Nonobstructive Urinary Retention: A Multicenter Study Assessing Predictors of Success. Neuromodulation. 2022.
15. Chan G, Qu LG, Gani J. Evaluation of pre-operative bladder contractility as a predictor of improved response rate to a staged trial of sacral neuromodulation in patients with detrusor underactivity. World J Urol. 2021;39(6):2113-9.
16. Han DH, Jeong YS, Choo MS, Lee KS. The efficacy of transurethral resection of the prostate in the patients with weak bladder contractility index. Urology. 2008;71(4):657-61.
17. Ou R, Pan C, Chen H, Wu S, Wei X, Deng X, et al. Urodynamically diagnosed detrusor hypocontractility: should transurethral resection of the prostate be contraindicated? Int Urol Nephrol. 2012;44(1):35-9.
18. Mitchell CR, Mynderse LA, Lightner DJ, Husmann DA, Krambeck AE. Efficacy of holmium laser enucleation of the prostate in patients with non-neurogenic impaired bladder contractility: results of a prospective trial. Urology. 2014;83(2):428-32.
19. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.
20. Thorner DA, Blaivas JG, Tsui JF, Kashan MY, Weinberger JM, Weiss JP. Outcomes of reduction cystoplasty in men with impaired detrusor contractility. Urology. 2014;83(4):882-6.
21. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. J Urol. 1993;150(5 Pt 2):1657-67.

CHAPTER 6: DETRUSOR ULTRASTRUCTURAL STUDIES IN OLDER PATIENTS WITH OVERACTIVE BLADDER – DO THEY HAVE FEATURES OF DEGENERATION AND DETRUSOR UNDERACTIVITY?

Introduction and objectives

The aging population

There is an aging population in Australia and worldwide.. The number of persons aged 60 years and over will be double from what it was in 2020 (1 billion) by 2050, representing an increase in proportion from 12% to 22%. The number of persons aged 80 years or older is expected to triple between 2020 and 2050, reaching 426 million. By 2030, 1 in 6 people in the world will be aged 60 years or over.(1)

Biologically, aging is a result of the impact of the accumulation of a wide variety of molecular and cellular damage over time. Older age is also characterized by the emergence of several complex health states commonly called geriatric syndromes. They are often the consequence of multiple underlying factors and include frailty, urinary incontinence, falls, delirium, and pressure ulcers.(1)

Bladder dysfunction and aging

It is well established that lower urinary tract symptoms increase in prevalence with aging.(2) In older men, benign prostatic hyperplasia is common with increasing age, as discussed previously in Chapters 4 and 5. In older men and women, overactive bladder syndrome (OAB) is a common bothersome lower urinary tract dysfunction. It is characterised by symptoms of urinary frequency, urgency, nocturia and sometimes also urinary incontinence, typically described as urinary urgency incontinence. For the older population, in combination with their other frailties, LUTS can cause significant risk of morbidity, including fall risk, and exacerbate issues of disturbed sleep, fatigue and other health implications.(3)

Ameda et al. reported that in older patients (mean 69 years), 40.9% were found to have detrusor overactivity, 31.1% have detrusor underactivity, and 10.8% have detrusor overactivity with detrusor underactivity on urodynamic studies.(4) Although the presence of detrusor overactivity increased with age, bladder contractility was not found to correlate with age.(4) It was interesting that the non-obstructed patient population was functionally distinct while symptomatically similar. The bladder is an “unreliable witness”, and patients can report symptoms which overlap with other lower urinary tract dysfunction diagnoses, making it difficult to properly diagnose based solely on symptomatology.(5) Therefore, age-related lower urinary tract functional changes warrant investigation and require differentiation between symptoms related to aging and those related to comorbid conditions. Treatment of non-obstructed patients with OAB based on symptomatology alone without further investigation may lead to inappropriate pharmacological therapy and unsuccessful outcomes.(4)

Co-existent overactive-underactive bladder

Co-existing overactive-underactive bladder (COUB) (with or without urodynamic evidence of DO and DU) has been proposed as a separate clinical syndrome because it differs from sole OAB and UAB and may not be the combination of both syndromes in the same patient.(6) LUTS are often classified into which symptoms are predominant, but as previously discussed, the bladder is an unreliable witness and symptoms may be shared among various distinct lower urinary tract dysfunction diagnoses.(5)

OAB syndrome, a storage dysfunction, may include urodynamic features of increased bladder sensation, detrusor overactivity and poor compliance, but all three may not necessarily coexist in the one patient.

UAB syndrome, a voiding dysfunction, may include urodynamic features of detrusor underactivity. It may also include a slow urinary flow or high post void residual, but is sometimes considered to be associated with reduced bladder sensation in the context of insensate chronic urinary retention.

Urodynamic studies can be helpful in delineating the underlying pathophysiologic mechanisms behind a patient’s presenting LUTS. COUB is an interesting condition to diagnose and treat and is under-

recognised. Patients may require a specific approach and considerations in treatment options beyond the treatment of single and in some ways opposing syndromes.(6)

Management of overactive bladder

OAB management may start with conservative strategies such as dietary modification, fluid management, and timed voiding, as well as bladder retraining. Overactive bladder can also be treated with medical therapy such as mirabegron (beta 3 agonist), or anticholinergic medications, which are often best avoided in the older population due to risk of anticholinergic side effects. These side effects include dry mouth, dry eyes, constipation, and cognitive side effects, which older patients are more susceptible to. Advanced therapies for overactive bladder include intra-detrusor onabotulinum toxin injections performed via a cystoscope, sacral neuromodulation implant, and percutaneous tibial nerve stimulation.

Anticholinergic medication and intra-detrusor onabotulinum toxin injections are common treatment options, but pose risk of urinary retention or worsening voiding function, which beta-3 agonists, sacral neuromodulators, and PTNS do not cause. The risk of urinary retention with anticholinergic medication is higher if the patient has elevated postvoid residual urine. The risk of retention in patients is also higher if the patient has concomitant bladder outlet obstruction (which may be undiagnosed) or detrusor underactivity.(7)

This section of the PhD aims to examine whether older patients with OAB may have OAB features on detrusor ultrastructure with concomitant DU features.

Methodology and patient cohort

Fourteen male patients over the age of 60 years with known overactive bladder on urodynamic studies were included in this section of the PhD project. Patients were excluded if their urodynamic parameters showed presence of bladder outlet obstruction (defined as BOOI > 40).

Age 60 years was chosen to be consistent with World Health Organisation data on aging in the population. Overactive bladder was defined by urodynamic diagnosis of at least one of the following three features:

1) Sensory overactive bladder (SOAB), comprised of
    a. Increased bladder sensation (IBS), and/or
    b. Urodynamic urinary urgency (UUU)
2) Presence of detrusor overactivity (DO)
3) Poorly compliant bladder (PCB).

For the purposes of this study, SOAB was considered present if the patient reported either IBS or UUU. Increased bladder sensation is defined as the complaint that the sensation of bladder filling occurs earlier or is more intense or persistent to that previously experienced. This differs from urgency by the fact that micturition can be postponed despite the desire to void.(8) IBS is still a matter of debate regarding its clinical relevance, urodynamic nature and underlying pathology. For the purposes of this study, increased bladder sensation (IBS) was defined as first desire to urinate at bladder volume <100ml.

Urinary urgency is defined as a compelling need to urinate which is difficult to defer (generally due to pain, pressure, and/or discomfort).(9) For the purposes of this study, UUU was considered present if the patient reported urinary urgency during the urodynamic study at a urodynamic bladder filling volume of less than 350ml.(10)

Detrusor overactivity was defined by the International Continence Society Standardization Sub-committee in 2002 as a urodynamic observation characterized by involuntary detrusor contractions during the filling phase that may be spontaneous or provoked.(11)

Bladder compliance is calculated as the change in volume divided by the change in detrusor pressure. Normal bladder compliance is defined as a value of >40 mL/cmH_2O, with values of <13 mL/cmH_2O representing severe compromise of bladder compliance.(12, 13) Patients with

normal bladder compliance <13 mL/H2O were given a score of 0, patients with moderately poor bladder compliance 14-40 mL/H2Owere were given a score of 1, and patients with severely poor compliance >40 mL/cmH2O were given a score of 2 in this domain.

All eligible patients who were going to undergo cystoscopy as part of their course of investigation and treatment were recruited into the study and consented for detrusor biopsy. The detrusor biopsy was taken at the time of cystoscopy and specimens were processed for transmission electron microscopy analysis according to the previously described protocol (see Chapter 3 Methodology for details of biopsy harvest and processing technique).

Previously established diagnostic criteria were used for ultrastructural analysis of myohypertrophy pattern (such as myocyte size and irregularity, myocyte cell separation, and collagenosis), degenerative features (such as electron density variation, vacuolation, and fragmentation) and hyperelastosis were identified. Scoring of degree of severity of features were recorded as 0, 1, 2, or 3 (0 = none, 1 = little, 2 = moderate, 3 = marked) by two observers (AC and VT/LC). Severity of the detrusor ultrastructural features was evaluated and correlated with urodynamic parameters and described.

Results

Fourteen male patients over 60 years of age with urodynamic features of overactive bladder and no urodynamic bladder outlet obstruction were identified for inclusion in this part of the study. Of these, two had normal bladder contractility (BCI >100) and may be considered as “controls” with only overactive bladder (patients 1 and 2). Twelve had weak bladder contractility (BCI <100) and therefore have the diagnosis of co-existent overactive-underactive bladder (patients 3-14).

The mean age was 80 years (IQR 73-86 years). 64% (9/14) men had increased bladder sensation (IBS), 43% (6/14) had detrusor overactivity, and 71% (10/14) had poorly compliant bladder. Four patients (patients 1-4) had presented with predominant overactive bladder storage symptoms such as urinary frequency, urgency, nocturia, and urinary urgency incontinence. Ten patients (patients 5-14) had presented with predominant voiding LUTS such as urinary retention, feeling of incomplete bladder emptying, slow urinary stream, and hesitancy.

Of the two patients with OAB only, one patient had IBS and PC without DO, and the other patient had DO only. For this group, mean BCI was 154 and VE 100%.

Of the two patients with COUB with predominantly storage LUTS, both patients had IBS and PC, and the other patient had DO. The two patients with COUB with predominantly storage LUTS had mean BCI 58 and mean VE 50%.

Of the ten patients with COUB with predominantly voiding LUTS, 60% (6/10) had IBS, 40% (4/10) had DO and 70% (7/10) had PC. Two patients had all 3 features of urodynamic OAB. The group of patients with COUB with predominantly voiding LUTS had mean BCI 27 and mean VE 18%.

Table 6.1: Urodynamic parameters of older males with OAB without BOO.

Patient	Age	Sex	IBS	DO	PC	VV	Qmax	Pdet at Qmax	PVR	BOOI	BCI	VE
1	82	M	0	1	0	360	27	44	0	-10	179	100
2	73	M	1	0	1	320	13	64	0	38	129	100
3	86	M	1	1	1	0	0	35	93	35	35	0
4	63	M	1	0	1	160	6	50	0	38	80	100
5	86	M	0	1	0	200	8	20	400	4	60	33
6	86	M	1	1	0	0	0	0	1200	0	0	0
7	70	M	1	0	0	320	8	50	0	34	90	100
8	63	M	1	1	1	0	0	0	100	0	0	0
9	90	M	1	0	1	100	10	0	200	-20	50	33
10	100	M	0	0	1	10	1	30	450	28	35	2
11	81	M	1	1	1	0	0	0	330	0	0	0
12	73	M	0	0	1	30	1	0	350	0	5	8
13	84	M	1	0	1	0	0	30	275	30	30	0
14	82	M	0	0	1	0	0	0	550	0	0	0

Age in years. M = male. IBS = increased bladder sensation, 0 = absent, 1 = present. DO = detrusor overactivity, 0 = absent, 1 = present. PC = poor compliance, 0 = absent, 1 =present. VV = voided volume (mL). Qmax = peak urinary flow rate (mL/s). pDet at Qmax = detrusor pressure at Qmax (cmH2O). PVR = post void residual urine (mL). BOOI = bladder outflow obstruction index, based on Abram-Griffiths number (AGN) for males, BOOI>40 represents obstructed. BCI = bladder contractility index, BCI<100 represents weak bladder contractility. VE = voiding efficiency (%).

Figure 6.1: Urodynamic study cystometrogram of patient with increased bladder sensation, detrusor overactivity, detrusor overactivity incontinence, poor compliance, and detrusor underactivity with inability to void. (patient number 3)

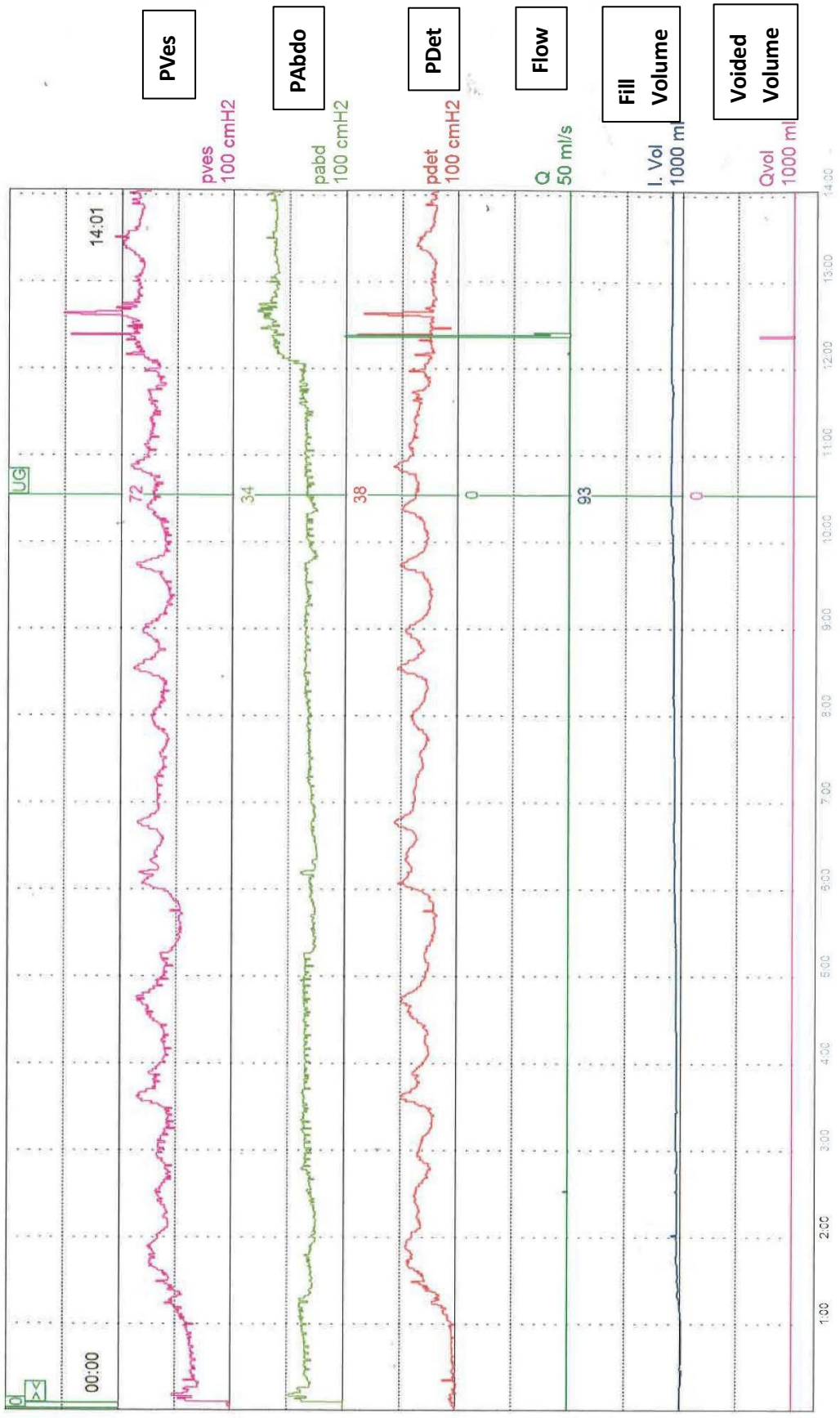
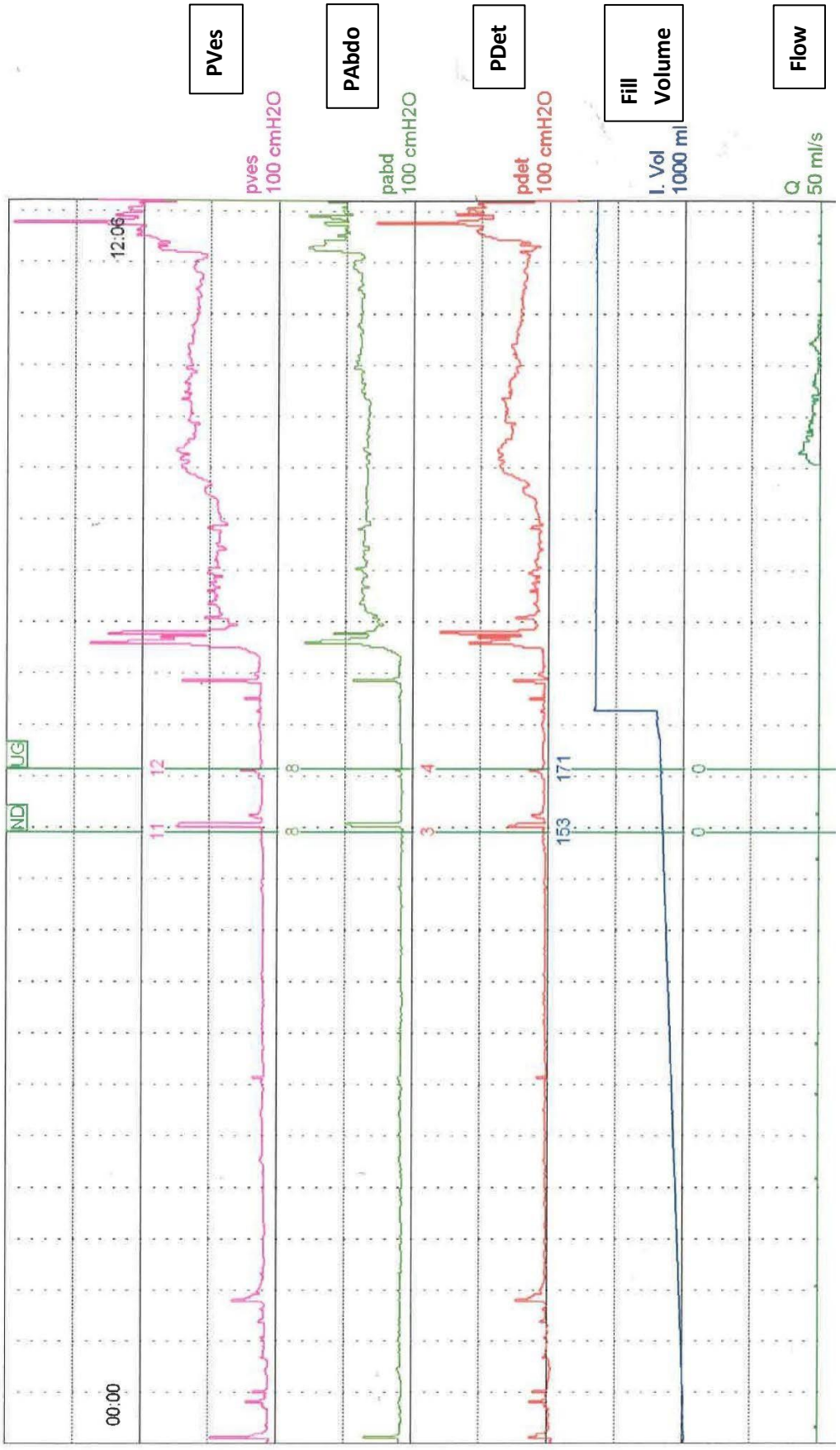


Figure 6.2: Urodynamic tracingof patient with increased bladder sensation, poor bladder compliance, and detrusor underactivity but complete bladder emptying. (patient number 4)



The detrusor ultrastructure features of this patient cohort were analysed and recorded. They are displayed in the table as follows.

Table 6.2: Older males with urodynamic OAB features correlated with detrusor ultrastructure data.

Patient	Age	IBS	DO	PC	Myohypertrophy 0123 (global impression)	Cell size, irregularity 0123	Increased separation of cells 0123	Increase in intercellular collagen 0123	Degenerative features 0123 (global impression)	Electron density variation 0123	Vacuolation, dense bodies 0123	Fragmentation, exclusion 0123	Hyperelastosis 0123
1	82	0	1	0	1	1	0	1	0	1	2	0	0
2	73	1	0	1	1	1	1	1	0	1	1	0	0
3	86	1	1	1	2	2	1	2	3	2	2	1	1
4	63	1	0	1	2	2	2	2	2	2	2	2	2
5	86	0	1	0	2	2	2	2	2	2	3	2	2
6	86	1	1	0	2	2	1	2	3	2	2	1	1

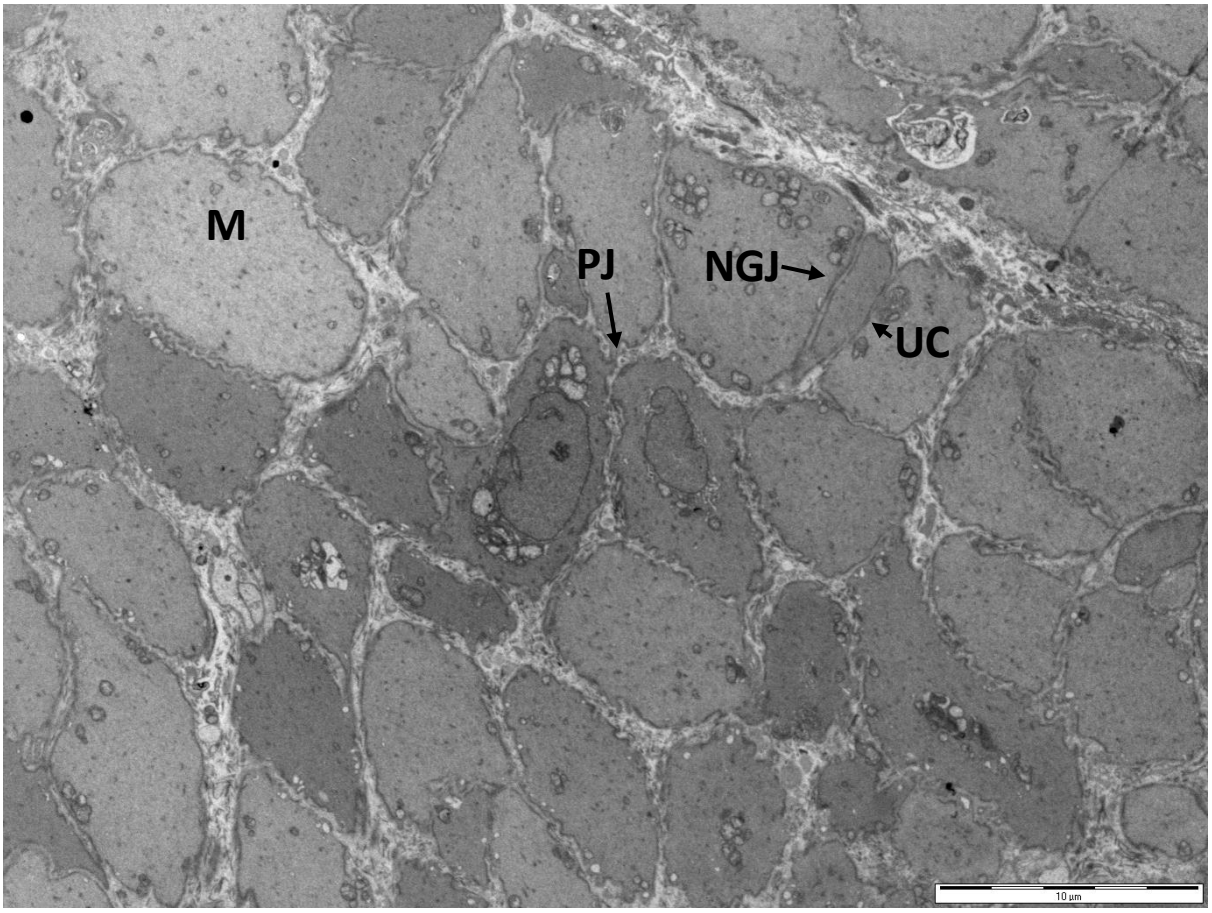
7	70	1	0	0	1	1	1	1	1	1	1	0	1
8	63	1	1	1	1	1	1	1	1	1	1	1	1
9	90	1	0	1	1	1	1	1	2	1	0	0	0
10	100	0	0	1	1	1	1	1	1	0	0	0	0
11	81	1	1	1	1	1	1	1	1	1	1	1	1
12	73	0	0	1	1	1	1	1	1	1	1	1	1
13	84	1	0	1	2	2	2	2	2	3	3	2	3
14	82	0	0	1	2	2	3	3	3	2	3	2	3

Correlations between structure and urodynamic function were observed. The two patients who had OAB only without DU showed mild hypohypertrophy changes (myohypertrophy global impression score 1) but their global impression scores for degeneration and hyperelastosis were zero. The two patients with predominantly storage LUTS (that is, clinical OAB syndrome) and urodynamic COUB scored moderately on myohypertrophy global impression scores, moderate to severe (2-3) on degeneration global impression scores, and mild to moderate on hyperelastosis scores. The OAB patients with concomitant DU appeared to have worse degeneration features and hyperelastosis features present, in contrast to the OAB patients without concomitant DU, who had very little to no degenerative features and no hyperelastosis.

In the group of patients with predominantly voiding LUTS and urodynamic COUB, those with DO (40%, 4/10) all had concomitant features of degeneration and hyperelastosis in addition to myohypertrophy. Not all patients with PC had hyperelastosis (29% did not have hyperelastosis), but the majority did. All patients with PC had degeneration features in addition to myohypertrophy.

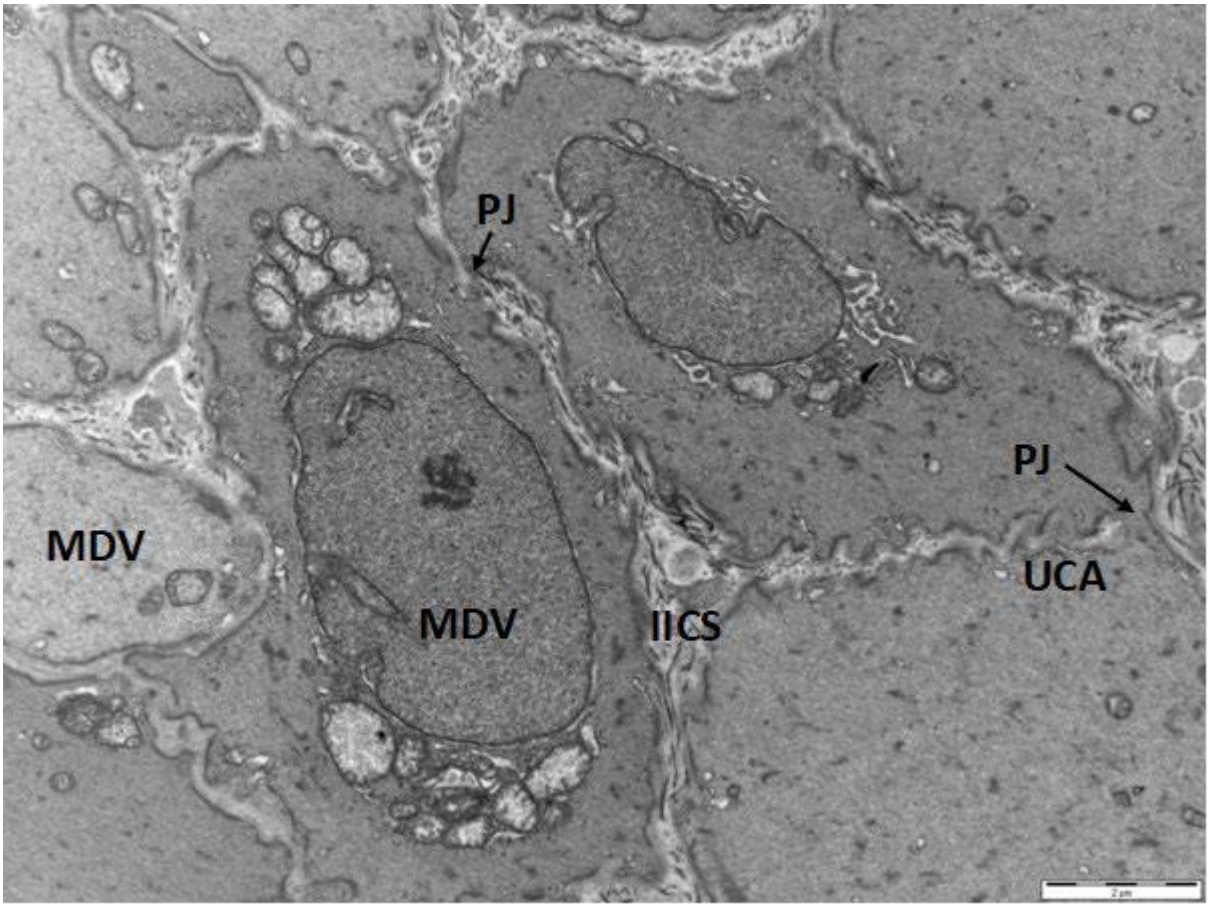
All patients with COUB had both myohypertrophy and degenerative features present on detrusor ultrastructural analysis, and most (83% (10/12) of patients) but not all had hyperelastosis as well. It appears that a significant number of nonobstructed patients with OAB have concomitant DU (that is, COUB). A significant number of older males with OAB also have detrusor ultrastructural features of degeneration which may correlate with DU. Regardless of whether patients presented with predominant storage LUTS or predominant voiding LUTS, many appeared to have features of degeneration.

Figure 6.3.1: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



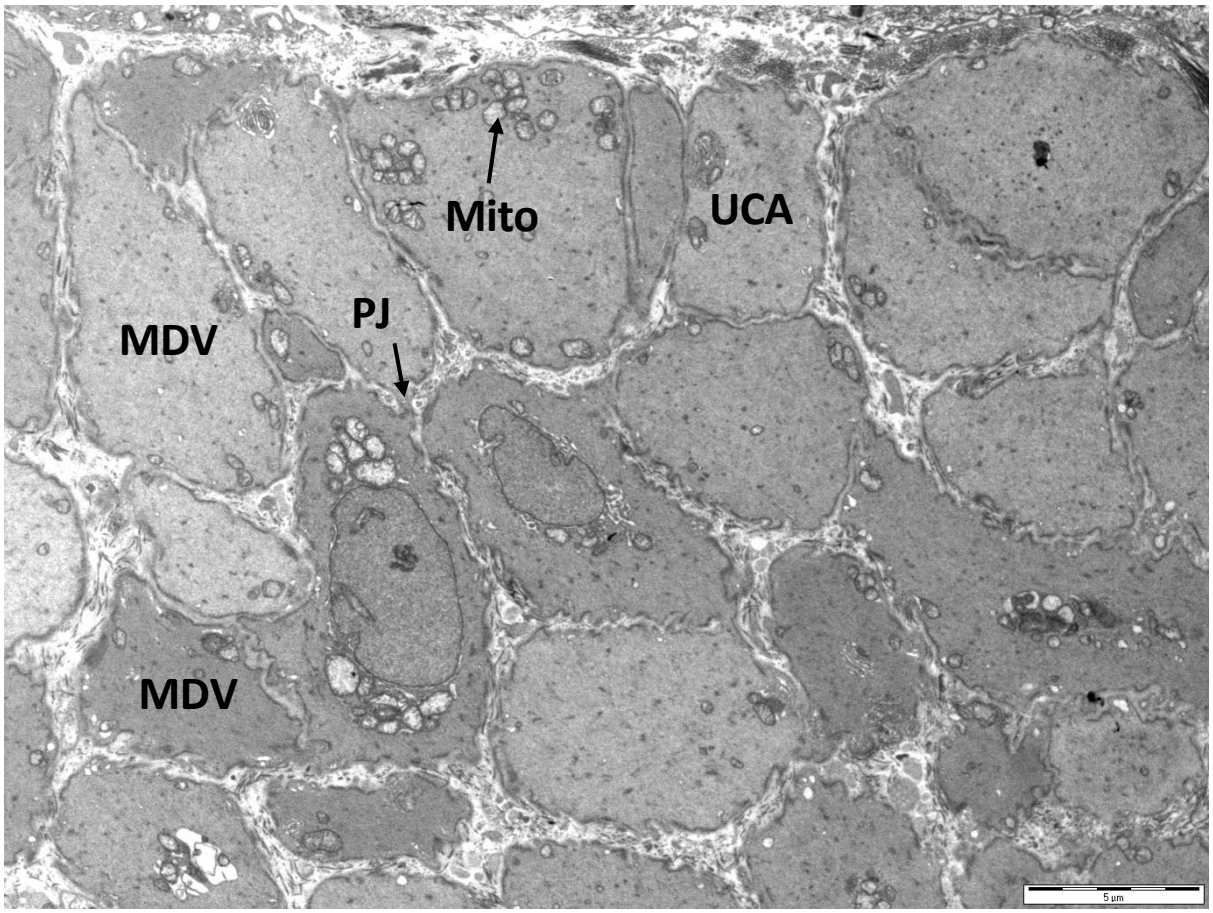
M = myocyte, NGJ = normal gap junction, PJ = protrusion junction, UC = ultraclose abutment.

Figure 6.3.129: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



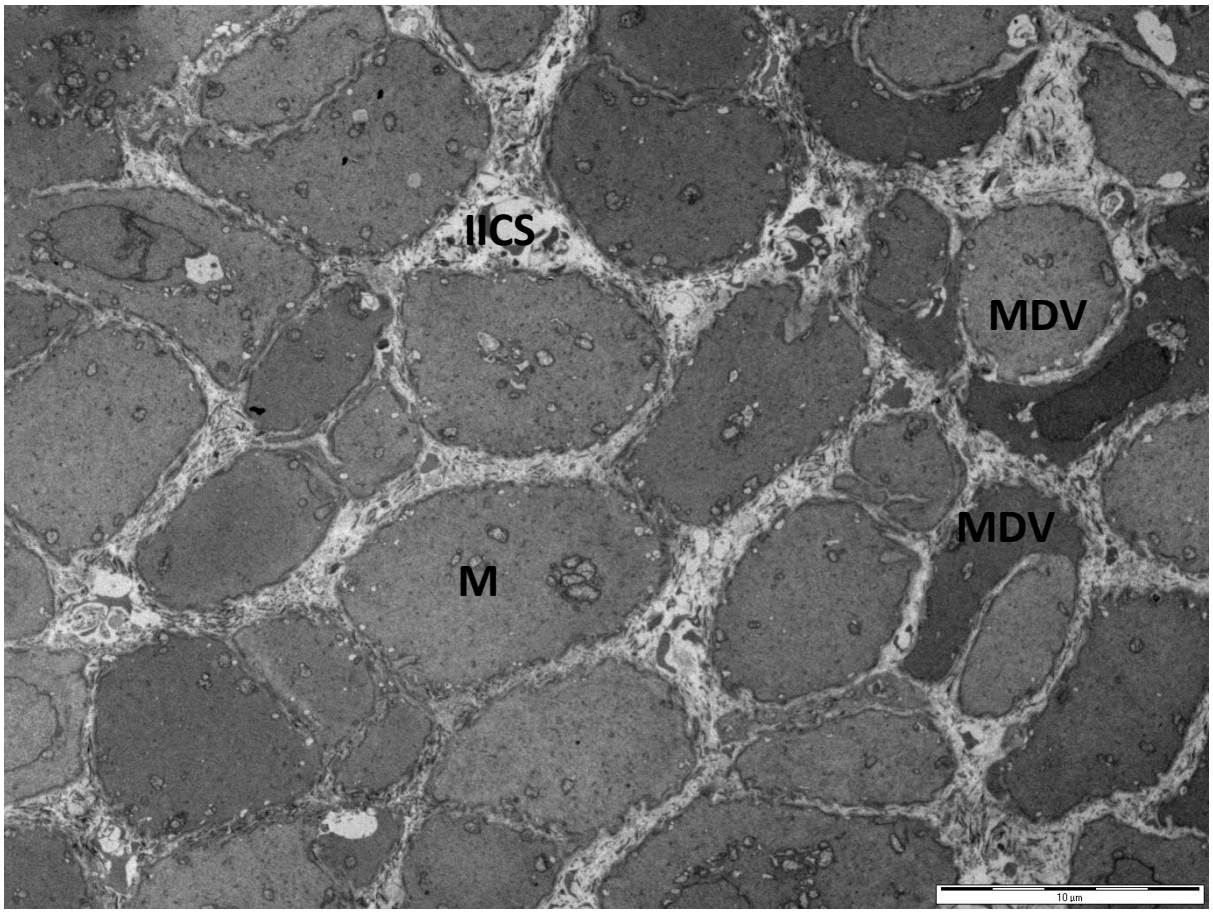
MDV = myocytes with electron density variation, IICS = increased intercellular separation, PJ = protrusion junction, UC = ultraclose abutment.

Figure 6.3.130: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



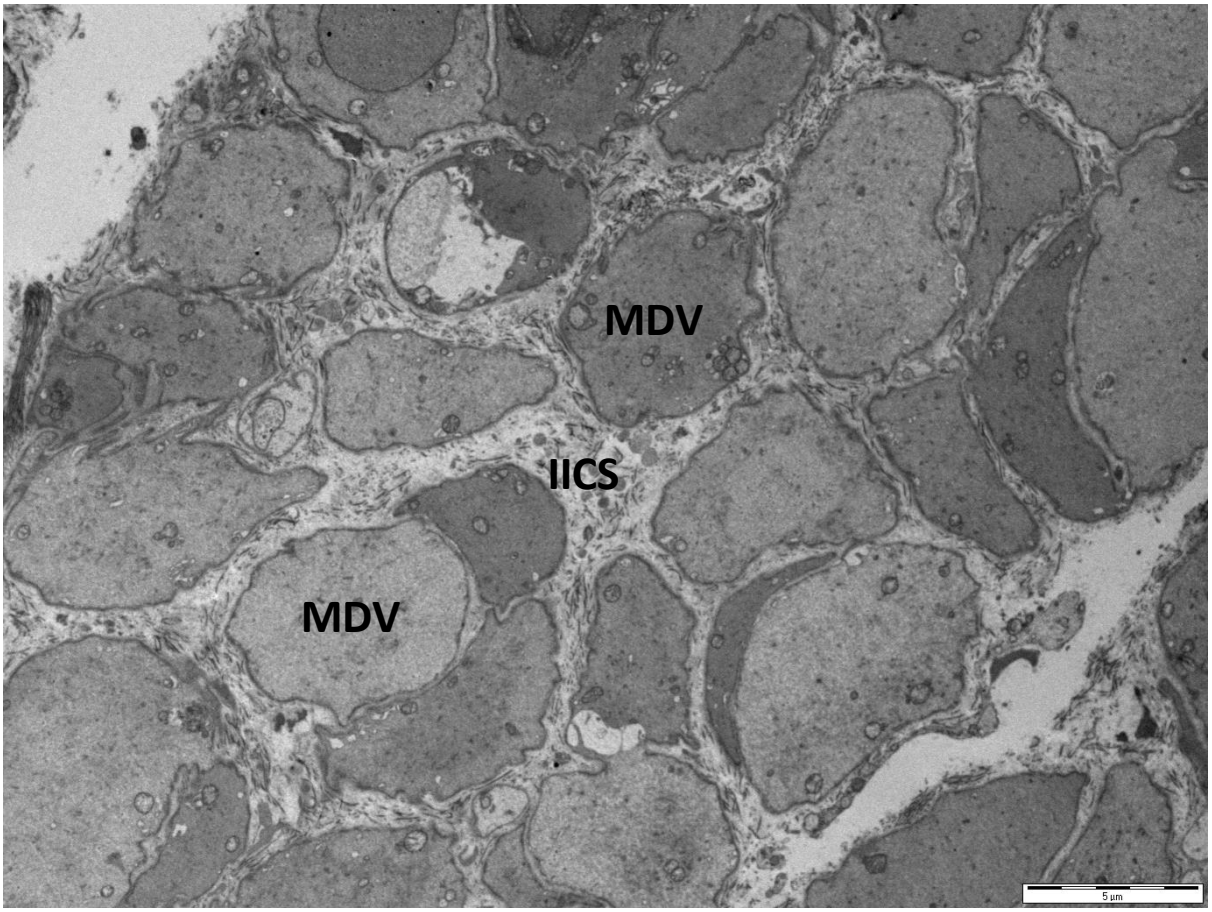
MDV = myocytes with electron density variation, Mito = mitochondria, UCA = ultraclos abutment.

Figure 6.3.131: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



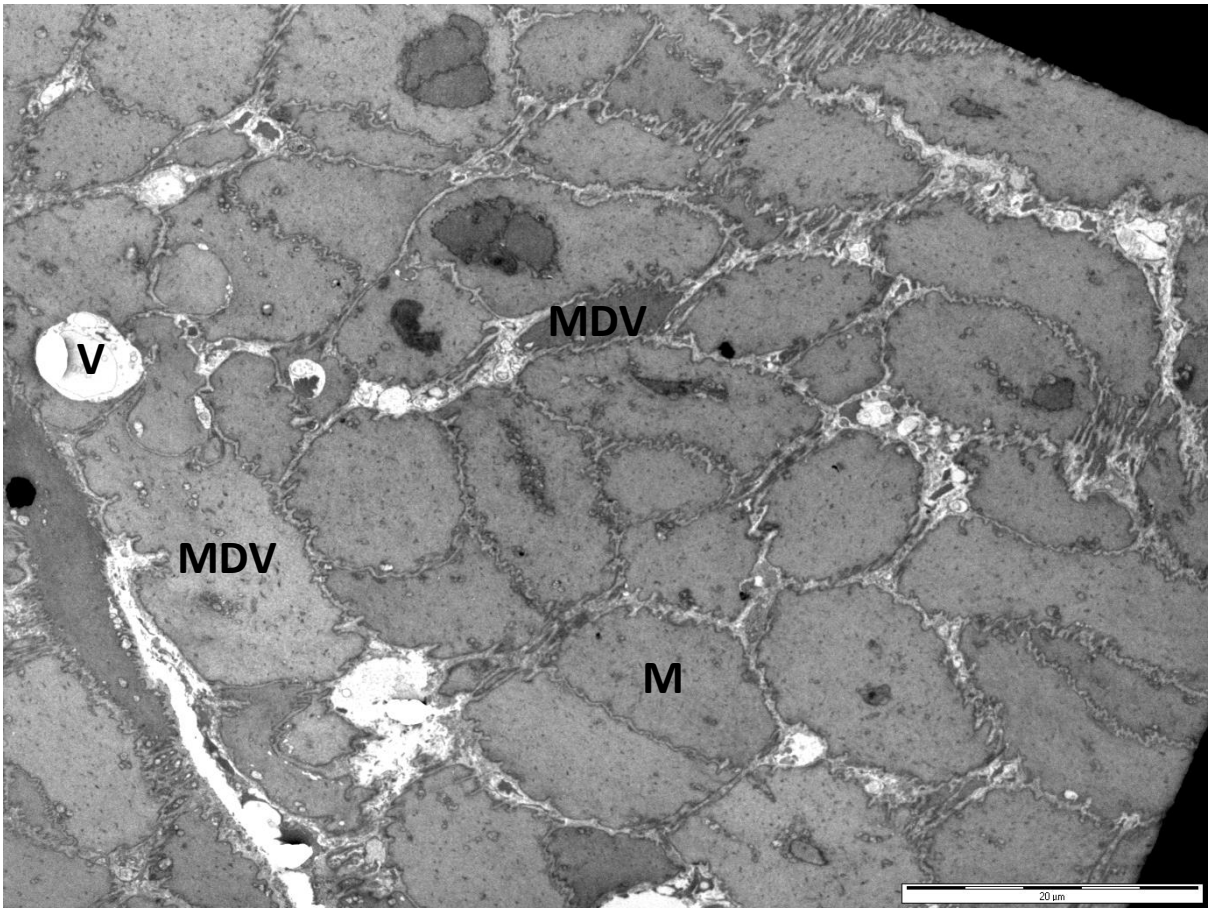
M = myocyte, MDV = myocytes with electron density variation, IICS = increased intercellular separation

Figure 6.3.132: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



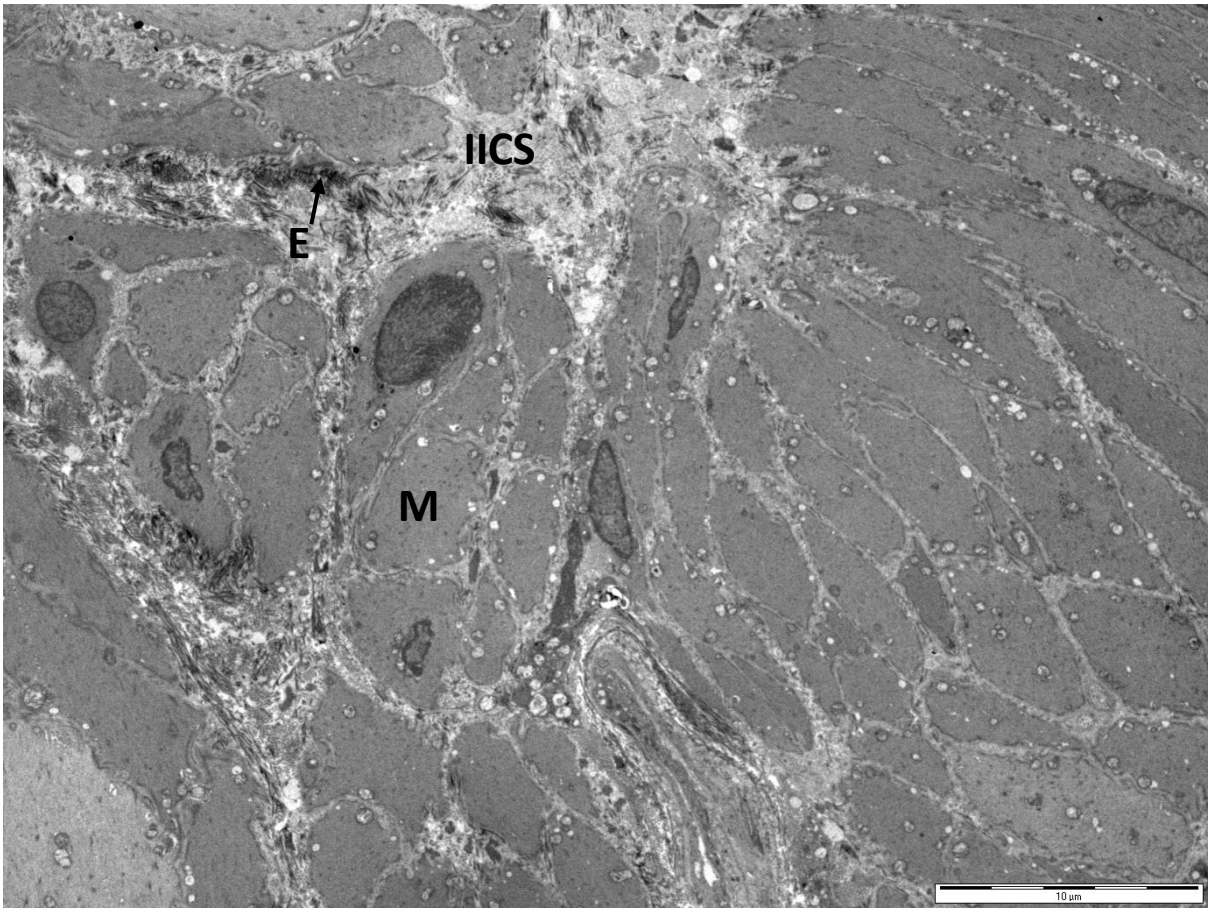
M = myocyte, MDV = myocytes with electron density variation, IICS = increased intercellular separation

Figure 6.3.133: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



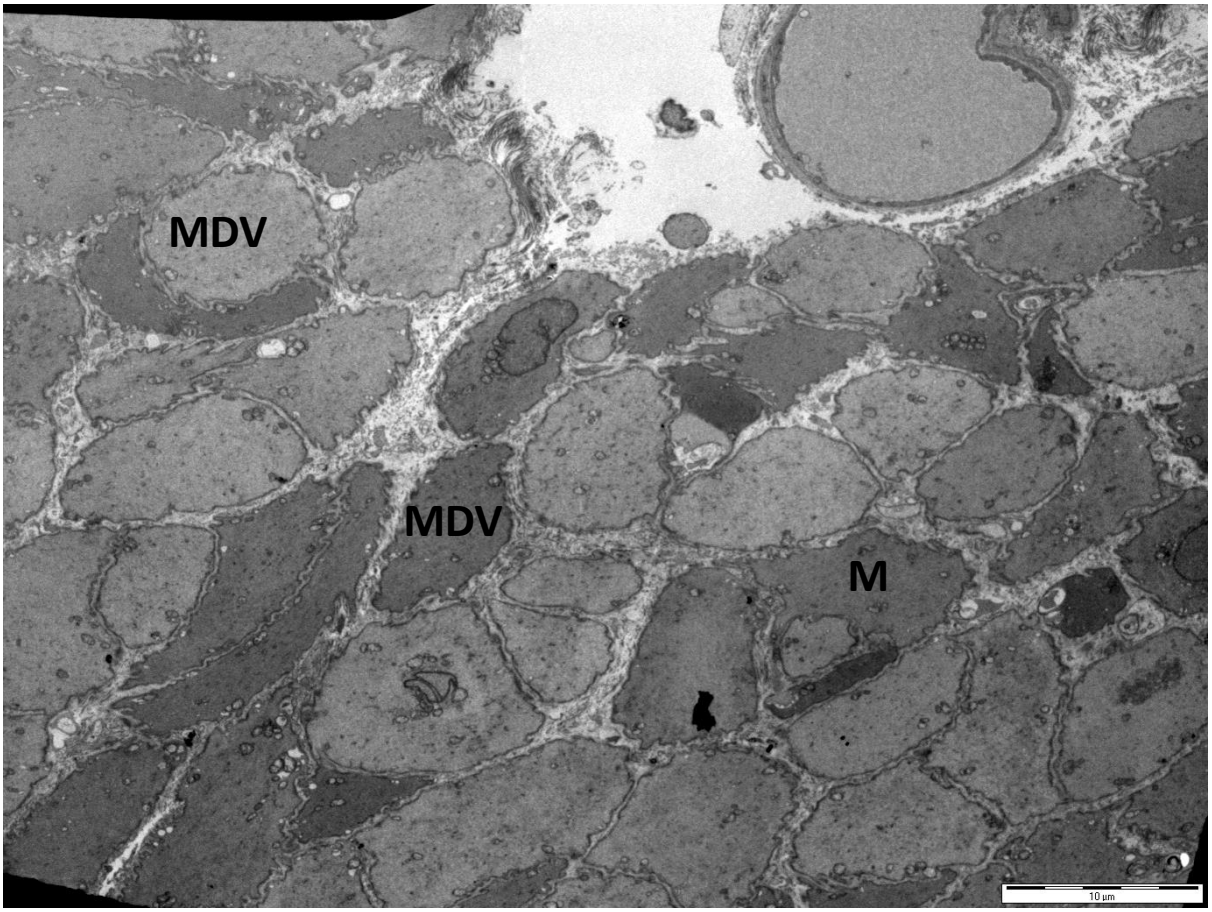
M = myocyte, MDV = myocytes with electron density variation, V = vacuolation.

Figure 6.3.134: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



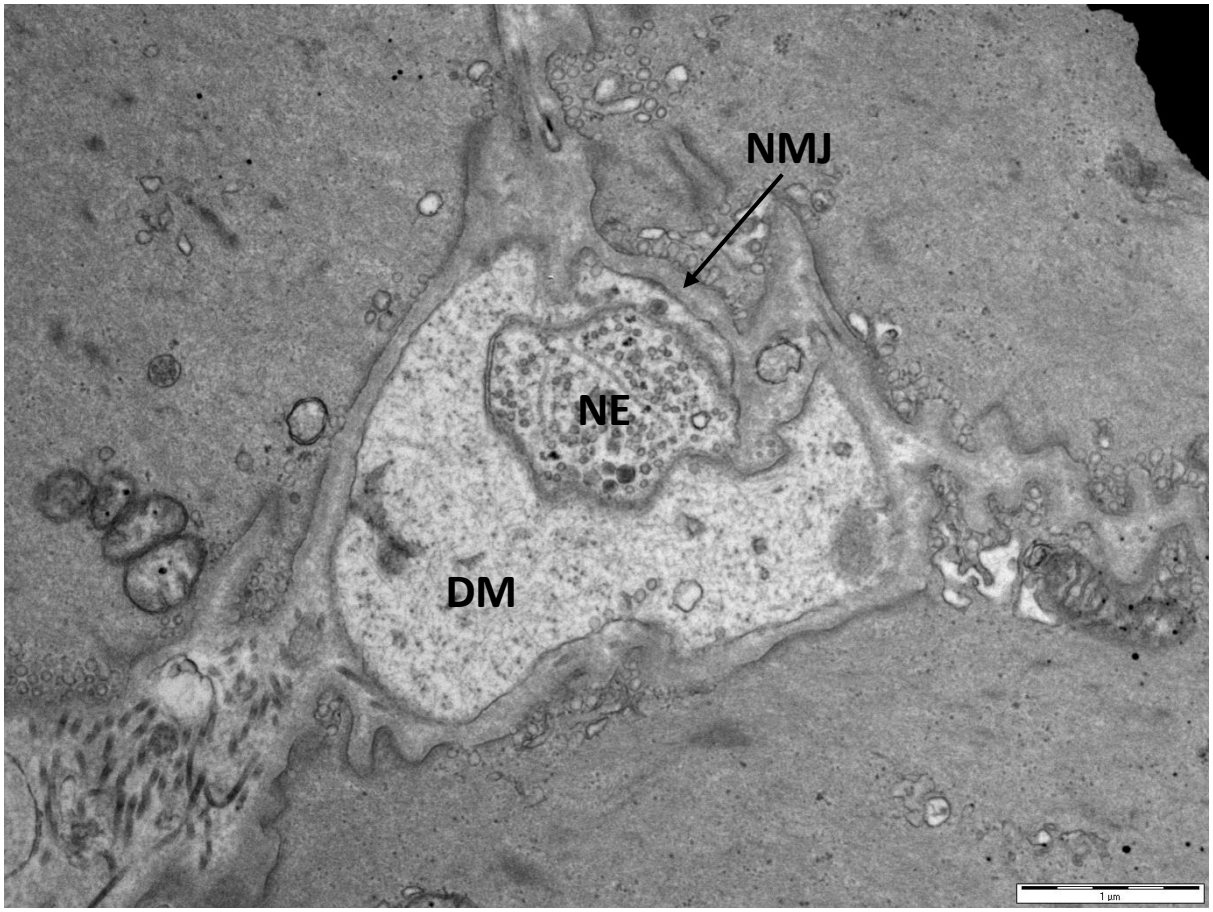
M = myocyte, E = elastin, IICS = increased intercellular collagen.

Figure 6.3.135: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



M = myocyte, MDV = myocytes with electron density variation.

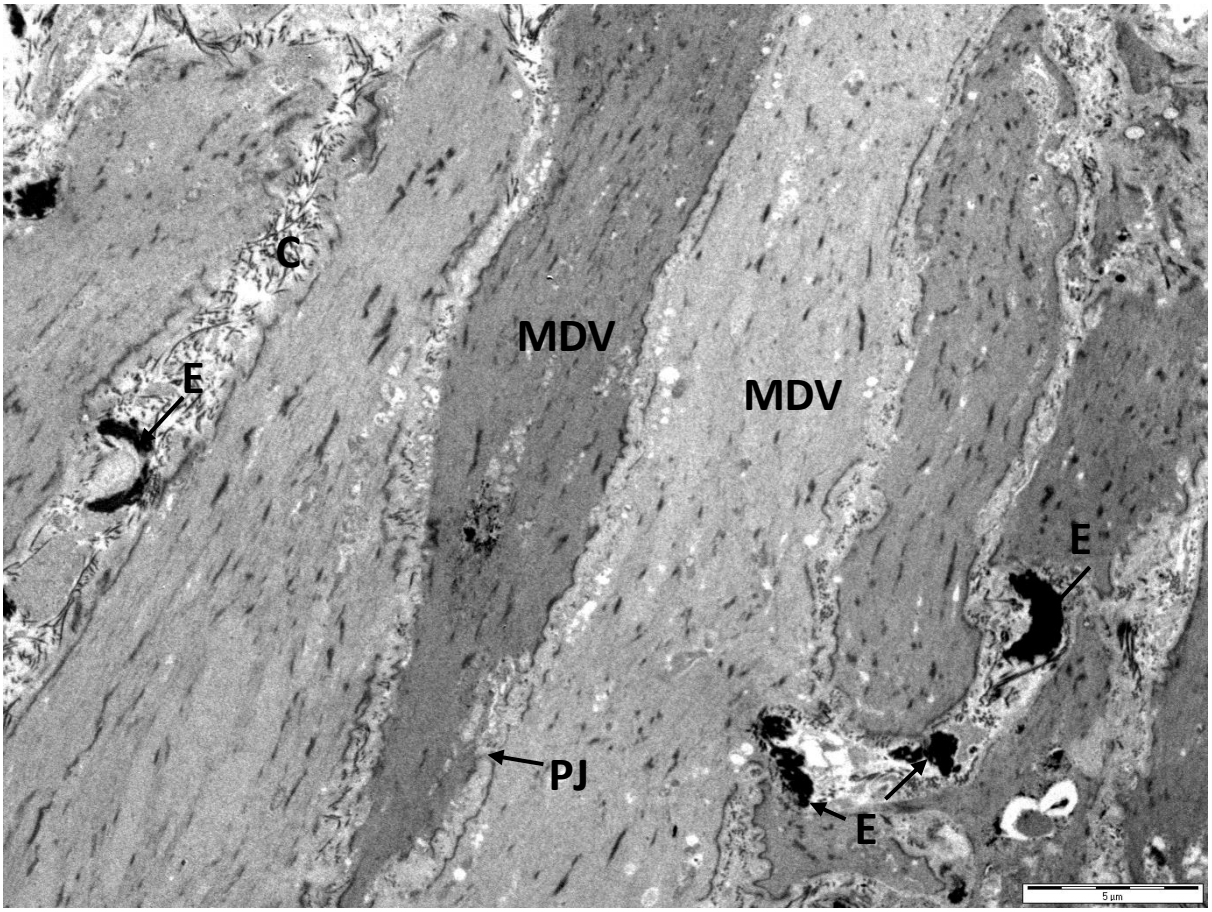
Figure 6.3.136: Micrograph of detrusor ultrastructure of male patient with predominant storage LUTS, urodynamic features of overactive bladder and detrusor underactivity. BCI<100, PVR 0ml. There are detrusor ultrastructure features of overactive bladder with abnormal junctions as well as degeneration. (patient number 4)



NMJ = neuromuscular junction, DM = degenerating myocyte, NE = nerve ending with neurotransmitter vesicles of different types as described by Elbadawi.

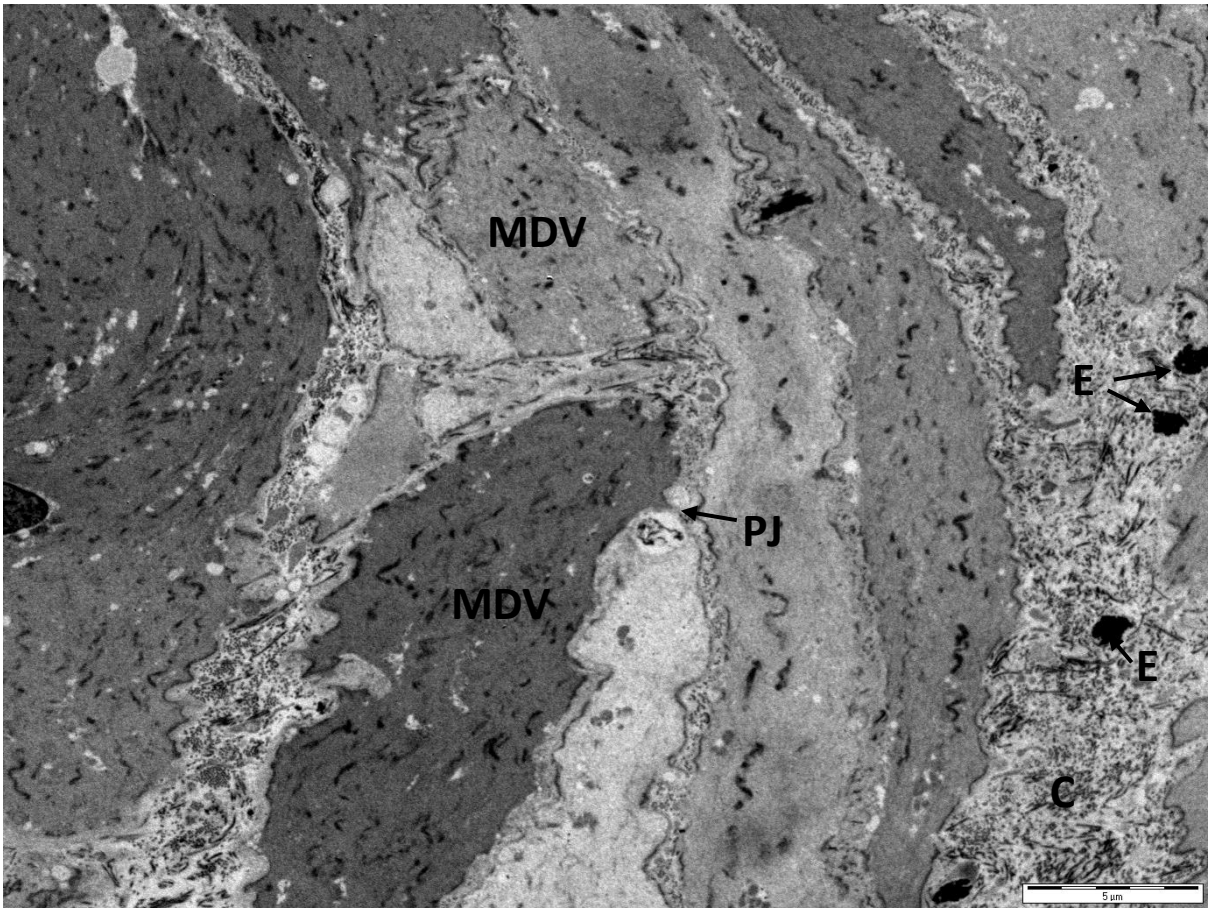
Figure 6.4.1: Micrograph of detrusor ultrastructure of male patient with predominant voiding LUTS and urodynamic features of coexistent overactive bladder and underactive bladder. (patient number 5)

His urodynamic study showed detrusor overactivity on filling. He voided 200ml with a Qmax of 8ml/s, BCI 60 and VE 33%, leaving 400ml PVR. Clinically, this patient was voiding enough spontaneously without requiring catheterisation on presentation, but eventually failed to void adequately and eventually required SPC on long term follow-up. His detrusor ultrastructure analyses showed features of abnormal junctions, myohypertrophy, degeneration, and hyperelastosis.



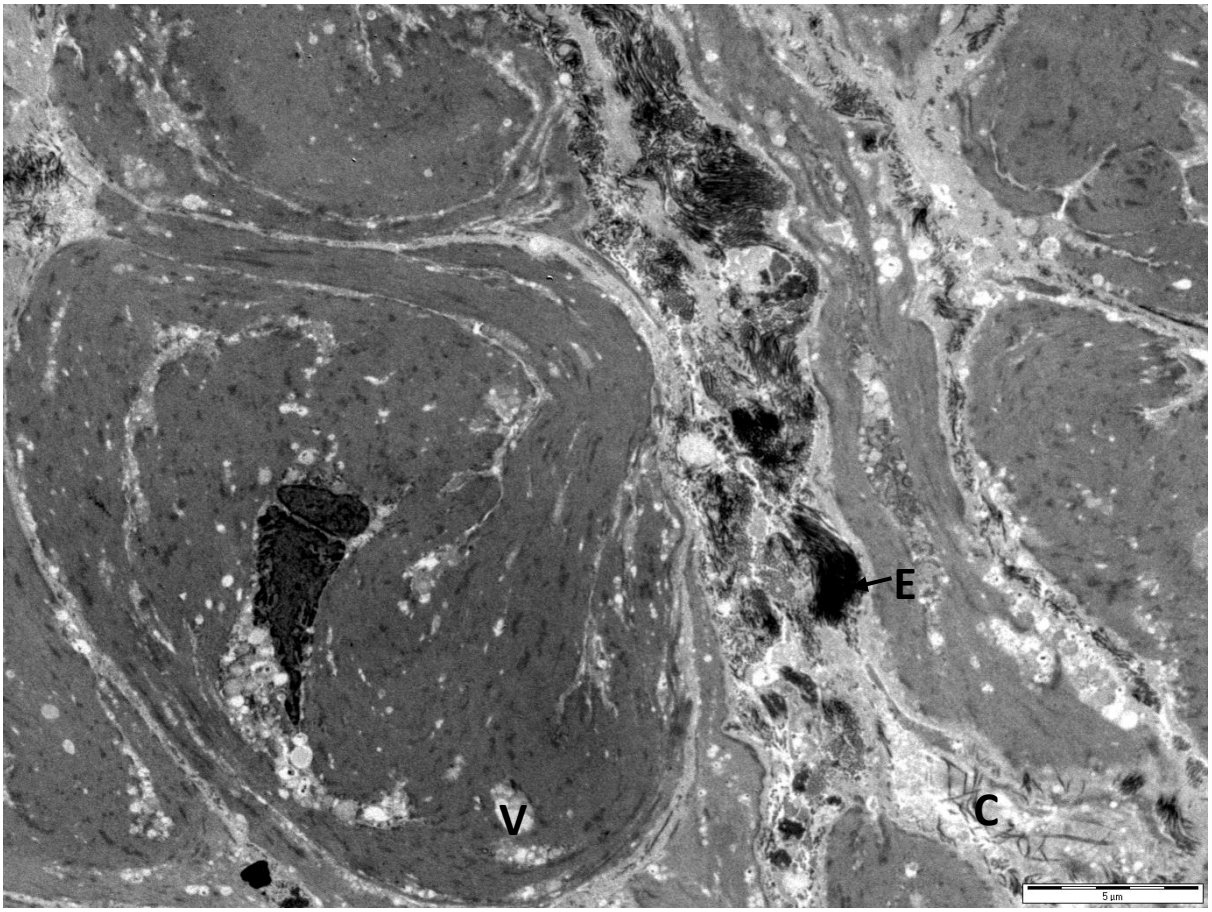
MDV = myocytes with electron density variation, E = elastin, C = collagen.

Figure 6.4.138: Micrograph of detrusor ultrastructure of male patient with predominant voiding LUTS, urodynamic features of overactive bladder and underactive detrusor, and detrusor ultrastructural features of abnormal junctions, degeneration and hyperelastosis. (patient number 5)



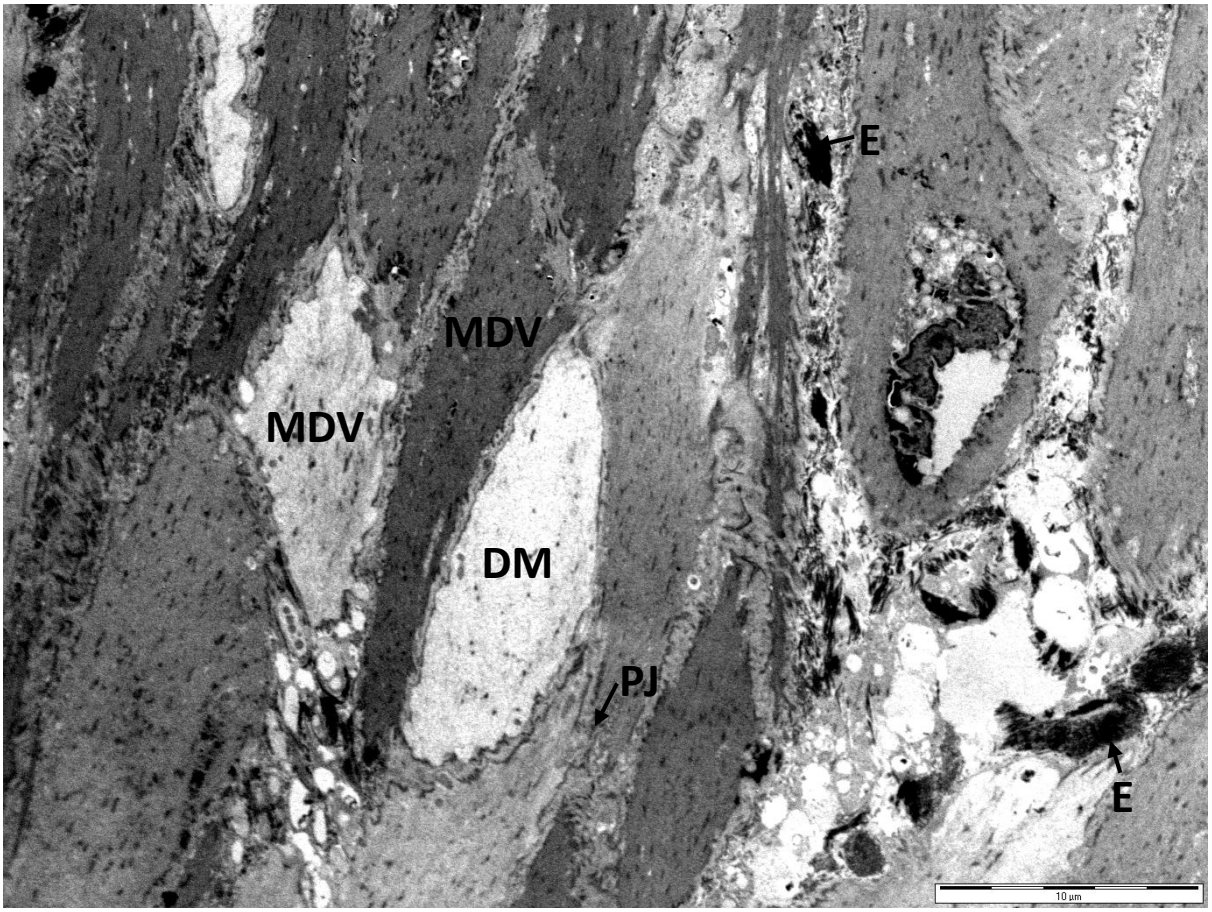
MDV = myocyte electron density variation, E = elastin.

Figure 6.4.139: Micrograph of detrusor ultrastructure of male patient with predominant voiding LUTS, urodynamic features of overactive bladder and underactive detrusor, and detrusor ultrastructural features of abnormal junctions, degeneration and hyperelastosis. (patient number 5)



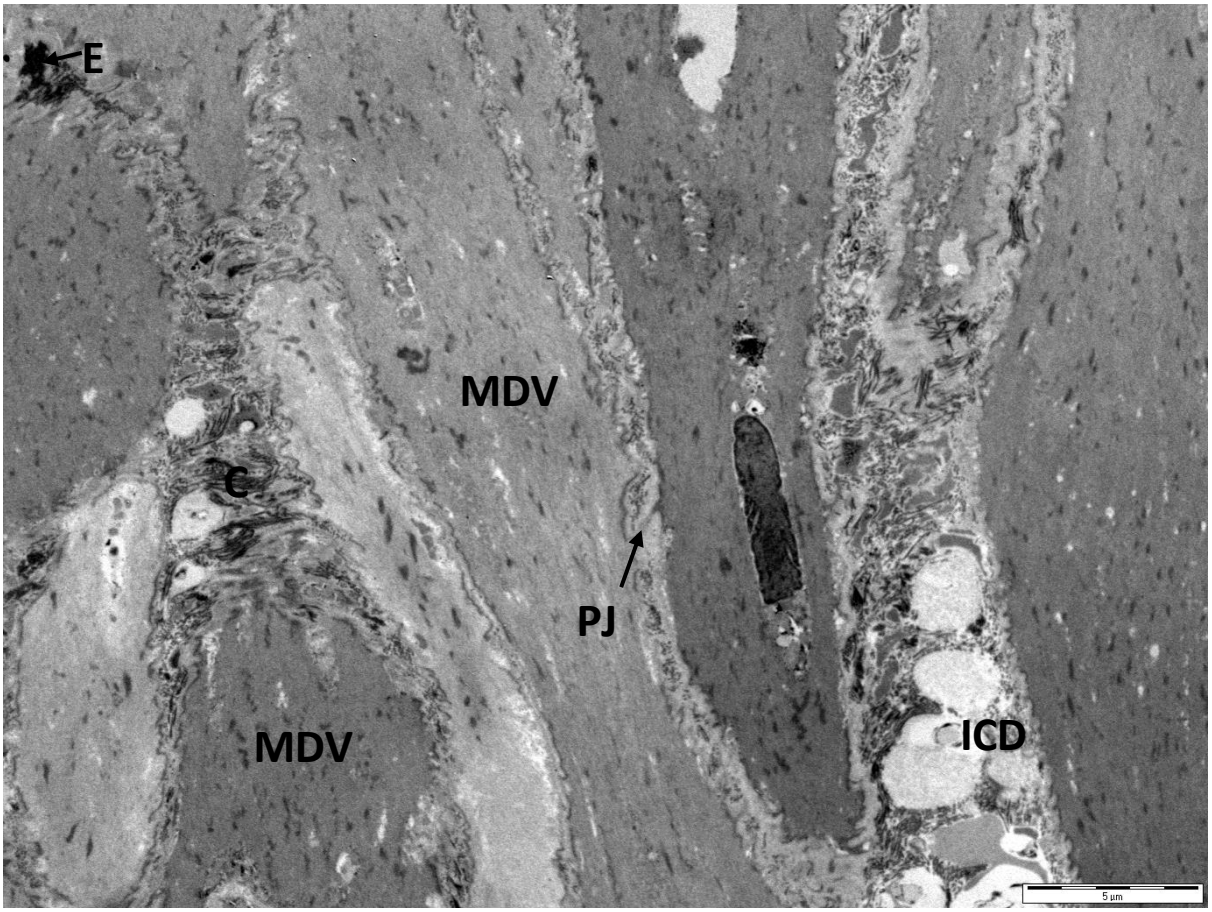
V = vacuolation, E = elastin, C = collagen.

Figure 6.4.140: Micrograph of detrusor ultrastructure of male patient with predominant voiding LUTS, urodynamic features of overactive bladder and underactive detrusor, and detrusor ultrastructural features of abnormal junctions, degeneration and hyperelastosis. (patient number 5)



MDV = myocyte electron density variation, DM = degenerating myocyte, E = elastin.

Figure 6.4.141: Micrograph of detrusor ultrastructure of male patient with predominant voiding LUTS, urodynamic features of overactive bladder and underactive detrusor, and detrusor ultrastructural features of abnormal junctions, degeneration and hyperelastosis. (patient number 5)



MDV = myocyte electron density variation, ICD = intercellular debris, C = collagen.

Discussion

There is selection bias in this cohort in that patients with more complex or mixed symptoms or who do not respond to initial therapies are more likely to undergo urodynamic study and subsequently be recruited into this detrusor ultrastructural analysis study. Notwithstanding, it appears that a significant number of non-obstructed patients with OAB have concomitant DU (that is, COUB). Additionally, a significant number of non-obstructed older males with OAB have detrusor ultrastructure features of degeneration and detrusor underactivity.

This study has demonstrated that symptomatology overlaps with different underlying urodynamic pathophysiology and that making a diagnosis of LUTD in older males based on symptomatology alone is fraught with inaccuracies. Furthermore, treating patients based on symptomatology may not be successful, as the mechanisms of treatment modalities may not be targeted appropriately to the physiological mechanisms underlying the LUTD diagnosis. For example, patient 4 had storage symptoms of frequency and urgency, but also poor urinary stream. The cystoscopy did not show anatomical cause for BOO. The detrusor ultrastructural features included moderate degeneration and hyperelastosis, which correlated with the urodynamics finding of DU, with BCI<100.

LUTD treatments may cause unwanted side effects if not appropriately matched to the underlying pathophysiology. An interesting case example is in the history of patient 3, who presented with storage LUTS, was treated based on symptoms with anticholinergic medication, and subsequently developed acute urinary retention. On urodynamic study, although the patient had no BOO, he had DU (BCI 35) and detrusor ultrastructural features showed severe degeneration.

It is of interest to examine the detrusor ultrastructure of older patients with a urodynamic diagnosis of overactive bladder for possible presence of occult features of degeneration and hypocontractile detrusor. Knowing the presence of such features could help guide decision making for treatment options (such as avoiding anticholinergic medication and onabotulinum toxin, which can cause retention risk or worsen voiding function) and prognostication of treatment success in older men with OAB. Detrusor ultrastructure analysis may help in risk profiling such patients, so that they can be counselled appropriately, and the clinician and patient may be better able to make an informed decision better individualised for the patient's pathophysiology. Structure seems correlated to function, and understanding of structure can help increase understanding of function.

There are still a number of concepts that are not well understood regarding COUB. The different urodynamic features in the OAB constellation of diagnoses, and whether they represent the same clinical or pathological condition in different points of a continuum or if they represent different diseases, is not well understood. The significance of detrusor overactivity positive OAB versus sensory (DO negative) OAB and how poor compliance fits into the constellation of features is not well characterised. That a patient can have concomitant DO and acontractile bladder during attempted voiding, such as in patients 6 and 11, is somewhat paradoxical, as it is known that the bladder as a muscle is able to contract, albeit not at the appropriate time or setting. Potential therapeutic targets may take advantage of information obtained from structural studies, hence future studies are warranted.

Conclusions

A significant number of older males (over 60 years of age) with OAB had COUB with concomitant occult features of degeneration and hypocontractile bladder in addition to myohypertrophy pattern features. It is possible that treatments which are known to cause risk of urinary retention and worsen voiding dysfunction may not be the treatment of choice for such patients, or at least should be used cautiously and with appropriate pre-treatment counselling. Detrusor ultrastructure analysis may assist decision making for such patients.

Although outside the scope of this study, future directions of research may investigate whether such features are also present in younger patients and whether there is a statistically significant correlation in a larger sample size. Additionally, further future research could be designed to investigate whether the presence of these degenerative features in patients with OAB correlate with clinical outcomes and treatment risks.

References

1. World-Health-Organisation. Ageing and Health. https://wwwwhoint/news-room/fact-sheets/detail/ageing-and-health. 2022.
2. Siroky MB. The aging bladder. Rev Urol. 2004;6 Suppl 1(Suppl 1):S3-7.
3. Chung A, Noguchi N, Chan L, Tse V. Voiding dysfunction in older men. Curr Opin Urol. 2016;26(2):177-83.
4. Ameda K, Sullivan MP, Bae RJ, Yalla SV. Urodynamic characterization of nonobstructive voiding dysfunction in symptomatic elderly men. J Urol. 1999;162(1):142-6.
5. Blaivas JG. The bladder is an unreliable witness. Neurourology and Urodynamics. 1996;15(5):443-5.
6. Mancini V, Tarcan T, Serati M, Wyndaele M, Carrieri G, Abrams P. Is coexistent overactive-underactive bladder (with or without detrusor overactivity and underactivity) a real clinical syndrome? ICI-RS 2019. Neurourol Urodyn. 2020;39 Suppl 3:S50-S9.
7. Verhamme KM, Sturkenboom MC, Stricker BH, Bosch R. Drug-induced urinary retention: incidence, management and prevention. Drug Saf. 2008;31(5):373-88.
8. Haylen BT, de Ridder D, Freeman RM, Swift SE, Berghmans B, Lee J, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. Int Urogynecol J. 2010;21(1):5-26.
9. Doggweiler R, Whitmore KE, Meijlink JM, Drake MJ, Frawley H, Nordling J, et al. A standard for terminology in chronic pelvic pain syndromes: A report from the chronic pelvic pain working group of the international continence society. Neurourol Urodyn. 2017;36(4):984-1008.
10. Tateno F, Sakakibara R, Aiba Y, Takahashi O, Shimizu A, Oki T. Increased bladder sensation without detrusor overactivity revisited: Use of a five-grade sensory measure. Low Urin Tract Symptoms. 2020;12(2):162-6.
11. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.
12. Toppercer A, Tetreault JP. Compliance of the bladder: an attempt to establish normal values. Urology. 1979;14(2):204-5.
13. Harris RL, Cundiff GW, Theofrastous JP, Bump RC. Bladder compliance in neurologically intact women. Neurourol Urodyn. 1996;15(5):483-8.

CHAPTER 7 – CLINICAL APPLICATIONS, FUTURE RESEARCH DIRECTIONS AND CONCLUDING REMARKS

Clinical applications

This chapter provides an overview of the key findings of the research project and the potential application of ultrastructural analyses in clinical practice. Several remaining gaps in knowledge are noted and suggestions made for future directions in research. Key learnings are summarised in concluding remarks.

Remarks on detrusor ultrastructure in female bladder outlet obstruction (chapter 4)

In the case of female bladder outlet obstruction, it was interesting to see that the detrusor ultrastructure features of the 'myohypertrophy' pattern (such as variation of myocyte size and shape, increased separation of cells, and increased intercellular collagen) were similar to those observed in male bladder outlet obstruction, and that the severity of detrusor ultrastructural features on qualitative assessment appears to correlate with duration and severity of obstruction. The pathophysiology of male BOO is often due to prostatomegaly, which has an insidious and gradual progression compared to aetiology in women who may have sustained BOO quite suddenly, such as after sling surgery.(1, 2)

Men may present later in their clinical course given the more gradual onset of symptoms, and are often treated with medication first, which may not be optimally relieve their bladder outlet obstruction, before reaching the decision for definitive surgery. Therefore, the bladder outlet obstruction is often present and sustained for a longer duration of time compared to with women before definitive treatment. It is also observed that men consult medical attention less than women and may present later than women to their doctor.(3) Nonetheless, the clear presence of ultrastructural changes in this small series of females with bladder outlet obstruction with similar severity compared to our male patients with BOO suggests that it is important that the diagnosis is made (such as in patients with voiding dysfunction after sling procedures) and treated accordingly.

We feel that bladder biopsy for detrusor ultrastructural analysis has a potential role in diagnosis of female bladder outlet obstruction, especially given that there is no clear consensus on urodynamic parameters for female BOO. As such, the technique may have clinical applications in female voiding dysfunction, where the diagnosis is equivocal, or where there is suspected concomitant detrusor underactivity. Detrusor biopsy ultrastructural analysis may potentially assist prognostication of natural history and treatment outcomes from a functional perspective. In treating bladder outlet obstruction with surgery, it is valuable to know whether the surgery will lead to desired outcomes. In men, it has been shown that detrusor ultrastructure features of degeneration are associated with worse voiding outcomes after BOO surgery.(4) Further studies are warranted to investigate whether this may be similar in women.

Remarks on detrusor ultrastructure in male detrusor underactivity (chapter 5)

Whilst voiding dysfunction in men is often due to bladder outlet obstruction, detrusor underactivity is not uncommon and is not as well understood as some other LUT dysfunctions.(5) Although it has been gaining more recognition in recent years, the definition of UAB and DU is relatively vague and limited in that although strength of detrusor contraction is reflected in indices such as the BCI, the duration of detrusor contraction is not well defined.(6, 7) It was interesting to note the detrusor ultrastructure findings of degeneration, including myocyte electron density variation, vacuolation and fragmentation, as well as hyperelastosis in some. A proportion of patients with detrusor underactivity also had urodynamic features of overactive bladder with detrusor ultrastructure features, such as abnormal gap junctions.

The results of our cohort of patients (the largest reported cohort in the literature) with non-neurogenic underactive bladder related to BOO showed that ultrastructural features were correlated with poor long term voiding outcomes. As such, there appears to be a role for detrusor biopsy ultrastructure analysis in not only diagnosing but also prognosticating intermediate post-treatment and long-term voiding outcomes, which may help in treatment decision making. Risk-benefit ratios are important

when considering surgery; avoidable surgery which exposes older patients to risks without much chance of success to gain is best not performed. Currently, DU treatment is quite limited and can be divided into two categories of approach: 1) to reduce outlet resistance such as by PF relaxation, alpha blocker medication, or bladder outlet surgery, and 2) to increase bladder contractility.(8) Medications such as bethanechol have not been shown to be effective.(9) The only treatment that exists is sacral neuromodulation (SNM) for "non-obstructive urinary retention", which is the modulation of spinal cord reflexes and brain networks by peripheral sensory and possibly motor neurons. However, the exact mechanism of action for SNM is still not fully elucidated.(10)

Where there is DU but still some detrusor contractility, such that the relief of bladder outlet obstruction (whether it be by prostatomegaly, or obstructive sling or other cause) or reduction of outlet resistance, may help the otherwise hypocontractile bladder achieve sufficient bladder emptying to allow the patient to be catheter-free or relieved of symptoms, this should be offered or at least considered.

Detrusor ultrastructural study may assist patient-selection of likely treatment responders. In the presence of end-organ failure (such as grossly fibrotic acontractile bladder), treatments trying to increase contractility may not be as effective.(11) For example, if detrusor ultrastructure features are a marker of end organ (that is, bladder) failure, then sacral neuromodulation should not be able to significantly improve function of the bladder in these circumstances.

Currently, the general thought is that if the urodynamic study shows hypocontractile bladder, sacral neuromodulation is tried, and if the urodynamic study shows acontractile bladder, then sacral neuromodulation is less likely to be successful and even possibly futile for achieving complete catheter freedom.(11) It would be interesting to understand whether important prognostic information for success of SNM can be obtained via detrusor ultrastructural analysis. If this were so, detrusor ultrastructural analysis may assist in prognosticating a patient's success with sacral neuromodulation and may also assist in selecting patients for a one-stage SNM device implantation if there is well-preserved detrusor architecture.

Remarks on structure in co-existent overactive-underactive bladder (chapter 6)

Co-existent overactive-underactive bladder (COUB) is an interesting and not uncommon LUTD. It is often considered as a combination of detrusor overactivity and detrusor underactivity (detrusor overactivity with impaired contractility), but has also been proposed as a unique clinical condition COUB with or without urodynamically proven detrusor overactivity or underactivity.(12, 13) On one hand, COUB may seem paradoxical. However, if one considers that OAB is a syndrome of storage function and UAB is a syndrome of voiding function, then both syndromes could coexist in the same patient where the LUT is functioning incorrectly in both phases of the micturition cycle.

If, somehow, the detrusor action in the storage phase can be harnessed and controlled and the detrusor inaction in the voiding phase can be reversed to the alternate phase of the micturition cycle, it may be closer to 'normal' function. Conceptually, DODU suggests that the problem may not be the detrusor muscle itself, but rather the activation/inhibition signalling or communication system that is malfunctioning. Given this potential theory, perhaps research should not only target the bladder as an end organ, but also target the messaging or communication pathways (neural or other) that relay information and instruction from brain to bladder and lower urinary tract or vice versa.

Onabotulinum toxin A intradetrusor injections is a treatment technique which acts on the bladder as an end-organ by inhibiting acetylcholine release from the presynaptic efferent nerves at the neuromuscular junctions in the detrusor.(14) It thereby "relaxes" the detrusor, effectively treating detrusor overactivity, but as a side effect can cause urinary retention or worsen voiding function. Caution is warranted for use of onabotulinum toxin A intradetrusor injections for patients with COUB, as there may be increased risk of post-treatment urinary retention or worsening voiding function in those with detrusor underactivity.(15) Unlike onabotulinum toxin A intradetrusor injections, SNM does not cause urinary retention or worsen voiding function as a potential side effect.

In analysing the detrusor ultrastructural features of older men with OAB, we found a significant number also have ultrastructural features of degeneration and hypocontractile detrusor. Perhaps this in part explains the anecdotal observation that older OAB patients may be more prone to urinary retention or voiding difficulties following onabotulinum toxin A intradetrusor injection therapy. As such, detrusor biopsy ultrastructural analysis may help identify patients with OAB who harbour occult

degenerative features associated with DU, and in doing so, assist in decision making and recommendations for optimal treatment. Detrusor biopsy ultrastructural analysis may help identify patients who would do poorly with one treatment modality, but may be better suited to another. Further research is warranted to investigate detrusor ultrastructure in COUB with LUTS treatment outcomes.

Future research directions

Scope of LUT dysfunctions with detrusor ultrastructural analysis

This PhD thesis has described detrusor ultrastructural features in females with bladder outlet obstruction (Chapter 4), males with detrusor underactivity (Chapter 5), and males with coexistent overactive-underactive bladders (Chapter 6), complementing previous work by our Institution. Further research is warranted for other population groups, including females with detrusor underactivity, females with coexistent overactive-underactive bladders, and neurogenic bladders. It would be interesting to study the detrusor ultrastructure in younger patient cohorts, including children with LUTD. Furthermore it would be interesting to examine whether features seen in ageing bladders are due to the ageing process itself or secondary to medical diseases such as metabolic syndrome, perhaps by examining a large cohort of older healthy humans versus humans with non-urological medical disease or metabolic syndrome.

Clarifying complexity and relationships between structure and function

The correlation of structure with function is very interesting. Though the relationship may simply be an association, the authors believe the correlation may occur in a causative way and that there are pathophysiological mechanisms which relate structure to function and vice versa.(16) The potential interplay of causation is fascinating to consider – are the functional issues a result of the development of structural changes, or are the structural changes a result of the functional state? These are questions that warrant further research. Features have been described in this PhD thesis which can be identified as relating to certain functional outcomes. However, there are also ultrastructural features which I have observed in our patients which I do not know how to interpret. For example, the scalloped edges of some myocytes were an interesting feature to observe, but to my knowledge, these have not yet been explained. I can only expect that somehow structure is related to function as a principle, which has been demonstrated in other aspects of the thesis.

Clinical applications of structural-functional relationships

Understanding structure to understand function
The design of this PhD is based on analysing structural features when the functional diagnosis by urodynamics (accepted as the 'gold standard') is known. The results presented do suggest that structural features may be more prognostic of clinical outcomes compared to the functional testing (urodynamics) parameters. As such structural information may allow us to better understand or predict function of the bladder and there is potential for clinical application of ultrastructural studies in helping clinicians arrive at a diagnosis without the patient needing to undergo invasive urodynamic studies or where urodynamic findings are inconclusive. Further studies are recommended to determine the sensitivity and specificity of detrusor ultrastructural analysis in diagnosing voiding dysfunction – I anticipate this direction of analysis would also be very useful in clinical practice. Furthermore, detrusor ultrastructural analysis may have a role in diagnosis and prognosis of LUTD in the context of natural history, as well as in prognostication of outcomes post-treatment, and hence also in assisting patient selection for treatments. Are there features which indicate end organ damage (that is, detrusor failure) and indicate an irreversible pattern of detrusor disease? It has been suggested that myogenic underactive bladder may bear resemblance to cardiac failure, and perhaps pharmacological therapeutic agents which are known to treat cardiac failure may have a role in bladder failure.(17)

Development of novel therapies which target structure to affect function
Are there treatment options which may be targeted at remodelling structure, for example, and subsequently impact and improve function? At present, most therapies for overactive bladder and underactive bladder target function with an unknown documented impact on structure; could the reverse approach be valid?.

Investigating the effects of LUTD treatment on LUT structure

How do LUTD treatments change structure, if they do so at all? If lower urinary tract dysfunction is treated and function improves, will there be potential remodelling and improvement in the structural features of the detrusor to accompany that? For example, it would be interesting to investigate whether OAB treatment with onabotulinum toxin A intradetrusor injections, sacral neuromodulation, and/or PTNS change the detrusor ultrastructure with time. It is possible that there is structural

Future research directions

remodelling, which may further improve and support functional parameters. This may provide insight into the mode of action of these treatment modalities, and help in investigating the role of multi-modal therapy and if the treatments have longer term effects that persist beyond whether the treatment is “present” or not. For example, may neuromodulation can be applied for a period of time until detrusor ultrastructure remodelling occurs and then stopped without the patient’s LUTD returning. Currently, SNM and PTNS are recommended as an ongoing treatments.

Concluding remarks

The field of detrusor ultrastructural study in humans with lower urinary tract dysfunction is a fascinating field of science, medicine, discovery, and potential.

In performing the detrusor ultrastructural analysis research for this PhD, we have described detrusor ultrastructural features in various groups of patients with voiding dysfunction, and have:

1) Identified ultrastructural changes in females with BOO that are similar to male BOO with moderately severe changes demonstrated after a relatively short duration of obstruction,
2) described detrusor ultrastructural features in male DU and demonstrated correlation of the myohypertrophy pattern with long term voiding outcomes, and
3) described detrusor ultrastructural features in male COUB and identified detrusor ultrastructural features of degeneration in this group.

We have also demonstrated a standardised methodology of obtaining, processing, and examining detrusor ultrastructure with a simplified ultrastructural analysis protocol that is applicable across a number of lower urinary tract dysfunctions. These lower urinary tract dysfunctions have included bladder outlet obstruction, detrusor underactivity, and coexistent overactive-underactive bladders.

Further research to validate our technique in other laboratories is something that would be useful, and we plan to collaborate with other laboratories both nationally and internationally regarding this.. Our experience has shown that there is much potential in detrusor biopsies for ultrastructural analysis being a diagnostic and prognostic tool in management of patients with lower urinary tract dysfunctions, and it would be important for standardisation of methodology to occur to help ensure that other clinicians and patients have access to the tool of detrusor ultrastructure analysis.

In conclusion, the study and correlation of ultrastructure and function of the lower urinary tract is an extremely fascinating area of research with potential for exciting clinical applications. We believe that detrusor ultrastructure studies not only improve our understanding of LUTD, but can also assist in management in this important area of health in the ageing population.

References

1. Tse V, Chan L. Outlet obstruction after sling surgery. BJU Int. 2011;108 Suppl 2:24-8.
2. Dmochowski RR. Bladder outlet obstruction: etiology and evaluation. Rev Urol. 2005;7 Suppl 6(Suppl 6):S3-S13.
3. Wang Y, Hunt K, Nazareth I, Freemantle N, Petersen I. Do men consult less than women? An analysis of routinely collected UK general practice data. BMJ Open. 2013;3(8):e003320.
4. Blatt AH, Brammah S, Tse V, Chan L. Transurethral prostate resection in patients with hypocontractile detrusor--what is the predictive value of ultrastructural detrusor changes? J Urol. 2012;188(6):2294-9.
5. Taylor JA, 3rd, Kuchel GA. Detrusor underactivity: Clinical features and pathogenesis of an underdiagnosed geriatric condition. J Am Geriatr Soc. 2006;54(12):1920-32.
6. Abrams P. Bladder outlet obstruction index, bladder contractility index and bladder voiding efficiency: three simple indices to define bladder voiding function. BJU Int. 1999;84(1):14-5.
7. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78.
8. Han DH, Jeong YS, Choo MS, Lee KS. The efficacy of transurethral resection of the prostate in the patients with weak bladder contractility index. Urology. 2008;71(4):657-61.
9. Barendrecht MM, Oelke M, Laguna MP, Michel MC. Is the use of parasympathomimetics for treating an underactive urinary bladder evidence-based? BJU Int. 2007;99(4):749-52.
10. De Wachter S, Vaganee D, Kessler TM. Sacral Neuromodulation: Mechanism of Action. Eur Urol Focus. 2020;6(5):823-5.
11. Ho FCS, He C, Yao HH, O'Connell HE, Gani J. Efficacy of sacral neuromodulation and percutaneous tibial nerve stimulation in the treatment of chronic nonobstructive urinary retention: A systematic review. Neurourol Urodyn. 2021;40(5):1078-88.
12. Mancini V, Tarcan T, Serati M, Wyndaele M, Carrieri G, Abrams P. Is coexistent overactive-underactive bladder (with or without detrusor overactivity and underactivity) a real clinical syndrome? ICI-RS 2019. Neurourol Urodyn. 2020;39 Suppl 3:S50-S9.
13. Resnick NM, Yalla SV. Detrusor hyperactivity with impaired contractile function. An unrecognized but common cause of incontinence in elderly patients. JAMA. 1987;257(22):3076-81.
14. Jhang JF, Kuo HC. Botulinum Toxin A and Lower Urinary Tract Dysfunction: Pathophysiology and Mechanisms of Action. Toxins (Basel). 2016;8(4):120.
15. Osborn DJ, Kaufman MR, Mock S, Guan MJ, Dmochowski RR, Reynolds WS. Urinary retention rates after intravesical onabotulinumtoxinA injection for idiopathic overactive bladder in clinical practice and predictors of this outcome. Neurourol Urodyn. 2015;34(7):675-8.
16. Tse V, Wills E, Szonyi G, Khadra MH. The application of ultrastructural studies in the diagnosis of bladder dysfunction in a clinical setting. J Urol. 2000;163(2):535-9.
17. Faria-Costa G, Charrua A, Martins-Silva C, Leite-Moreira A, Antunes-Lopes T. Myogenic Underactive Bladder and Heart Failure Resemblance: A Novel Role for SGLT2 Inhibition? Eur Urol Focus. 2022;8(6):1783-6.

APPENDIX

INTERNATIONAL PROSTATE SYMPTOM SCORE (I-PSS)

Patient Name: Date:	Not At All	Less Than 1 Time In 5	Less Than Half The Time	About Half The Time	More Than Half The Time	Almost Always	YOUR SCORE
1. Incomplete Emptying Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinating?	0	1	2	3	4	5	
2. Frequency Over the past month, how often have you had to urinate again less than two hours after you have finished urinating?	0	1	2	3	4	5	
3. Intermittency Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Urgency Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Weak Stream Over the last month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Straining Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	
	None	**Once**	**Twice**	**3 times**	**4 times**	**5 or more**	**YOUR SCORE**
7. Nocturia Over the past month how many times did you most typically get up each night to urinate from the time you went to bed until the time you got up in the morning?	0	1	2	3	4	5	
Total I-PSS Score							

Quality of Life due to Urinary Symptoms	Delighted	Pleased	Mostly satisfied	Mixed	Mostly unhappy	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?	0	1	2	3	4	5	6

The I-PSS is based on the answers to seven questions concerning urinary symptoms. Each question is assigned points from 0 to 5 indicating increasing severity of the particular symptom. The total score can therefore range from 0 to 35 (asymptomatic to very symptomatic).

Although there are presently no standard recommendations into grading patients with mild, moderate or severe symptoms, patients can be tentatively classified as follows: **0 - 7 = mildly symptomatic; 8 - 19 = moderately symptomatic; 20 - 35 = severely symptomatic.**

The International Consensus Committee (ICC) recommends the use of only a single question to assess the patient's quality of life. The answers to this question range from "delighted" to "terrible" or 0 to 6. Although this single question may or may not capture the global impact of BPH symptoms on quality of life, it may serve as a valuable starting point for doctor-patient conversation.

APPENDIX

RESEARCH STUDY INTO THE ULTRASTRUCTURAL BASIS OF VOIDING DYSFUNCTION

INFORMATION FOR PARTICIPANTS

You are invited to take part in a research study into the changes within the bladder in patients with voiding dysfunction. The aim is to increase our understanding of bladder disorders as many people, especially the elderly, suffer from frequency, urgency and urge incontinence or difficulty emptying. The causes of these conditions, overactive and underactive bladder, are often unknown. The study aims to investigate the structure of bladder muscles with electron microscopy.

Procedures

You would already have had a urodynamics test before you were asked to participate in this study. After the urodynamics, if your urologist thinks that an inspection of your bladder (cystoscopy) is necessary for your treatment, a bladder biopsy will be taken. This will be performed under a general anaesthetic. The biopsy involves taking a 1-2 mm sample of your bladder muscle. Parts of this sample will later be inspected under an electron microscope to examine the structure of your bladder muscle.

Possible Discomforts, Side Effects and Risks

The risks of this procedure are minor and short-lasting, and include a burning sensation when passing urine, urine infection and a small chance of passing blood-stained urine. There is a less than 1% chance of causing a small tear in the bladder which may then be treated by leaving a catheter tube in the bladder for 3 days.

Confidentiality

If you consent to take part in this study, your hospital medical records may be inspected by your health carers, by regulatory authorities or by the Human Research Ethics Committee. By signing the attached consent form, you are giving permission for this to be done. All details obtained by those named will remain confidential. A report of this study may be submitted for publication, but individual participants will not be identifiable in such a report.

Potential Benefits

While we intend that this research study furthers medical knowledge and may improve management of this condition in the future, it may not be of direct benefit to you. You will not be notified of the results but your treating clinician however may with your permission ask for a report.

Withdrawal from the study

Participation in this study is entirely voluntary. You are in no way obliged to participate and - if you do participate - you can withdraw at any time. Whatever your decision, please be assured that it will not affect your medical treatment or your relationship with the medical staff.

Compensation

Every reasonable precaution will be taken to ensure your safety during the course of the study. In the event that you suffer injury as a result of participating in this research project, hospital care and treatment will be provided at no extra cost to you.

Further Information

When you have read this information, your doctors will discuss it with you further and answer any questions you may have. If you would like to know more, please feel free to contact us on 9767 6292. This information sheet is for you to keep.

This study has been approved by the Human Research Ethics Committee – Concord Hospital of the Sydney Local Health District. If you have any concerns or complaints about the conduct of the research study, you may contact the Secretary of the Concord Hospital Human Research Ethics Committee, on (02) 9767 5622.

PARTICIPANT CONSENT FORM

RESEARCH STUDY INTO THE ULTRASTRUCTURAL BASIS OF VOIDING DYSFUNCTION

I, ...[name]

of..... [address]

have read and understood the Information for Participants for the above named research study and have discussed the study with

……...

- I have been made aware of the procedures involved in the study, including any known or expected inconvenience, risk, discomfort or potential side effect and of their implications as far as they are currently known by the researchers.
- I understand that, during the course of this study, my medical records may be accessed by the researchers, by regulatory authorities or by the Ethics Committee approving the research in order to verify results and determine that the study is being carried out correctly.
- I freely choose to participate in this study and understand that I can withdraw at any time.
- I also understand that the research study is strictly confidential.
- I hereby agree to participate in this research study.

Name (Please Print): ..... .

Signature: ..... **Date:** .....

Name of Person who conducted informed consent discussion (Please Print):

……

Signature of Person who conducted informed consent discussion:

……

APPENDIX 3

EM SCORING SHEET

EM identification code:…….

Ultrastructure feature	**Score (0123)**
Myohypertrophy 0123 (global impression)	
Cell size, irregularity 0123	
Increased separation of cells 0123	
Increase in intercellular collagen 0123	
Degenerative features 0123 (global impression)	
Electron density variation 0123	
Vacuolation, dense bodies 0123	
Fragmentation, exclusion bodies 0123	
Hyperelastosis 0123	