THESIS TITLE:

Tibial Nerve Stimulation for the Management of Overactive Bladder and the Measurement of Urinary Neurotrophins as a Biomarker of Response to Treatment

MR JAI H SETH BSc (Hons) MBBS MSc MRCS FRCS

UCL Student Number: 950466

Department of Uro-Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London

Submitted as partial fulfillment of the requirements for the MD(Res) in Urology

Institute of Urology, University College London (UCL)

2017

Signed Declaration

I, Jai Seth confirm that the work presented in this thesis is of my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Jai H Seth

ACKNOWLEDGEMENTS

I wish to express my sincere and utmost thanks to Dr Jalesh Panicker, for his support, oversight, supervision, encouragement, and guidance during the entire project and preparation of this thesis. He has read and helped direct, plan and correct previous drafts of this thesis.

This thesis would not have been possible without the support of Professor Clare Fowler who gave me the opportunity to join the 'Uro-Neurology' team at Queen Square and offered me a research fellow position which made this possible for me. It was an honour and privilege to have worked under her wing of support and direction.

I am indebted to my fellow colleagues Mr Arun Sahai has also helped and guided me immensely with the background research, and Professor Dasgupta for his invaluable support for my acquisition of the position as research fellow.

I am indebted to 'The Urology Foundation' who awarded me with a research scholarship, which funded my salary and allowed me to complete this project. This was instrumental to the whole project and could not have been completed without their support.

The study described in Chapter Three of this thesis was also supported by an educational grant from the InComb project and the Institute of Neurology, Queen Square.

Firstkind Ltd also provided funding for the running of the trial to evaluate a novel form of transcutaneous tibial nerve stimulation, which is described in Chapter Four.

A heartfelt acknowledgement and thanks go to Mr Marcus Oppenheim for the assistance he has provided me. He taught me many of the techniques required, and helped set up the project and has assisted with study critique and planning the method development. This study would not have been possible without his expert tutelage and patience.

I am grateful to Dr John Land and Mr Geoff Lynes who hosted me in the Neurometabolic Unit, where I spent many months performing the experiments involved for these chapters. I am especially thankful for their advice when things frequently did not go as planned.

Ms Collette Haslam, Ms Gwen Gonzales and Ms Juliana Ochulor were all instrumental in recruiting patients for the study, and their wholesome support throughout the research period. We shared many memorable moments during the entire research period.

Last but not least, I would like to thank my family at home starting with my wife Gauri and my baby sons Kai and Josh who are my inspirations. Gauri, as an academic trainee kindly took the time to proof read the thesis, thank you for this. I thank my parents, for their tremendous undivided support and motivation throughout my life and education and am so grateful for their ongoing love and devotion. I owe great thanks to my parents-in-law who through looking after Gauri, Kai and Josh, have provided me with the opportunity to complete this write up. This thesis is dedicated to my loving family.

TABLE OF CONTENTS

	Page
Acknowledgements	3
List of tables, figures & appendices	6
Abbreviations	14
Chapter ONE	16
Chapter TWO	84
Chapter THREE	125
Chapter FOUR	198
References	259
Thesis Corrections	271
Appendices	291

List of Tables

CHAPTER ONE

Table 1: Percutaneous Tibial Nerve Stimulation (PTNS) versus Sacral Nerve Stimulation (SNM).

Table two: A summary of all clinical studies investigating PTNS in patients with idiopathic and neuropathic Overactive Bladder (OAB).

Table three: The spread of diagnoses in patients receiving PTNS.

Table four: Baseline demographics according to the disease group of patients attending PTNS (n=74).

Table five: The Comparison of the mean sub-scores for individual domains on the ICIQ-OAB and ICIQ-LUTSqol at baseline.

Table six: Mean change in the measured parameters from baseline to 12 weeks for all patients.

Table seven: Absolute changes in key factors: overall and according to disease group.

Table eight: Logistic regression analysis investigating the relationship between baseline factors and changes in key variables and returning for top-ups.

Table nine: Results of the patient satisfaction survey.

CHAPTER TWO

Table ten: Summary of all the published studies that have measured urinary NGF (uNGF) levels in patients with various bladder dysfunctions.

Table eleven: Summary of studies evaluating urinary BDNF in patients.

CHAPTER THREE

Table twelve: Advantages and limitations of urodynamic testing.

Table thirteen: Plate plan one.

Table fourteen: The absorbances from the plate reader for plate one.

Table fifteen: Plate plan three.

Table sixteen: The absorbances for plate three.

Table seventeen: Plate plan four.

Table eighteen: The absorbances for plate four.

Table nineteen: Characteristics of the three groups.

Table twenty: Unadjusted linear regression analysis to explore factors related to the log NGF/Cr levels.

Table twenty-one: Unadjusted linear regression analysis to explore factors related to the log BDNF/Cr levels.

CHAPTER FOUR

Table twenty-two: Distribution of diagnoses in the two treatment arms.

Table twenty-three: Reasons why 14 patients failed screening.

Table twenty-four: Device related and non-device related reasons for patient withdrawal from the study.

Table twenty-five: Adverse events – classified into either related or unrelated to the device.

Table twenty-six: Demographics of the responders and non-responders.

Table twenty-seven: Study characteristics at baseline according to diagnosis.

Table twenty-eight: Mean (SD) absolute changes in the ICIQ questionnaire and bladder diary outcomes from baseline to week 12 according to diagnosis.

Table twenty-nine: Change in perceived day frequency according to baseline characteristics.

Table thirty: Change in perceived nocturia according to baseline characteristics.

Table thirty-one: Change in perceived urgency according to baseline characteristics.

Table thirty-two: Change in perceived urge leakage according to baseline characteristics.

Table thirty-three: Change in ICIQ OAB total according to baseline characteristics.

Table thirty-four: Change in ICIQ QoL total according to baseline characteristics.

Table thirty-five: Change in mean voided volume according to baseline characteristics.

Table thirty-six: Change in 24 hour bladder frequency according to baseline characteristics.

Table thirty-seven: Change in mean urge according to baseline characteristics.

Table thirty-eight: Change in mean leakages according to baseline characteristics.

Table thirty-nine: Change in leakage severity according to baseline characteristics.

Table fourty and fourty-one: The absolute change in ICIQ-LUTSqol and ICIQ-OAB score over the visits.

Table fourty-two below: Potential benefits of Transcutaneous Posterior Tibial Nerve Stimulation.

List of Figures

CHAPTER ONE

Figure one: The course of the Tibial nerve as it travels in the lower limb.

Figure two: The course of the posterior tibial nerve at the ankle.

Figure three: The questionnaire tools that have received grade A recommendation from the third ICI.

Figure four: Study timeline.

Figure five: Flow chart illustrating the patient pathway for the study.

CHAPTER TWO

Figure six [1]: The afferent fibres leave the bladder and travel to the spinal cord with the neurotransmitter substances.

Figure seven [2]: The proposed complex interaction of afferent receptors and the different acting neurotransmitter substances.

Figure eight [3]: A model illustrating possible chemical interactions between urothelial cells, afferent nerves, efferent nerves and myofibroblasts in the urinary bladder.

Figure nine [4]: Provoked NGF release from target cells occurs under certain circumstances, which sensitizes afferent nerves, enhances synaptic transmission and produces OAB symptoms.

CHAPTER THREE

Figure ten: The sandwich assay.

Figure eleven: An example of a plate format with two standard columns.

Figure twelve: The linear relationship between the standard dilutions of NGF concentrations and absorbances.

Figure thirteen: The linear relationship between the standard NGF concentrations and absorbances are demonstrated.

Figure fourteen above: This graph shows the deterioration of the absorbances from the 125pg/ml, 250pg/ml and IQC dilution of NGF standard over a two month period.

Figure fifteen: Bar chart showing the comparable ELISA results from two different operators.

Figure sixteen: Correlation between y-axis: urinary NGF/Cr levels (pg/µmol) and x-axis: OAB score for the patients with MS reporting OAB symptoms (n=67).

Figure seventeen: The plot of urinary BDNF/Cr levels (pg/µmol) (y-axis) versus OAB score (x-axis) for the patients with MS and OAB symptoms (n=67).

Figure eighteen: Receiver operator curves for urinary NGF/Creat and OAB score for the MS patients with and without OAB symptoms (n=77).

Figure nineteen: Receiver Operator Curves (ROC) for urinary BDNF/Creat and OAB score for MS patients with and without OAB symptoms (n=77).

Figure twenty: The correlation between EDSS and OAB score in MS patients both with and without OAB symptoms.

Figure twenty-one: Interactions between neurotrophins and the immune cells

CHAPTER FOUR

Figure twenty-two: Summary flow chart of patients enrolling into the study.

Figure twenty-three: Graph to show the change in ICIQ-OAB score among the MS patients with differing EDSS scores. X-axis EDSS score.

Figure twenty-four: Graph to show the mean ICIQ-OAB total scores in responders versus non-responders.

Figure twenty-five: Graph to show the mean ICIQ-LUTSqol total scores in responders versus non-responders.

Figure twenty-six: Patient satisfaction survey.

Figure twenty-seven: Change in NGF/Cr levels over the course of treatment in patients with MS.

Figure twenty-eight: Change in NGF/Cr levels over the course of treatment in patients with idiopathic OAB.

Figure twenty-nine: Change in BDNF/Cr levels over the course of treatment in patients with MS.

Figure thirty: Change in BDNF/Cr levels over the course of treatment in patients with idiopathic OAB.

List of photographs

Photograph One: The loaded plate with ELISA.

Photograph Two: Polystyrene and polypropylene tubes.

List of Appendices

1. NHS REC approvals

2. UCLH R&D approvals

3. Patient consent forms

4. Patient information leaflet

5. Letter to GP

6. Urinary Symptom Profile questionnaire

7. ICIQ-OAB questionnaire

8. ICIQ-LUTSqol questionnaire

9. Bladder diary

10. GRA and patient satisfaction

11. Likert scale

12. Manufacturer ELISA protocols

ABBREVIATIONS

List of Abbreviations

AE	Adverse Event
ATP	Adenosine triphostphate
BDNF	Brain derived neurotrophic factor
BoNT/A	Botulinum toxin type A
BOO	Bladder outflow obstruction
BPE	Benign prostate enlargement
CGRP	Calcitonin gene related peptide
CISC	Clean Intermittent Self-Catheterisation
CMG	Cystometrogram
Cr	Creatinine
CRF	Case Report Form
CSF	Cerebrospinal fluid
CVA	Cerebrovascular Accident
DO	Detrusor Overactivity
DSD	Detrusor-sphincter dyssynergia
DU	Detrusor Underactivity
ELISA	Enzyme linked immunosorbent assay
FU	Follow up
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
HRA	Honorary Research Assistant
IB	Investigator's Brochure
IC	Informed Consent
ICS	International continence society
ICIQ-OAB	International consultation on incontinence questionnaire – OAB
ICIQ-LUTSqol	International consultation on incontinence questionnaire - LUTSqol
IDO	Idiopathic detrusor overactivity
IPSS	International Prostatic Symptom Score
JBRU	Joint UCLH/UCL Biomedical Research Unit
LUTs	Lower Urinary Tract Symptoms
LUTD	Lower Urinary Tract Dysfunction
MS	Multiple Sclerosis
NHNN	National Hospital for Neurology & Neurosurgery
NHS	National Health Service
NHS R&D	National Health Service Research and Development Unit
NGF	Nerve Growth Factor
NDO	Neurogenic detrusor overactivity
NLUTD	Neurogenic lower urinary tract dysfunction
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
OAB	Overactive Bladder
Participant	An individual who consents to take part in a study

PI	Principal Investigator
PIL	Patient Information Leaflet
PTNS	Percutaneous Tibial Nerve Stimulation
P2X3	Purinergic receptor
QOL	Quality Of Life
RCT	Randomized Controlled Trial
REC	Research Ethics Committee
SCI	Spinal cord injury
SP	Substance P
SOP	Standard Operating Procedure
TRKA	Tropomysin-related kinase receptor A
TRPV1	Transient vanilloid receptor potential 1
TTNS	Transcutaneous Tibial Nerve Stimulation
U	Units
UDS	Urodynamic study
uNGF	Urinary NGF
uBDNF	Urinary BDNF
UCL	University College of London
UCLH	University College of London Hospitals NHS Trust
UI	Urge incontinence
USS	Urgency Severity Scale
UTI	Urinary tract infection

All abbreviations used should be listed and defined

CHAPTER ONE

Chapter synopsis

The overactive bladder (OAB) is a prevalent syndrome of urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence. OAB significantly affects patient quality of life. This chapter is a prospective evaluation of Percutaneous Tibial Nerve Stimulation (PTNS) as a treatment for overactive bladder syndrome. This treatment was introduced as a service in the department of Uro-Neurology, and this chapter analyses the first 18-months of experience, in a heterogenous group of patients that have been referred to a tertiary neurological centre. Within the chapter there is a literature review that explores the evidence behind PTNS, and the outcomes of this cohort of patients are discussed. The chapter also discusses the challenges with objectively assessing patient outcomes. The overactive bladder syndrome is a patient reported symptom profile and outcomes are inherently subjective and fully reliant on the accuracy of patient reporting.

Tibial nerve stimulation for the management of the neurogenic and idiopathic overactive bladder: A safe and effective treatment

Abstract

Background

Percutaneous Tibial Nerve Stimulation (PTNS) is a minimally invasive technique of neuromodulation for treating overactive bladder (OAB) symptoms. The aim of this study was to assess safety and efficacy of PTNS in patients with neurological disease reporting overactive bladder symptoms.

Methods

In this prospective evaluation, patients finding first-line treatments for OAB either ineffective or intolerable underwent a standard 12-week course of PTNS (Urgent PC, Uroplasty). Urinary symptoms were evaluated using standardised questionnaires (ICIQ-OAB and ICIQ-LUTSqol) and bladder diaries. Patients opting to continue treatment after 12 weeks returned for top-up sessions.

Results

Seventy-four patients (22 males and 52 females) with mean age of 56.0 (95% CI: 52.2, 59.8) years, were included in this cohort. Twenty-five patients (33.8%) had idiopathic OAB, 19 (25.7%) had multiple sclerosis (MS), and 30 (40.5%) other neurological diagnoses at entry. Sixty-four patients (86%) completed 12 weeks of treatment. Significant improvements were noted in mean ICIQ-OAB scores of -5.1 (SD: 10.4) (p=0.003), ICIQ-LUTSqol quality of life scores -22.4 (SD:44.3) (p=0.001), 24-hour bladder frequency -1.2 (SD: 2.2) (p=0.001), and

severity of incontinent episodes -0.2 (SD: 0.4) (p=0.004). Patients found treatment comfortable and no adverse effects were reported. Twenty-four out of fifty-three patients (45%), who were invited for top-up therapy returned with mean top-up frequency of 44.4 days (7-155 days). Patients who reported improvements in OAB symptoms, leakage severity and quality of life at week 12, as well as patients with MS, were more likely to return for top-up sessions.

Conclusions

The safety and efficacy of PTNS for the management of OAB is comparable between patients with idiopathic and neuropathic OAB. Significant improvements in overactive bladder symptoms and quality of life were observed, with minimal adverse effects.

INTRODUCTION

The Overactive Bladder (OAB) syndrome is defined by the International Continence Society (ICS) as a syndrome of urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence (UUI), in the absence of urinary tract infection or other obvious pathology [6]. OAB symptoms affect all age groups with increasing prevalence with age, and therefore relavent with an ageing UK population. It has significantly negative impacts on quality of life affecting sleep, sexual activity, mental health, work productivity and may also increase social isolation [7]. Elderly patients with OAB are reported to have increased numbers of falls, fractures, and skin infections. Patients can be difficult to screen for, with and although many patients seek treatment it is felt a significant proportion of patients do not receive it [8].

The cost of this condition is difficult to calculate accurately as one must consider the cost of treatment, including medications or surgical intervention, cost of continence products such as pads and liners, and the treatment of other collateral medical issues it can cause, aswell as the loss productivity of work and earnings that the patient is unable to sustain. For individual patients, this is a condition which has worse quality of life scores than those with asthma, diabetes, hypertension and depression [8].

In the United States a cost of illness study estimated the national cost of OAB with urgency incontinence in 2007 at $65.9 billion, with predicted costs of $76.2 billion in 2015 and $82.6 billion in 2020 [9]. Prevalence of OAB is underestimated, although the National Overactive Bladder Evaluation study have suggested 17% of men and women are affected in the United States, and from 12-17% in six European nations [10-12]. The prediction of those affected is thought to rise to 20.1% by 2018 [13].

Management of OAB

The first line of treatment for the OAB involves conservative measures such as behavioural modifications with fluid and caffeine restriction, pelvic floor exercises and medical therapy [14]. Clinicians however frequently face patients who have failed to respond to these initial measures. The success of antimuscarinic therapy, although effective for many patients, may be misrepresented in clinical trial results which tend to have a short duration of follow-up (3months), with intensive follow-up regimes and incentives, which may falsely elevate adherence [15]. In longer term follow-up studies only 18.2% of patients continued with treatment after six months, due to poor treatment efficacy, cost, adverse effect profiles or inadequate follow-up regimes [16].

Other potential treatment options include sacral neuromodulation (SNM), a form of central nerve stimulation, which is a clinically effective alternative to drug therapy, being efficacious in up to 80% of patients [17]. However important considerations are its cost, need for specialist surgery and the associated potential complications. Although less invasive than augmentation cystoplasty, implantable devices are susceptible to infections in 5%, pain at surgery site in 25%, lead migrations in 16%, haematoma formation, subsequent revision surgeries for repositioning in 15%, and wound problems in 7%. These complications include a re-operation rate of 33% [17]. SNM may therefore be unsuitable for many elderly patients who may be frail and have several co-morbidities or cognitive impairment. Implants are also contraindicated in patients who may need MRI scanning, which is a consideration especially for patients with Multiple Sclerosis (MS) who may need regular follow up scanning.

OnabotulinumtoxinA (BTX-A) is another option, which has recently acquired its licence in the UK for its use in patients with idiopathic and neuropathic bladder dysfunction after the acquisition of high level evidence for its success [18, 19]. However this is currently not offered by many primary care trusts due to costs, and its availability remains limited. Patients can also be dissuaded from pursuing this line of management due to its inherent risks of voiding dysfunction and the need for subsequent clean intermittent self-catheterisation (CISC). Also its effectiveness is only for a limited duration, and patients ultimately require repeated injections. This leaves a gap in the available management options between antimuscarinic medications and more invasive treatments such as BTX-A, SNM and ultimately invasive surgical options to physically increase the bladder storage capacity such as clam-ileocystoplasty. Such an alternative treatment should be efficacious whilst combining other characteristics such as cost-effectiveness and being minimally invasive with little adverse events.

Neuroceuticals

For what is largely a functional lower urinary tract disorder as opposed to an anatomical one, surgery may not be the most suitable solution. The use of 'Neuroceuticals' is a form of treatment that uses electrical stimulation to target specific nerve fibres to manage a number of different conditions. This stimulation modulates neural impulses that control bodily functions, and is aiming to repair lost function. Researchers across many disciplines are exploring the use of this technique for example to encourage insulin release from cells to treat diabetes, to regulate food intake for obesity, and to rebalance vascular smooth muscle tone for patients with hypertension or pulmonary diseases [20]. Circuits of action potentials, the catalyst for electrical impulses, are essential for the normal functioning and communications between all organs. These nerve fibres are all interconnected and organized in bundles

allowing for pinpoint localization of therapy. Currently delivery of electrical stimulation is initially being harnessed in devices that are being designed for these specific purposes. For example pacemakers and defibrillators for cardiac muscle stimulation, deep brain stimulators for patients with Parkinson's Disease and depression, sacral nerve stimulators for patients with bladder dysfunction, and vagus nerve stimulators for patients with epilepsy or rheumatoid arthritis [20]. However these devices are still at a preliminary stage in that they still do not target individual cells within these circuits. Within bundles adjacent individual nerve fibres may be carrying signals in opposing directions, or fibres originating from different organs leading to side effects by stimulating collateral fibres and dampening the clinical efficacy. Devices of today are not as yet producing naturalistic patterns of action potentials, and currently only provide simple waveforms that can either block or stimulate nerves rather than reproduce dynamic activity on a millisecond scale [20].

The mechanisms of action for neuromodulation is unclear, but is thought to have a combination of a direct effect on the bladder and a central effect on the micturition centres of the brain. It has been observed that nerves can change their function and signalling in response to disease, injury and repeated electrical stimulation, which is coined in the phrase neural plasticity. The aim of electrical nerve stimulation in the lower urinary tract is to modulate this plasticity in order to restore bladder dysfunction. Neuromodulation can either be targeted at the level of the central or peripheral nerves. Sacral nerve stimulation is an FDA approved treatment, applied centrally, to manage OAB and urinary retention.

Peripheral Nerve Stimulation

Dorsal Penile Nerve Stimulation

Peripheral nerve stimulation has been subject to interest and study for some time. Experimental animal studies have investigated the use of a range of peripheral nerves to target the bladder. Electrical stimulation of the dorsal penile nerve (DPN) in anaesthetised cats had clear effects on the micturition reflex [21]. It was observed that stimulation at a lower frequency of 5-10Hz, inhibited distension evoked bladder contractions, promoted urinary storage, and increased the bladder capacity. Whereas stimulation at 33-40Hz augmented the distension-evoked contractions and when the bladder was filled above a threshold volume, stimulation at 20-40Hz activated the micturition reflex and elicited detrusor contractions increasing voiding efficiency compared to the distension-evoked voiding. This ability to promote detrusor contractions through DPN stimulation was preserved following acute spinal transection, suggesting the ability of genital afferents in modulating the micturition reflex and potential use of restoring bladder control through nerve stimulation. The frequency of delivery was also clearly important for the type of effect induced. When applied to human patients with detrusor overactivity (DO), dorsal genital nerve stimulation has lead to an improvement in bladder capacity, reduction in urgency and incontinence episodes [22].

Clitoral Nerve Stimulation

An early rat study showed that pelvic or hypogastric nerve stimulation, could evoke responses in the postganglionic nerves that supply the penis and clitoris [23]. More recently it has been shown that in 4 women with spinal cord injury, short duration electrical stimulation of the clitoral nerve provided significant improvements in bladder capacities and reduction of maximum bladder pressures [24].

Pudendal Nerve Stimulation

In the same way, pudendal nerve stimulation (PDNS) has also been explored in patients with OAB and DO [25]. One of the early reported studies recruited 29 patients who had failed conservative treatments. PDNS was experimented using either intravaginal or intra-anal transcutaneous electrodes. Some patients also had additional stimulation through a single needle electrode placed directly into the pudendal nerve. Stimulation was administered with amplitudes determined by the patient's pain threshold. Voltage pulses at 10Hz were used, at one-week intervals for four weeks in total. All patients showed significant improvements in functional bladder capacity and some reduction in the frequency of micturition. Two patients who were unaffected by the external electrodes, responded well to the direct needle electrode suggesting the mode of delivery may be more effective using direct needle puncture onto the nerve for some patients.

Later another study examined the use of chronic PDNS, in 84 patients who had either Interstitial Cystitis (IC), or OAB refractory to sacral neuromodulation (SNM) [26]. Ninety-three percent of those who had failed SNM responded to PDNS. At median follow up of 24.1 months, significant improvements were seen in frequency, voided volume, incontinence, urgency, and IC Symptom scores. This study shows that chronic PDNS is a potential alternative for patients refractory to other therapies.

The safety and efficacy of these forms of peripheral nerve stimulation are yet to be backed by evidence from phase 3 clinical trials. It has been noted during OAB drug trials that the placebo effect can effect up to 64% of patients for urinary incontinence symptoms [27]. One form of peripheral nerve stimulation that has an emerging evidence base is Percutaneous Tibial Nerve Stimulation (PTNS). This chapter focuses on the use of PTNS for the OAB.

Percutaneous Tibial Nerve Stimulation

Percutaneous Tibial Nerve Stimulation (PTNS) is another peripheral method of stimulating nerves for controlling bladder function. is This less invasive, office based treatment applies electrical stimulation in a retrograde manner through the tibial nerve, to indirectly modulate the sacral plexus.

Curently being explored as an alternative for patients, PTNS may have the qualities to fill the gap in the treatment armentarium [28]. It is felt that PTNS could offer a suitable alternative for patients unable to tolerate side effects of antimuscarinic medications (such as dry mouth, dry eyes, constipation and cognitive CNS effects), and those refractory to behavioural modifications, and those not willing to consider more invasive options such as the surgical implantation of a SNM device, or more complex reconstructive surgery.

The inherent advantages that PTNS could offer over SNM, are summarised in table 1 below.

Table 1: Percutaneous Tibial Nerve Stimulation (PTNS) versus Sacral Nerve Stimulation (SNM).

	PTNS	SNS
Advantages of PTNS	Non-invasive; Fine gauge needle placed into the medial aspect of ankle	Invasive; tined lead placed through S3 foramina
	No anaesthesia required	Requires general anaesthesia
	Side-effect profile minimal, include discomfort at needle insertion site, one report of haematoma formation at the ankle	Side-effects, which may require surgical revision: • implantable devices are susceptible to infections in 5%, • pain at surgery site in 25%, • lead migrations in 16%, • haematoma formation, • subsequent revision surgeries for repositioning in 15%, and • wound problems in

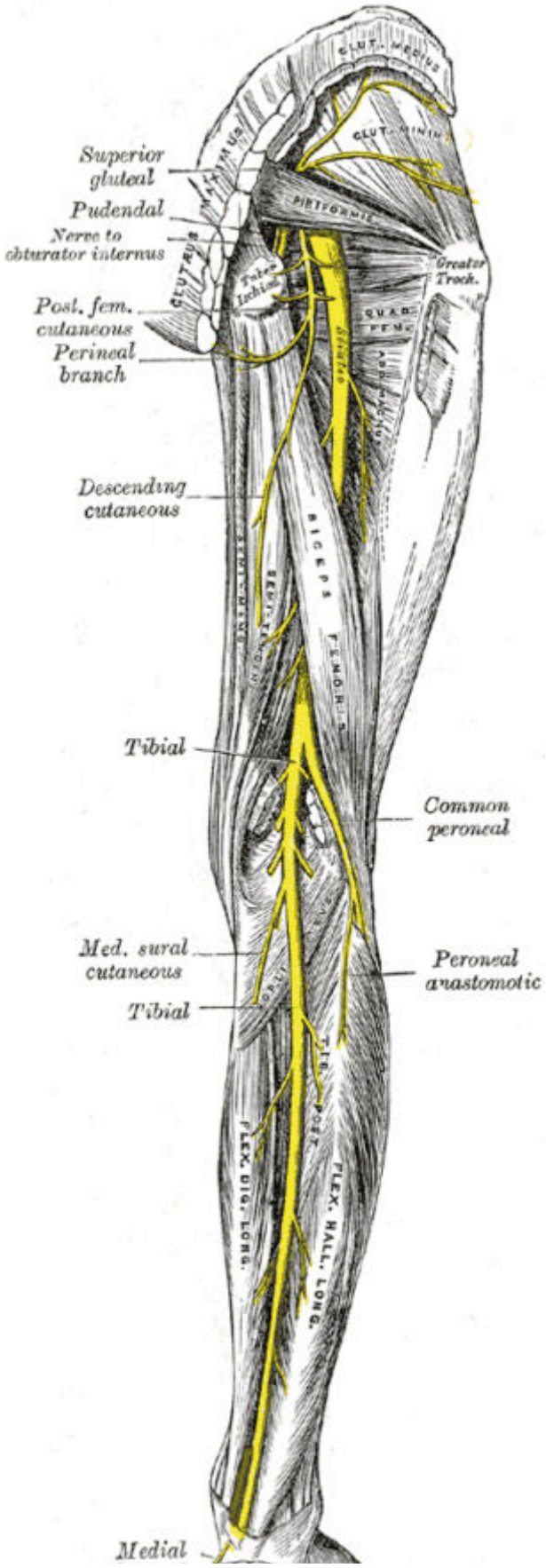
		7%. • These complications include a re-operation rate of 33%
	Proven against sham	Unproven against sham
	PTNS appears significantly less costly [29], however more health economic studies required for accurate cost effectiveness	Health economic studies required for cost effectiveness
Advantages of SNS	Requires 12 weekly visits to the outpatients	Fewer visits however follow up will be long term
	Long term data not available	5 year data available [30]
	Mechanism of action unclear	Mechanism of action unclear
	Not able to cure symptoms, possibly less efficacious than SNS [31] - however no head to head studies	Possible to cure patient symptoms
	Intermittent stimulation	Continuous stimulation

PTNS - Mechanism of action

The location of the posterior tibial nerve for stimulation is behind and above the medial malleolus of the ankle. This point coincides with 'spleen-6' the traditional Chinese acupuncture site for the treatment of urological complaints, pelvic disorders and dysmenorrhoea.

PTNS involves the stimulation of the posterior tibial nerve, which is a mixed sensory motor nerve, formed of axons derived from the L4 to S3 spinal nerve roots, comprising the outflow of sacral nerves, which innervate the bladder, urinary sphincter and pelvic floor. These fibres provide autonomic and somatic innervation to the lower urinary tract (bladder and urethral sphincter), large bowel and pelvic floor. PTNS inhibits bladder activity by depolarizing the lumbar afferent and somatic sacral nerves and ultimately rebalancing the inhibitory and excitatory impulses that control bladder function. This promotes inhibitory somatic afferent signalling, which is largely deficient in the OAB. These afferents project to the pontine micturition centre and with chronic stimulation, can lead to changes in the suprapontine regions that finally modulate micturition reflexes [32]. Afferent nerve stimulation provides central inhibition of the preganglionic bladder motor nerves through a direct route in the sacral spinal cord. In animal models, it has been shown that neuromodulation can lead to hypertrophy of the external urethral sphincter and consequently greater urethral closure pressures and hence promoting the guarding reflex [32]. Figure one below is a schematic of the course of the tibial nerve travelling down the lower limb.

Figure one: The course of the Tibial nerve as it travels in the lower limb (Grey's Anatomy).



Three main pathways have been debated for mechanism of action [33]:

1. Direct stimulation of the hypogastric nerve through activation of sympathetic fibres at low bladder filling volumes
2. Direct stimulation of the nuclei of the pudendal nerve in the spinal cord at maximal bladder filling
3. Supraspinal inhibition of the detrusor

This treatment therefore requires the existence of uninterrupted spinal inhibitory pathways, which are capable of preventing a detrusor contraction.

It has been shown in an animal model that intravesical acetic acid instillation in the rat, resulted in a rise in c-fos expression (a marker of neuronal metabolic activity) in the sacral cord, and this was significantly reduced after PTNS treatment of the hind leg [34]. Another study in rats demonstrated that after PTNS, the mast cell count in the bladder diminished [35]. Tai et al showed in cats that irritation induced bladder overactivity can be suppressed by tibial nerve stimulation [36]. A 30 minute stimulation at low and high frequencies (5Hz and 30Hz) was able to produce prolonged inhibition of bladder activity and increased bladder capacity for 2hours or more.

PTNS – Background of Clinical studies

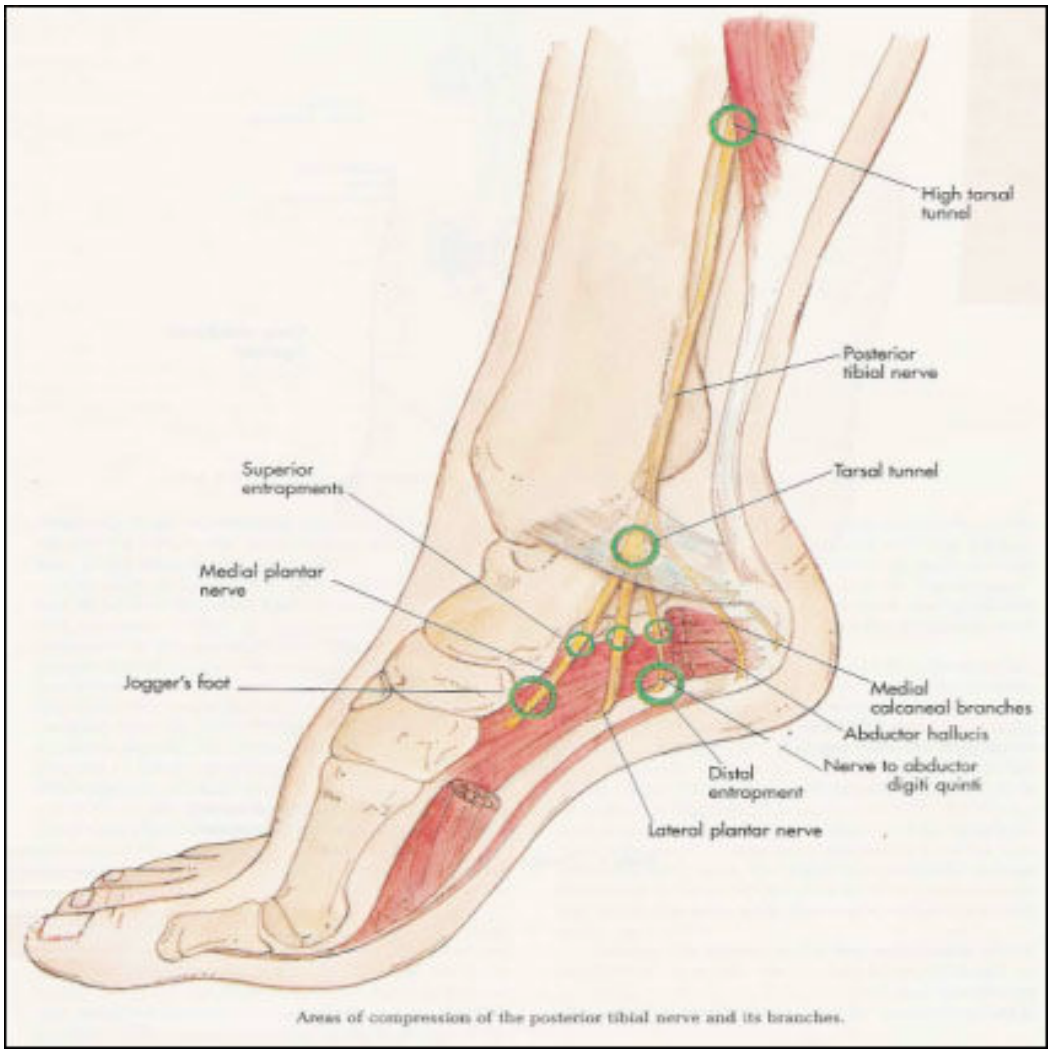
Despite the lack of clarity about it mechanism of action, PTNS is being adopted as a potential treatment for the OAB, and other conditions such as non-obstructive urinary retention, neuropathic bladder, paediatric voiding dysfunction, and painful bladder syndrome, although it still requires further validation for all of the above. McPherson in 1966 demonstrated that in

cats stimulation of the posterior tibial nerve inhibited bladder contractions [37]. Further efforts to stimulate this nerve for bladder dysfunction stem back to McGuire in 1983 [38]. In his publication, inspired by effects of acupuncture at the tibial and peroneal nerves, McGuire described electrical stimulation of the tibial nerve in 22 patients with bladder complaints secondary to a variety of conditions including neurological diseases, interstitial and radiation cystitis. The results were declared 'astonishingly good' when eight of eleven patients with DO became 'dry' using urodynamic parameters.

Also in the 1980s, Stoller began studies on pig-tailed monkeys that showed intermittent PTNS could inhibit bladder instability and urge incontinence [39]. Over the next decade, at the Univerity of California and San Francisco, Stoller reported in a guest lecture that intermittent percutaneous stimulation of the tibial nerve in a group of 98 patients proved its safety and potential efficacy. PTNS became more recognised after this as Stoller afferent nerve stimulation (SANS).

SANS technique was described using a 34-guage needle, which is placed bilaterally, three finger-breadths above the medial malleolus and posterior to the tibia. The location of the posterior tibial nerve at the ankle is illustrated in figure two below. A grounding adhesive electrode is placed near the medial calcaneus. Electrical stimulation is then administered via a 9-V battery-powered generator, at an amplitude of 0.5 to 10mA, with a fixed pulse width (200μs) and frequency (20Hz). The confirmation that the needle is in the correct place is by evoking plantar flexion of the toes or toe fanning once the nerve is being stimulated.

Figure two: The course of the posterior tibial nerve at the ankle.



Areas of compression of the posterior tibial nerve and its branches.

Govier et al following this performed another of the earlier multicentre studies on 53 patients with OAB in the United States. Patients received weekly treatments for 12 weeks. Seventy-one patients were classified to have treatment success, with overall reductions of 25%, 21% and 35% for mean daytime, night-time voiding frequency, and urge incontinence.

Based on this data, regulatory clearances were achieved for PTNS with FDA-approval in 2000 for urinary urgency, frequency and urge incontinence. In 2010 this was updated to include OAB. A PTNS device received the CE mark for this purpose in 2005, since which Uroplasty have marketed the Urgent PC Neuromodulation System, the only device commercially available today. This has subsequently been the device used in the pivotal phase III study, 'The Study of Urgent PC vs Sham Effectiveness in Treatment of Overactive Bladder Symptoms' (SUmiT) [40]. PTNS has also acquired approval from the National Institute of Clinical Excellence (NICE) within the guidance for the management of urinary incontinence in women. Although approved, the data concerning the patient selection criteria and the longevity of treatment is still limited.

A published systematic review examining PTNS for adults with OAB, identified four randomized controlled trials and six prospective observational cohort studies investigating this treatment. PTNS was found to be efficacious compared to control groups for reducing urinary frequency and urge incontinence episodes [41]. Most studies include a follow-up period of 12 weeks.

A published multicentre, randomized study known as the OrBIT (Overactive Bladder Innovative Therapy) trial, examined the head to head use of PTNS versus extended release

tolterodine [42]. This was conducted over 11 centres in the United States, and the primary endpoint was the frequency of urination per 24-hour period, after 12 weeks of treatment. Secondary endpoints included the mean change in the number of urge incontinence episodes, number of voids causing waking, volume voided, and number of urgency episodes using the Indevus Urgency Severity Scale. This information was captured using voiding diaries, OAB-q questionnaires and a Global Response Assessment (GRA) scale. The results reported 100 patients randomized, with 50 patients in each of the two arms. The GRA showed that 79.5% of patients from the PTNS arm and 54.8% in the tolterodine arm self-reported to have been either cured or improved. Significant improvements were seen in all OAB symptom subset scores after 12-weeks treatment for both groups, and there was no significant difference between groups. The lack of a placebo arm in this study leaves uncertainty about how much the elevated improvements noted in the GRA from the PTNS group is related to placebo effect.

Another randomized study comparing PTNS to solifenacin has also recently been published [43]. This was a randomized crossover study of 40 patients who had four weeks of therapy with 5mg oral solifenacin followed by a 3-month washout period before having a 6-week regime of PTNS treatment, which entailed twice weekly 30-minute sessions. The results of this study suggest that both PTNS and solifenacin provide improvements in all subscores of OAB, however PTNS was shown to be superior to solifenacin in particular with regards to the number of episodes of daily micturition and quality of life improvements. The dosing of 10mg of solifenacin was not used in this study, and the effect of an increased dosage may have influenced the results.

The same group that conducted the OrBIT study went on to develop a validated sham and published the results of the first multicentre, double-blind, placebo-controlled randomised trial involving 220 patients with idiopathic OAB [40]. This is known as the SUmiT Trial and compared the efficacy of 12 weekly, 30 minute stimulations of PTNS against a sham treatment [40]. Quality of life questionnaires and voiding diaries were completed at baseline and after 12-weeks of treatment. A responder was defined by the reporting of a moderate or marked improvement of bladder symptoms on a 7-level GRA. The GRA is a subjective outcome that considers patient perceived improvement. Secondary endpoints included any change in a specific subset of symptom scores (either urgency, frequency, and urge incontinence), voiding diary parameters measured over 3-days, OABq scores and quality of life scores using the SF-36 [40]. To blind the treatment, patients were seated in a recliner, and their feet were draped, away from view. In the sham group, a dummy needle was placed, where the needle retracted into the sheath but also gave the sensation of a pin-prick, with no skin puncture taking place, and therefore the tibial nerve could not be stimulated.

The results at 13-weeks, for the primary end point, shows that 54.5% and 20.9% of patients from the treatment and sham group respectively achieved a moderate or marked improvement in the GRA, which was statistically significant ($p<0.001$). For secondary end points of individual subset symptom scores for urinary urgency, frequency and urge incontinence, there were significant improvements in the PTNS arm compared to sham. Interestingly with the three-day voiding diary parameters for mean daytime and night-time urinary frequency, there were significant improvements from baseline to 13 weeks in both the PTNS and sham arms. The change in OAB-q symptom scores and quality of life scores from baseline to 13-weeks, suggested significant improvements in the PTNS group compared to sham. The authors concluded this study, which is the first to include a realistic sham arm, provides level-one evidence that PTNS is a safe and effective treatment for OAB.

A smaller randomized placebo controlled, double-blind study of 35 female patients with proven DO has also been reported [44]. Seventeen patients completed the treatment arm of PTNS, and fifteen received a placebo treatment of having a needle placed into the medial aspect of the gastrocnemius muscle. The stimulator was switched on for 30 seconds in the sham group so patients could feel a minor electrical sensation in the skin, but this was then turned off for the remainder of the 30-minute treatment. Patients in both arms received three 30-minute treatments per week, with 12 sessions in total. The number of patients who had a 50% or greater reduction of incontinence episodes was 12 (71%) in the treatment arm and 0 (0%) in the sham arm. Significant improvements were also seen in the treatment arm for the number of micturitions, voided volumes and I-QOL scores, with no improvements noted at all in the sham arm.

Table two summarises all of the clinical studies evaluating PTNS for the OAB.

Table two: A summary of all clinical studies investigating PTNS in patients with idiopathic and neuropathic OAB.

Study	Population and Number of patients	Study design and Treatment Regime	Criteria for success	Results
1. Peters et al, 2010. SUmiT study [40].	Patients with idiopathic OAB (220) randomized between PTNS versus a sham treatment.	**Multicentre, double blind, placebo controlled RCT.** Once weekly for 12 weekly x 30minute sessions. Current level of 0.5 to 9mA at 20Hz.	Primary Endpoint: Responder defined as reporting symptoms to moderately or markedly improve on a 7-level global response assessment (GRA). Secondary Endpoints: GRA subset symptom components, Voiding diary parameters, OAB-q symptom severity score, SF-36 general health quality of life score	54.5% PTNS arm and 20.9% of sham arm reporting moderate or marked improvement. Significant voiding diary parameter improvements compared with sham.
2. Finazzi-Agro et al, 2010 [44].	Female patients with DO (35) randomized between PTNS versus a sham treatment.	**Double blind, Placebo controlled RCT** 12 x 30minute sessions, performed three times a week Low voltage stimulator (9V),	Primary outcome: Percentage of responders in the two groups. (Patients with 50% or greater reduction in urge incontinence episodes). Secondary outcome: number of incontinence	71% of the PTNS arm and 0% of the sham arm were considered responders. Statistically significant improvements were seen in the bladder diary and quality of life scores in the PTNS arm but not in the

		using a stimulation current of 0-10 mA and a pulse width of 200 mseconds increased until toe flexion/fanning was noticeable.	episodes, micturitions, mean voided volume (from voiding diary) and I-QOL score.	placebo arm.
3. Peters et al, 2009. OrBIT study [42].	OrBIT (Overactive Bladder Innovative Therapy) trial. 100 adults with urinary frequency were randomised to 12 weeks of PTNS or 4mg tolterodine daily.	**Multicentre, non-blinded, non-placebo controlled RCT** 12 weekly 30-minute sessions of PTNS delivered.	Primary objective: Compare efficacy of PTNS to tolterodine in reducing the 24 hour urinary frequency (from voiding diaries). Secondary endpoints: mean change in urinary urge incontinence episodes over 24 hours, nocturia, volume voiding per day, urgency from Indevus Urgency Severity Scale, improvement in OAB-q scale and using a GRA scale.	Global response assessment (a subjective asessment) demonstrated 79.5% of patients in the PTNS arm reporting cure or improvement compared to 54.8% of patients on tolterodine arm. Objective assessments showed significant improvements in both arms that were comparable.
4. Vecchioli-Scaldazza et al, 2013 [43].	Women with OAB (40) crossover between Solifenacin	**Non-placebo, randomised controlled crossover study** 30-minutes of	Primary endpoint: Reduction in the number of voids/24hours. Secondary endpoints: reduction in number of	Reduction in bladder diary parameter of daily micturitions, nocturia, and urge incontinence in both groups. However PTNS

	Succinate (SS) 5mg (4 weeks) and 6 weeks of twice weekly PTNS with 3 months washout in-between.	PTNS twice a week for 6-weeks. Crossover study, randomised.	episodes of urge incontinence, nocturia, changes in voided volume, patient perception of intensity of urgency and quality of life using OAB Questionnaire Short Form.	showed greater effectiveness in patient perception of urgency and quality of life.
5. Karademir et al, 2005 [45].	Patients with DO (43) randomized between SANS alone (Group 1), 8 weekly 60 minute sessions, vs SANS, 8 weekly 60 minute sessions plus oxybutynin 5mg daily (Group 2)	**Non-placebo controlled RCT (combination study)** SANS was applied at 0.2ms at a frequency of 20Hz, with adjustable amplitude within the range of 0.5-10mA.	Patients were evaluated with the Bristol Urinary Questionnaire and a voiding diary. Improvements in symptoms >70%, 35-70% and <35% were considered as complete, partial and non-responses respectively.	Treatment response rate of 61% and 83.2% for groups 1 and 2 respectively. 36.7% and 46.1% reduction of frequency and urgency in Group 1 whilst 44.2% and 61.1% reduction in Group 2. However no statistically significant difference between the two groups.
6. Klinger et al, 2000 [46].	15 patients with DO (11 women and 4 men)	**Open label urodynamic study** 12 x 30 minute stimulations, at a rate of 4 stimulations per week, with a 9V monopolar	Complete response: Day voids ≤8, nocturia ≤2, pad test 0-1g, DO improved. Partial response: Day voids 8-10, nocturia >2, pad test 2-10g, DO improved.	Seven (47%) showed a complete response and considered cured. Three (20%) had significant improvement. Five (33%) were classified as non-responders. Urodynamic DO was

		generator. Stimulation was selected with a range of 0.5-10mA, fixed pulse width of 200ms and a frequency of 20Hz. Mean follow up was 10.9 months.	Non-response: Day voids >10, nocturia >2, pad test >10g, DO unchanged.	eliminated in 77% of patients. Significant increases were seen in urodynamic parameters such as bladder capacity, volume at first bladder sensation, and bladder volume at normal desire to void. Mean reduction in day and night voids from 16.1 to 8.3 and 4.4 to 1.4 respectively.
7. Vandoninck et al, 2003 [47].	90 Patients with OAB	**Urodynamic Study, open labelled, non-placebo** 12 sessions of PTNS.	Primary outcome: reduction in number of leakage episodes of ≥50% per 24 hours. Secondary outcomes: Patient's request for continuation of treatment was subjective assessment (considered positive responders). Bladder diary parameters Incontinence specific QOL Short Form-36 for general wellbeing. Urodynamic data after 12 PTNS sessions –	Objective success rate at 56% achieving primary outcome. Subjective success rate was 64%. Voiding diary and quality of life scores improved significantly. DO could only be diminished in a few cases. Increases in bladder capacity were significant. Patients without DO at baseline were 1.7 times more likely to respond.

			volume at which DO occurred. (DO defined as an increase of more than 15cm H20 or lower amplitude if accompanied with distinct sensation of urgency).	
8. Vandoninck et al, 2003 [48].	35 patients with urge incontinence (10 male, 25 female)	**Multicentre, open label** 12 weekly sessions of PTNS Needle and electrode connected to a low voltage (9V) electrical stimulator Current 0-10mA applied with a fixed frequency of 20Hz and a pulse width of 200microseconds. The former was increased until curling of the big toe or fanning of all toes were seen, at a well tolerable level.	Frequency/Volume charts were completed, Incontinence-QOL questionnaire, and SF-36 at 0 and 12 weeks. Subjective success was willingness to continue treatment. Objective success was defined as a decrease (to < 50%) in total number of leakage episodes.	22 patients (63%) reported subjective success 24 (70%) showed a ≥50% reduction in total number of leakage episides, QOL parameters improved significantly.

9. Van Balken et al, 2001 [49].	37 patients with OAB (10 male and 27 female) 12 patients with non-obstructive urinary retention (5 men and 7 women)	**Multicentre, open label study** 12 weekly sessions of PTNS performed, each for 30 minutes. Low voltage stimulator (9V), adjustable pulse intensity of 0 to 10 mA, a fixed pulse width of 200microseconds and a frequency of 20Hz. Amplitude is slowly increased until large toe curls or toes fan.	Success was defined as patient request for further treatment. Voiding diaries were collected, SF-36 used to assess quality of life.	OAB patients: Significant reduction in leakage episodes, number of pads, voiding frequency and nocturia, increase in mean voided volume. Retention patients: Reduction in number of catheterisations, increased mean volume voided.
10. Govier et al, 2001 [50]	52 patients with OAB	**Multicentre, open label, non-randomised** 12 weekly sessions of PTNS for 30minutes. Stimulation was titrated from 0-10mA, with a fixed pulse width pf 200 microseconds at a frequency of 20Hz.	Primary objective to demonstrate safety and efficacy (using 3-day bladder diaries) Secondary objectives were assessed using impact of incontinence on health related quality of life outcomes (SF-36, Short form McGill Pain Questionnaire).	71% were classified as a treatment success and started on long-term treatment. 25% and 21% reduction in mean day and night time voids. Urge incontinence episodes were reduced by 35%. Significant improvements in QOL indices.

11. Amarenco et al, 2003 [51]	44 patients with OAB (29 women and 15 men). 13 patients had DO secondary to Multiple Sclerosis, 15 with spinal cord injury, 9 with Parkinson's disease, and 7 with idiopathic DO.	**Prospective study** Examination of the acute urodynamic effect of PTNS. Stimulation delivered in continuous mode at 10Hz frequency and 200 milliseconds wide. Intensity was chosed under the threshold determining motor contraction.	Volume comparisons were made at first involuntary detrusor contraction (IDC) and at maximum cystometric capacity (MCC). Test was considered positive if first IDC and/or MCC increased 100ml or 50% during stimulation.	Mean volume at first IDC was 162.9mls pre-stimulation and 232.1 during PTNS. Mean MCC was 221mls pre-stimulation and 277.4 during PTNS. Significant improvements in first IDC volume and MCC were seen with PTNS. The test was considered positive in 22/44 patients.
12. Van der Pal et al, 2006 [52]	30 patients with urge incontinence (4 men and 26 women).	**Prospective study** Low voltage stimulator (Urgent PC) Adjustable stimulation intensity 0-10mA and fixed stimulation parameters e.g pulse width of 200ms and frequency of 20Hz. Amplitude was slowly increased to	Bladder diaries, SF-36 and incontinence specific QOL were recorded at baseline and after PTNS.	Significant improvements were seen with reduction of SF-36 domain, which correlated with a significant reduction in the number of pads required.

		achieve motor signs with big toe flexion or toe fanning. PTNS was performed for 30 minutes for 12 sessions, three times a week for four weeks.		
13. Van Balken et al, 2006 [53].	132 patients (51 men and 81 women). 83 with OAB, 16 with non-obstructive urinary retention, and 33 with chronic pelvic pain.	**Multicentre, non-randomised** Stimulation had an adjustable pulse intensity from 0-10mA, a fixed pulse width of 200microseconds and a frequency of 20Hz. The stimulator contained a 9V battery (Urgent PC), and motor response in the feet were sought for an adequate response. PTNS was delivered weekly for 12 weeks, in 30 minute sessions.	Subjective response was the patients request for further chronic treatment. Objective success was a decrease in symptoms of over 50% for number of voids/24 hours, number of incontinence episodes, visual analogue score for pain, number of catheterisations/24hours)	Objective success seen in 32.6% and subjective success in 51.5%. A total low score at baseline in the SF-36 questionnaire, especially the mental component showing poor mental health, proved to be predictive for not obtaining objective or subjective success. Mental health should be considered when selecting patients for this therapy.

14. Van der Pal et al, 2006 [52].	11 patients with OAB	**Prospective study** All patients had been successfully treated with PTNS having ≥50% fewer incontinence episodes and ≥50% lower voiding frequency at the end of the treament regime (noted as T1). All patients then stopped treatment for 6 weeks and completed the bladder diaries and QOL questionnaires after this T2. After this they restarted PTNS treatment and were re-assessed (T3).	The first objective was defined as ≥50% increase in incontinence episodes and /or voiding frequency at T2. The second objective was ≥50% fewer incontinence episodes and/or voiding frequency at T3.	At T2, 7/11 patients had ≥50% increase in incontinence episodes and /or voiding frequency. Performance had signficantly deteriorated once PTNS was stopped. At T3, nine patients had ≥50% fewer incontinence episodes and/or voiding frequency. Other bladder diary parameters had improved significantly once back on PTNS. It was concluded that continuous therapy is required for OAB patients, and that efficacy can be reproduced in patients previously treated successfully.
15. Nuhoglu et al, 2006 [54].	35 patients with OAB refractory to oxybutynin therapy.	**Prospective study** SANS weekly for 10 weeks, 30 minutes a session. Utitlised Urosurge SANS device,	Three-day voiding diaries were recorded and urodynamics was performed at baseline, after treatment and at one year.	19 (54%) of patients had complete recovery after treatment. Significant improvements were noted in the QOL and urgency core, with increased voided

		monopolar generator, working at 20Hz frequency, with 200msn pulse width and 0-10mA adjustable pulse intensity, using a 9V battery.		volumes. Eight out of 19 subjects maintained complete recovery at one year follow up. Significant improvements were observed in urodynamic parameters after treatment such as volume and pressure at IDC, and MCC. However uninhibited IDC disappeared in no patients.
16. Kabay et al, 2009 [55].	19 patients with MS and NDO (6 men and 13 women).	**Prospective study** Urodynamics were performed at baseline and after 12 weeks of weekly PTNS. Stimulation was delivered at 200μsecond pulses at a pulse rate of 20Hz.	Urodynamic data, voiding diaries, and pad tests were performed. Complete response was considered as a ≥50% decrease in urgency, urinary incontinence, daytime frequency, nocturia and pad test. A decrease between 25% and 50% was considered a partial response. A decrease below 25% was considered as non-response.	Significant increases in mean volume and maximum pressure at first IDC, and MCC were observed. Improvements were also seen with mean maximum pressure at MCC, maximal flow rate. Detrusor pressure at maximal flow improved in 3 out of 5 patients with detrusor sphincter dyssynergia (DSD). Clinical complete response was seen in 33.3%, 40%, 57.9%, 75% and 90% for

				urgency, urinary incontinence, daytime frequency, nocturia and pad test respectively. Use of PTNS in MS patients with NDO is promising from this study.
17. Gobbi et al, 2011 [56].	21 patients with MS and LUTS, unresponsive to anticholinergics.	**Open label, prospective study** 12 weekly sessions of PTNS, for 30 minutes, at a current level of 0.5-9mA, at 200μs pulses, with a pulse rate of 20Hz, based on motor sensory responses in the foot.	3 day bladder diaries were collected, symptoms were assessed with patient perception urgency scale, and King's Health QOL questionnaire before, during and after treatment. A visual analogue scale (VAS) was used (indicated no improvement to 10 indicating complete cure). Treatment satisfaction was defined as a VAS score greater than 5, or a reduction of LUTS ≥50%.	Significant reduction of daytime frequency, nocturia and mean post void residual. 89% of patients reported 70% satisfaction with the treatment. Significant QOL improvements were seen in the King's Health QOL domains.
18. Kabay et al, 2009 [57].	29 patients with MS and NDO. (12 men and 17 women).	**Prospective study** PTNS delivered at a pulse rate of 20Hz, at 200μs	Acute effects of PTNS were assessed with urodynamics.	There were significant increases in mean volume at first IDC, and mean MCC.

		pulses.		
19. Fjorback et al, 2007 [58].	8 patients with MS and DO.	**Prospective study** 20Hz, at 200μs pulses, PTNS was applied bilaterally.	Urodynamic study assessing acute effects of PTNS on DO.	PTNS failed to suppress DO in all patients.
20. Zecca et al, 2014 [59],	83 patients with MS and LUTS.	**Prospective study** 30 minutes stimulation weekly for 12 weeks. Current level of 0.5-9mA using charge-compensated 200μs pulses and a pulse rate of 20Hz.	Assessment of sensory and motor response in the feet and correlation with clinical outcome. Objective parameters included 3 day voiding diary, Kings Health Questionnaire and treatment satisfaction VAS scale.	51 out of 83 (61%) patients reported an improvement in LUTS of ≥50%. All patients showed a motor and or sensory response. 64%, 6% and 30% exhibited a sensory only, motor only and a combined sensory-motor response respectively. The presence of either was not associated with sensory or motor impairment or spasticity. Those with a sensory only and combined sensory motor response were associated with a significantly better outcome than a motor response alone. Over 90% of responders showed a sensory response.

21. Zecca et al, 2014 [60].	84 patients with Multiple Sclerosis and mixed LUTS (voiding and storage symptoms)	**Open label, Multicenter, Prospective Study.** 12 weekly sessions of PTNS Responders defined as having (≥50%) improvement of LUTS entered a maintenance phase. Greatest frequency was every two weeks.	Assessed with 3-day bladder diary and patient perception of bladder condition (PPBC) questionnaire before and after 12 weeks. Treatment satisfaction was assessed using a 7-level GRA scale. Urodynamics were perfomed before the start and after 24 months of PTNS.	89% of patients responded to the initial 12-week therapy. Prolonged PTNS lead to persistent improvement of LUTS in patients with MS.

PTNS in neurological patients

The majority of publications regarding PTNS relate to idiopathic OAB. Fjorback investigated the acute effects of bilateral PTNS in a group of eight patients with MS and NDO. Bilateral stimulation was used to maximize the number of depolarized afferent tibial nerve fibres. Slow fill cystometry was performed in these patients to confirm DO, and this was then repeated during stimulation with PTNS. The second cystometry during PTNS however failed to demonstrate suppression of either phasic or terminal DO in these patients [58].

Kabay et al. studied PTNS in neurological patients [55, 57, 61]. In one study of 29 patients with MS and proven DO, the acute effects of PTNS were investigated. A 50% increase in the bladder volume at first involuntary detrusor contraction (1st IDCV) was seen in 18 patients during PTNS, from a mean of 138 to 230mls. A 50% improvement in the MCC was also seen in 14 patients with a mean increase from 194 to 286mls during stimulation [57]. Stimulation was applied in 200 microsecond pulses at a pulse rate of 20 Hz. Kabay et al went on further to study the effects before and after 12 weeks of PTNS, again in patients with MS. Nineteen patients were recruited, and PTNS was delivered in the same manner as the previous study. The intensity setting was chosen as the intensity immediately under the threshold for determining motor contraction. The stimulation amplitude was set at the maximum tolerable level according to the individual subject. It was again shown that there were significant increases in the mean volume at 1st IDCV from 124 to 218mls after PTNS, and MCC improved from 200mls to 267mls after PTNS. There were also significant improvements in the PdetQmax (cmH20) from baseline at 36mls to 25mls after PTNS.

Kabay et al also examined the acute urodynamic effects of PTNS in a group of 32 patients with Parkinson's disease with DO. Again electrical stimulation at 200μs pulses at a rate of 20Hz were applied, at the amplitude that was most tolerable for evoking toe flexion or fanning. During stimulation the mean volume at first intradetrusor contraction (IDCV) increased from 145ml to 245ml, whilst the mean MCC increased from 205ml to 301ml.

As mentioned above out of the few PTNS studies reporting on patients with neuropathic bladder dysfunction, seven have included patients with Multiple Sclerosis (MS) [51, 55-60]. It has been reported that between 50% to 100% of all patients with MS will complain of LUTS and poor QOL [62] [63]. As the neurological pathology progresses, voiding dysfunction may worsen and more resistant to treatment. This can lead to more severe DO, poor bladder emptying, voiding difficulties, recurrent urinary tract infections, which can ultimately trigger further relapses causing spasticity, immobility, and cognitive impairment [57].

As with idiopathic OAB, the treatment options are limited. Neurological patients also often fail to respond to drug therapy. In addition the evidence for SNM for patients with MS is weak and is not recommended [64]. It is thought that surgical implantation of such a device may also be futile as patients are prone to neurological relapses at which stage the device loses its efficacy.

The availability of a non-invasive, easily applicable, low risk treatment such as PTNS for patients with MS would be significantly beneficial. The experience of PTNS in these patients needs further study and is the focus of this study.

Aims and objectives

The aim of this study was to assess safety and efficacy of PTNS in patients with reporting OAB symptoms attending a tertiary neurological referral centre.

The objectives of the study were to prospectively evaluate the efficacy, safety and impact on quality of life of PTNS in patients with a heterogenous cohort of neurological disease including MS and idiopathic OAB with LUTS refractory to other therapies such as antimuscarinic therapy. Idiopathic and neuropathic groups of patients were also compared for any difference in response to PTNS.

The patient population included those that were attending the outpatients in the Department of Uro-Neurology, which serves a heterogenous group of neurological patients. In June 2012, the department began offering PTNS as a service. This service prospectively collected data through an audit and there were two patient groups within this study;

1. Patients with idiopathic OAB, or
2. Patients with neuropathic OAB secondary to Multiple Sclerosis or other neurological diseases.

This study followed up patients who were treated with PTNS and compared the clinical response to this treatment between these groups. Factors that predict whether patients return for top-up treatments were also analysed.

MATERIALS AND METHODS

The National Hospital for Neurology and Neurosurgery is a tertiary referral centre for patients with all neurological conditions. Patients seen in the Department of Uro-Neurology are offered a range of therapeutic options including medications, intradetrusor injections of BTX-A and SNM. A nurse led clinical service offering PTNS was introduced in June 2012.

PTNS was conducted in an outpatients setting in a single centre over the period of 18 months (June 2012 to December 2013). Consecutive patients with refractory OAB, regardless of primary pathology, were offered PTNS after appropriate counselling with department information leaflets. Patients were excluded if they were unable to attend weekly appointments, or diagnosed to have a urinary tract infection with urine dipstick testing.
Patients were reviewed and assessed by a clinician in the outpatients, and suitable patients for PTNS were selected. Patients attended weekly for their treatments, and completed bladder diaries and symptom questionnaires at baseline and end of study.

The procedure consists of introducing a 34 gauge needle inserted at 60 degree angle approximately 5 cm cephalad to the medial malleolus and slightly posterior to the tibia. PTNS lead connected to the Urgent PC stimulator (Urgent PC, Uroplasty, Minnetonka, USA Surface electrode is placed on the ipsilateral calcaneus, 20 Hz) and a current strength ranging from 0.5 to 9 mA is delivered based on an individual subject's motor (flexion of the big toe or fanning of the toes) and sensory responses across the sole of the foot. Patients were only included into the study if they had a positive sensory and/or motor response to the stimulation

in the feet. This is important as a previous study has demonstrated that efficacy is associated with an appropriate presence sensory/motor response [59].

The standard protocol consists of once weekly, 30-minute sessions of stimulation over a twelve-week period. .

Questionnaires

Many questionnaire tools exist to evaluate the severity of the different aspects of LUTS. Some are designed to assess OAB symptoms, others are more focused on urinary incontinence and its impact on QOL [65]. Questionnaire tools that are highly recommended by the International Continence Society (ICS) at the third consultation receive a Grade A rating [65]. This rating suggests that evidence has demonstrated the validity, reliability and responsiveness to change following standard psychometric testing. The questionnaire tools chosen for this study are the ICIQ-OAB (which is based on the ICS*male* and BFLUTS) and the ICIQ-LUTSqol which is based on the King's Health Questionnaire, both of which have received grade A rating. The different types of ICIQ modular questionnaires that have received grade A rating are listed in the figure three below [65, 66]:

Figure three: Enlists the questionnaire tools that have received grade A recommendation from the third ICI.

Fully validated modules, derivations and recommendation grade from third ICI

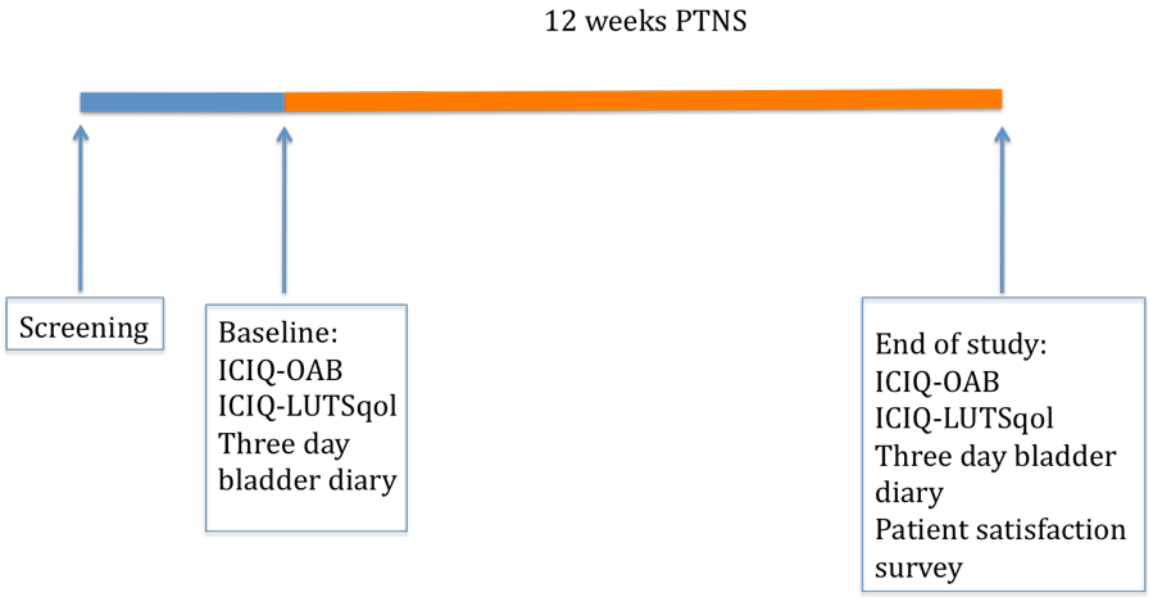
Module Name and Derivation	Assessment Area	ICI Recommendation Grade
ICIQ-MLUTS (ICS*male* Short Form[8])	Urinary symptoms (male)	A
ICIQ-FLUTS (BFLUTS Short Form[9])	Urinary symptoms (female)	A
ICIQ-UI Short Form[1]	Urinary incontinence short form	A
ICIQ-N (ICS*male*[2]/BFLUTS[3])	Nocturia	A
ICIQ-OAB (ICS*male*[2]/BFLUTS[3])	Overactive bladder	A
ICIQ-MLUTS Long Form (ICS*male*[2])	Urinary symptoms long form (male)	A
ICIQ-FLUTS Long Form (BFLUTS[3])	Urinary symptoms long form (female)	A
ICIQ-LUTSqol (KHQ[4])	Urinary symptoms QOL	A
ICIQ-UIqol (I-QOL[5])	Urinary incontinence QOL	A
ICIQ-OABqol (OABq[6])	Overactive bladder QOL	A
ICIQ-Nqol (N-QOL[7])	Nocturia QOL	Not incontinence
ICIQ-MLUTSsex (ICS*male*[2])	Sexual matters related to urinary symptoms (male)	A
ICIQ-FLUTSsex (BFLUTS[3])	Sexual matters related to urinary symptoms (female)	

Study timeline

The duration of treatment varied in previous published studies, from between 4 to 12-weeks. This study design included a 12-week treatment regime to be comparative with the SUmiT study, which is the largest placebo controlled study to date.

The first 20 patients completed questionnaires and bladder diaries at baseline, week 4, 8 and 12. After an initial analysis, it was evident that patients were experiencing benefit beyond 8 weeks upto 12-weeks, and so the remainder of patients continued to complete 12-weeks of treatment as art of the study design. Questionnaire tools and bladder diary collection was performed at baseline and end of study (week-12). The study timeline is illustrated in figure four below.

Figure four: Study timeline.



Primary Outcome

The change in the ICIQ-OAB and ICIQ-LUTSqol questionnaire scores, and the 3-day bladder diary parameters over twelve weeks of treatment was the primary outcome. Outcomes were the change in OAB symptoms, LUTS-related quality of life, and individual symptoms (urinary urgency, 24-hour frequency, daytime frequency, night-time frequency, leakage episodes and severity) as assessed on the bladder diary at week-12.

Secondary Outcome

Secondary outcomes were patient satisfaction and request to continue with the treatment, as measured by a more subjective Satisfaction Survey administered at the end of treatment at week-12. Sub-analyses were performed to identify the factors that could predict which patients would opt for top-up treatment after the study. Idiopathic and neuropathic groups of patients were also compared for any difference in response to PTNS.

Reviewing the literature, various studies use completely different definitions of a successful response. Some studies suggest the use of ≥50% improvement in OAB symptom scores, whilst others make use of the patient reported GRA. Some discussion focuses on whether a mean improvement in scores of ≥50% actually correlates with patient satisfaction. Therefore we included the use of a satisfaction survey, which included a question of whether the patient would be interested to continue the treatment by attending for further top-up treatments. This would allow us to identify those who responded to therapy.

Subjects reporting benefit at 12-weeks were invited to return for top up PTNS sessions based upon self-reported recurrence of OAB symptoms. Subjective reporting using patient reported outcomes (PRO) has been recommended in the evaluation of treatments for OAB, as this is a symptom syndrome [67]. The diagnosis and management almost entirely judged by patient's self reporting on symptoms and their impact on their QOL and well-being.

Paired t-tests were used to see whether baseline levels in each factor differed from 12-week measurements. Count data was compared using chi-squared tests or Fisher's exact test as appropriate. The distribution of continuous variables was examined and Students t-tests/ANOVAs or Kruskal Wallis tests/Mann Whitney U tests were applied as appropriate. Linear regression was used to explore the relationship between baseline factors and the change in OAB symptoms and logistic regression analysis was used to identify the relationship between pre-specified variables and coming for a top-up. Data analysis was done using Stata 13.1. All statistical tests are two-sided and a p-value<0.05 was considered to be significant.

RESULTS

Of 74 consecutive patients (22 male, 52 female; mean (95% CI) age 56.0 (52.2, 59.8) years) 25 (33.8%) had idiopathic OAB and 49 had neuropathic OAB. Of the neuropathic group, 19 (25.7%) had multiple sclerosis (MS) and 30 (40.5%) had other neurological conditions enlisted in table 3 below. As can be seen from the flow chart below, sixty-four (86%) patients completed 12-weeks of treatment. There were no statistically significant differences between the diagnosis groups in age, gender or baseline questionnaires scores (see table four).

Figure five: Flow chart illustrating the patient pathway for the study.

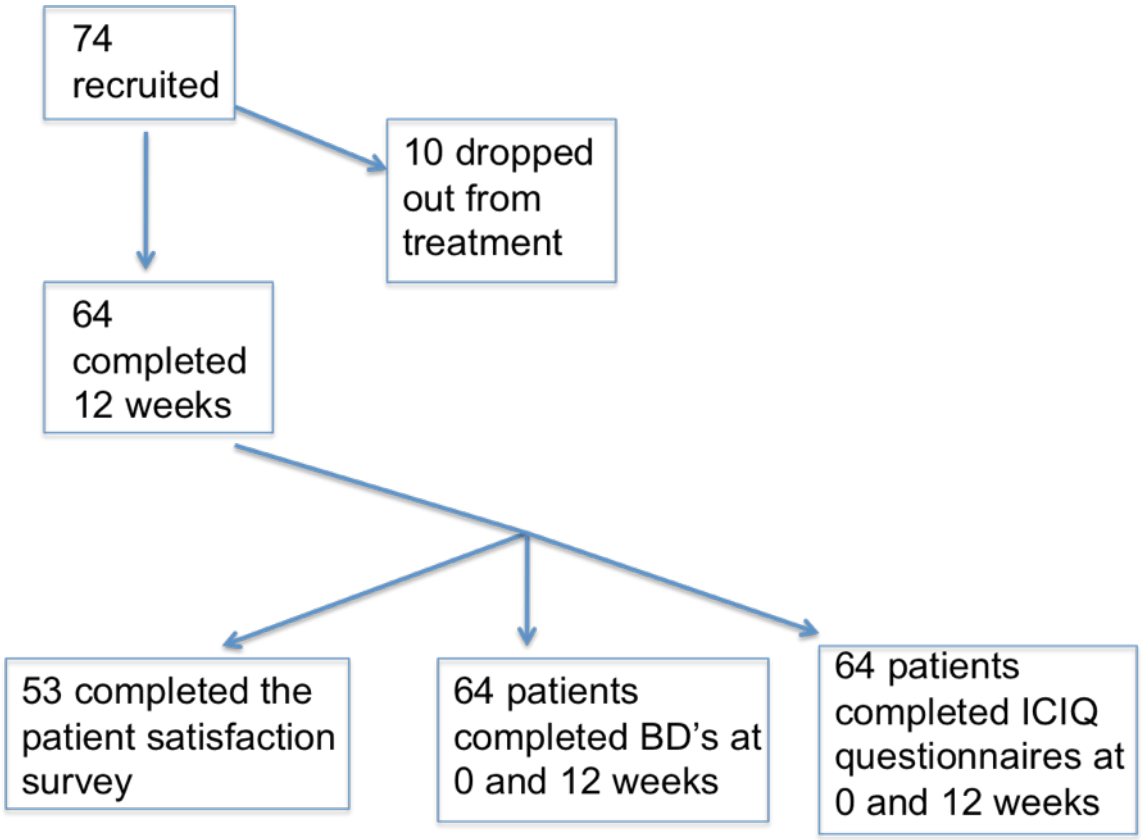


Table three: The spread of diagnoses in patients receiving PTNS.

Diagnosis	**Number of patients**
Idiopathic	25
Multiple sclerosis (MS)	19
Parkinson's disease	6
Fowlers syndrome	4
Parkinsonian syndrome	1
Transverse myelitis	1
Adrenomyeloneuropathy	2
Adrenomyeloneuropathy carrier	1
Carnitine palmitoyltransferase II (CPT II) deficiency	1
Limbic encephalitis and subdural haematoma	1
Poliomyelitis	1
Cauda equina, lumbar decompression	1
Cervical and lumbar stenosis	1
Peripheral neuropathy, lumbar canal stenosis	1
Pure autonomic failure	1
Familial dysautonomia, central sleep apnoea	1
Friedrichs ataxia	1

Spinal cerebellar ataxia type 3	1
Alpha-actin deficiency	1
Myasthenia gravis	1
Systemic lupus erythematosus with cerebral involvement	1
Epilepsy	1
Post-traumatic epilepsy, migraine	1
Total	74

Table four: Baseline demographics according to the disease group of patients attending PTNS (n=74).

Variable	Overall N=74	Idiopaths N=25	Neuropathic OAB N=49	P-value
Gender; n male (%)	22 (29.7%)	6 (24.0%)	16 (32.7%)	0.74
Age; mean (SD)	56.0 (16.1)	58.3 (17.1)	53.7 (15.1)	0.65
ICIQ-OAB total score; mean (SD)	40.4 (9.8)	41.1 (11.6)	40.2 (8.9)	0.91
ICIQ-LUTSqol total score; Mean (SD)	187.3 (51.3)	186.9 (53.0)	187.6 (49.5)	0.97
24h bladder freq (BD) score; Mean (SD)	11.7 (3.9)	12.6 (4.1)	10.8 (3.8)	0.39
Mean bladder urge (BD) score; Mean (SD)	2.5 (1.0)	2.6 (1.3)	2.3 (0.9)	0.30
Mean no. leakages (BD); mean (SD)	3.3 (4.9)	3.5 (5.9)	3 (3.8)	0.57
Bladder leakage severity (BD); mean (SD)	0.9 (0.9)	0.8 (0.9)	1.0 (0.8)	0.72

MS: Multiple Sclerosis

BD: Bladder diary

Values expressed as mean (SD)

Mean bladder urge: 0; nil, 1;mild, 2; moderate, 3; severe urge

Bladder leakage severity; 0; mild, 1; moderate, 2; severe leakage severity

The table above shows no significant differences for the baseline parameters between the three patient groups. P-values compare the symptom scores at baseline for each disease group.

Table five: Shows the comparison of the mean sub-scores for individual domains on the ICIQ-OAB and ICIQ-LUTSqol at baseline.

Parameter	Overall	Idiopathic OAB	Neuropathic OAB	Range
Mean Urge (ICIQ-OAB subscore) (SD)	2.7 (0.9)	2.8 (0.9)	2.7 (0.9)	0: never 1: occasionally 2: sometimes 3: most of the time 4: all of the time
Mean Urge leakage (ICIQ-OAB) (SD)	2.3 (1.1)	2.5 (1.3)	2.3 (1.0)	0: never 1: occasionally 2: sometimes 3: most of the time 4: all of the time
Mean Frequency (ICIQ-OAB) (SD)	1.9 (1.2)	1.8 (1.2)	2.0 (1.3)	0: 1 to 6 times 1: 7 to 8 times 2: 9 to 10 times 3: 11 to 12 times 4: 13 or more times

Mean Nocturia (ICIQ-OAB) (SD)	2.6 (1.1)	2.8 (1.1)	2.5 (1.1)	0: none 1: one 2: two 3: three 4: four or more
ICIQ-OAB A; mean (SD)	9.6 (2.5)	10.1 (2.9)	9.3 (2.3)	0-16
ICIQ-OAB B; mean (SD)	30.6 (8.2)	30.9 (9.4)	30.5 (7.8)	0-40
OAB Symptoms (ICIQ-OAB total A+B); mean (SD)	40.4 (9.8)	41.1 (11.6)	40.1 (9.0)	0-56
ICIQ-LUTSqol A; mean (SD)	56.6 (11.0)	56.6 (11.1)	56.6 (11.1)	0-76
ICIQ-LUTSqol B; mean (SD)	130.2 (41.8)	130.3 (43.2)	130.2 (41.8)	0-200
ICIQ-LUTSqol total (A+B); mean (SD)	186.9 (48.5)	186.9 (53.0)	186.9 (47.1)	0-276

ICIQ question format [66]

A: assesses the presence or absence of a symptom and its severity

B: is a separate scale to assess associated bother

BD: bladder diary

SD: Standard deviation

For all patients combined, significant improvements were noted after 12 weeks of treatment with mean decrease in total ICIQ-OAB scores of -5.1 (SD: 10.4) (p=0.003), total ICIQ-LUTSqol scores -22.4 (SD: 44.3) (p=0.001), and reduced bladder diary parameters of 24-hour urinary frequency -1.2 (SD: 2.2) (p=0.001), number of incontinence episodes -0.4 (SD: 1.9) (p=0.16) and severity -0.2 (SD: 0.4) (p=0.004) of incontinent episodes. P values have been calculated using paired ttests. This is shown in table six below.

Table six: The mean change in the measured parameters from baseline to 12 weeks for all patients.

Variable	N	Mean baseline level (SD)*	Mean week 12 level (SD)	Mean change from baseline to week 12 (SD)	P-value
OAB symptoms (ICIQ-OAB)	43	40 (9.7)	34.9 (13.2)	-5.1 (10.4)	0.003
Quality of life (ICIQ-LUTSqoL)	46	188.5 (47.9)	166.1 (58.9)	-22.4 (44.3)	0.001
24h freq**uency (BD)**	39	11.6 (3.8)	10.3 (3.7)	-1.2 (2.2)	0.001
Urge **(BD)**	40	2.5 (1.0)	2.3 (1.0)	-0.2 (0.8)	0.16
Number of incontinent episodes (BD)	37	3.3 (4.6)	2.9 (4.5)	-0.4 (1.9)	0.16
Incontinence severity (BD)	38	0.9 (0.8)	0.7 (0.8)	-0.2 (0.4)	0.004

* Only including individuals with follow up values available

BD: Bladder Diary

ICIQ: International consultation on incontinence questionnaire

Table seven: Absolute changes in key factors: overall and according to disease group.

Variable	Overall N=74	Idiopaths N=25	All neuropathic OAB N=49	MS N=19	Other Neurological conditions N=30	P-value
ICIQ-OAB total difference; median (IQR)	-4.8 (-14.2, 3)	-5.5 (-19, 3.5)	-4 (-9.3, 2.5)	-7 (-11, -1)	-1 (-7.5, 6)	0.19
ICIQ-LUTSqol total difference; median (IQR)	-25 (-57, 11.4)	-16 (-62, 18)	-34 (-53, 4.8)	-57 (-79, 3)	-11 (-27, 6.5)	0.35
24h bladder freq Difference (BD); median (IQR)	-1.3 (-3.3, 0.4)	-1.7 (-3.7, 0.3)	-0.85 (-2.8, 0.4)	-1.7 (-3, 0)	0 (-2.5, 0.7)	0.50
Mean bladder urge difference (BD); median (IQR)	-0.2 (-0.8, 0.2)	-0.3 (-1.1, 0.1)	0 (-0.4, 0.2)	0 (-0.5, 0.1)	0 (-0.3, 0.3)	0.35
Mean no. leakages difference; median (IQR)	-0.5 (-2, 0.1)	-0.7 (-3, 0)	-0.2 (-1, 0.2)	0 (-0.7, 0)	-0.3 (-1.3, 0.3)	0.36
Bladder leakage severity difference; median (IQR)	-0.1 (-0.3, 0.1)	0 (-0.2, 0)	-0.2 (-5.5, 0.1)	-0.4 (-0.8, 0)	0 (-0.3, 0.1)	0.09

BD: Bladder diary

IQR: Inter-quartile range

Table seven above shows the absolute changes for the ICIQ questionnaires or bladder diary parameters. The improvement in symptoms were not significantly different between the three individual groups by 12 weeks suggesting the treatment was as efficacious in each patient group. There was a trend for the MS group to improve more than the other neuropathic patients, from the change in ICIQ-OAB and ICIQ-LUTSqol scores.

For percentage improvements, all patients combined (both idiopathic and neuropathic OAB), regarding the ICIQ-OAB subscores, there was a 27 % improvement in urgency and frequency at 12 weeks compared to baseline, 20.8 % improvement in nocturia, and 24 % improvement in the total ICIQ-OAB, whilst there was a 21.5 % improvement in the total ICIQ-LUTSqol score.

Continuation of treatment with top-ups

After the first twenty patients had completed 12-weeks of treatment, we included the patient satisfaction survey, which included the question whether patients would opt to continue for top-up therapy. Thirty-five out of fifty three patients (66 %), opted to continue treatment after completing 12-weeks. Twenty-four out of fifty-three patients actually returned (45%) and eight had idiopathic OAB, and 16 neuropathic OAB. The mean top-up interval was 44.4 days (7-155 days), with a mean top up frequency of 1.1top ups/month. It has been described in the literature that once PTNS treatment has stopped, symptoms regress. It is not clear when they regress and but some studies have reported recurrence at 6-weeks to 3-months post end of treatment [52, 54]. This study correlates with the literature and maintenance treatment is clearly necessary for continued benefit.

Patients reporting less of an improvement in ICIQ-LUTSqol, ICIQ-OAB, and less improvement in leakage severity had significantly lower odds of returning for top-up therapy. MS patients were 2.92 (95% CI: 0.85, 10.09) times more likely to return back for top ups ($p=0.01$) compared to those with other neurological diagnoses.

Table eight: Logistic regression analysis investigating the relationship between baseline factors and changes in key variables and returning for top-ups.

There were a total of 24/53 (45%) people who came back for topups. An Odds ratio of >1 suggests a patient is more likely to return for a top-up with an improvement in this parameter. Any factor with a value of <1 means they are less likely to return for a top-up.

	Unadjusted Analysis		**Adjusted Analysis**	
Variable	**Odds ratio (95% CI)**	**P-value**	**Odds ratio (95% CI)**	**P-value**
Diagnosis;		0.01		0.04
Idiopathic	Ref		Ref	
MS	2.92 (0.85, 10.09)		10.90 (0.43, 274.63)	
Other Neuro	0.43 (0.12, 1.52)		0.19 (0.03, 1.18)	
Age; (per 5 year increase)	0.91 (0.78, 1.06)	0.22	-	-
Gender; (female versus male)	1.04 (0.36, 3.03)	0.94	-	-
Baseline ICIQ-OAB; (per 1 unit increase)	1.00 (0.99, 1.01)	0.95	-	-
Baseline ICIQ-LUTSqol; (per 1 unit increase)	0.86 (0.73, 1.02)	0.09	-	-
Baseline 24h bladder freq; (per 1 unit increase)	1.03 (0.76, 1.40)	0.83	-	-
Baseline mean bladder urge; (per 1 unit increase)	1.00 (0.56, 1.79)	0.99	-	-
Baseline mean no leakages; (per 1 unit increase)	0.93 (0.80, 1.09)	0.38	-	-
Baseline leakage severity; (per 1 unit increase)	1.22 (0.57, 2.57)	0.61	-	-
ICIQ-OAB change; (per 1 unit increase)	0.93 (0.87, 0.99)	0.03	1.01 (0.90, 1.12)	0.86
ICIQ-LUTSqol change; (per 1 unit increase)	0.98 (0.96, 0.99)	0.007	0.96 (0.94, 0.99)	0.02
24h bladder freq change; (per 1 unit increase)	0.95 (0.71, 1.28)	0.75	-	-
Mean bladder urge change; (per 1 unit increase)	0.38 (0.13, 1.10)	0.07	-	-
Mean no leakages change; (per	0.81 (0.54, 1.21)	0.30	-	-

1 unit increase)				
Leakage severity change; (per 1 unit increase)	0.93 (0.87, 0.99)	0.03	-	-

Satisfaction survey

Fifty three patients completed the PTNS satisfaction-survey, patients found treatment comfortable on a 7-point Likert scale (1-poor 7- high), the mean comfort score was 5.98±1.15, mean symptom improvement was 3.96± 2.15, mean treatment satisfaction was 4.36±2.45, mean recommendation to a friend score of 5.71±1.69).

Table nine: Results of the patient satisfaction survey.

Parameter	Satisfaction Score (7-point Likert scale (1-poor, 7- high))
Comfortability of treatment	6.0
Recommend treatment to a friend	5.7
Mean symptom improvement	4.0

Adverse effects

No significant adverse effects were reported. Five patients experienced mild discomfort at the site of needle insertion, however this did not affect the compliance to the treatment.

DISCUSSION

PTNS is one of the few neuromodulative techniques that has been studied through a placebo controlled, randomised phase III multicentre study. The literature suggests that once the stimulation is discontinued, symptoms tend to recur, and that OAB is a chronic syndrome that requires long-term or lifelong therapy [68]. Therefore the use of a minimally invasive, cost-effective form of intermittent stimulation, with efficacious outcomes is highly desirable. Compared to the use of BTX-A, the efficacy of which has been proven in numerous high volume, multicentre, double blind, placebo controlled, randomised trials, demonstrating the level I evidence for its use, the data on PTNS is significantly lacking. The evidence is lacking for patients with neuropathic OAB, in particular patients with MS, where there may be disease specific factors that make the OAB symptoms more aggressive and resistant to treatment.

OAB symptoms in these patients themselves can also exacerbate fatigue, spasticity, disability and mental health that possibly increases the impact of OAB and incontinence on QOL, that justifies considering these patients as a separate entity to those with idiopathic OAB.

This is one of the few studies to examine the use of PTNS in patients with neurological diseases, with only four previous studies reporting results in patients with MS [51, 55-57], and the first to compare response with patients with idiopathic OAB. At baseline this patient cohort was comparable to those patients included in the SuMIT Study, with moderate OAB, with a 24 hour urinary frequency at 12.6 and 12.4, number of daily incontinence episodes at 3.2 and 3 respectively.

For all patients in this study grouped together, significant improvements were seen for daytime frequency and the ICIQ-OAB and ICIQ-LUTSqol scores, of -1.2 (p=0.001), -5.1 (p=0.003) and -22.4 (p=0.001) respectively.

Using a subjective question at the end of 12-weeks of treatment, patients were asked if they would like to continue with treatment. Thirty-five out of fifty three patients (66%) answered they would be keen to continue with treatment, however only 24 (45%) of the patients actually returned for further top up treatments. The reason for this is unknown, however many patients were travelling far distances to reach the hospital and this is especially difficult for those with poor mobility, and reliant on patient transport to attend appointments.

Although these are small numbers, it appears that there was a trend for the absolute improvements in ICIQ-OAB and ICIQ-LUTSqol to be more evident in the patients with MS compared to the other neuropathic or idiopathic group, however this was not to significance. Furthermore patients with MS were significantly more likely to request further top-up treatment. This requires further investigation in a larger study with a higher volume of patients. This may reflect the difference in the underlying disease process between these two groups. Perhaps the interrupted neural pathways that occur in MS may be more responsive to the sacral afferent remodeling that occurs with PTNS. It is also possible that patients with idiopathic OAB have a more severe or alternative pathology that is less correctable through the mode of action of PTNS. Furthermore it may be that MS patients have less treatment expectation, due to the numerous co-morbidities that they face with their condition [69].
Baseline clinical scores were not able to predict from this cohort which patients were more likely to attend for top-up therapy. However patient performance and the response to the first 12-weeks of treatment can predict those who will return for continuation treatment.

Study Limitations

Ten patients (13.5%) were lost to follow up and did not complete the 12-weeks of PTNS treatment. It is unknown whether this was due to lack of efficacy or adverse effects of the treatment.

BD parameters did not change significantly such as the mean and maximum voided volume. There is a lack of evidence for the precision of bladder diaries and they continue to be used as an outcome measure in clinical studies of incontinence. They can be difficult to complete for patients due to the inconvenience of having to measure every single void and record every urge, leakage and volume of fluid that is consumed. From patient feedback some found the BD particularly difficult to adhere to during weekdays due to the lack of discretion available at the workplace and having to carry a measuring jug for frequent toilet visits, leading to poor diary compliance. Other patients have difficulties with memory and as a result forget to complete voiding episodes, especially at night. Those who reserve capturing BD visits for the weekend only, also have inherent inaccuracies as patients when at home on weekend have different drinking habits to when they have to leave the house during the working week, and may therefore not be a true reflection of the working week when symptoms are more problematic. For future studies, portable electronic bladder diaries have been shown to potentially be superior to paper diaries, as patients are able to input data in real-time and found these easy to use [70]. Although this still requires further investigation, it may provide a superior alternative to accurately witness to the daily bladder behaviour.

The evaluation is limited to the 12-weeks of therapy, and therefore follow up was not studied. For a true representation of clinical efficacy, patients will be followed up for another 12-months to capture the top up process. Very little information is known about the long-term efficacy of repeated treatments. Macdiarmid reported on thirty-three patients from the Overactive Bladder Innovative Therapy Trial who were offered to complete 12-months worth of PTNS [68]. Subjects received a mean of 12.1 treatments each, with a mean of 21-days between treatments. This showed that 94 and 96% of responders had sustained benefit at 6 and 12-months respectively. At end of study, 12-months, there were reported mean improvements in bladder diary parameters, increased voided volumes compared to baseline assessments, and a significantly improved OAB symptom severity score compared to baseline, 12-weeks and 6-months. This suggests that PTNS can sustain its durability through a 12-month period.

The STEP study identified that at least 75% of the patients who had a positive response to the first 12-weeks of therapy, went on to have sustained symptom improvements for up to three years with an average top up treatment of one per month [71].

The assessment tools used were largely subjective and focused on PRO measures. From the literature, the definitions of successful PTNS treatment have differed amongst the various authors, and therefore more difficult to compare studies. There currently is a lack of formal guidance to judge OAB treatment response. A systematic-review examined 75 clinical trials that evaluated behavioural, medical or neural stimulation treatments for OAB [72]. Considerable heterogeneity was evident between these studies. Some authors used percentage improvements regarding a threshold of 50-100% as a response, whilst others used absolute changes in symptom frequency, or PROs that measured general satisfaction or symptom bother. There was also heterogeneity among patients for what constitutes a treatment success

or failure. PROs are important however as they can demonstrate whether an observed change is clinically meaningful to the patient.

Cystometry was not performed on patients after starting treatment to ascertain objective changes objective in urodynamic parameters, however the experience from the literature is that whether DO appears to diminish or not, urodynamic parameters may not necessarily correlate with clinical findings. The role of urodynamics prior to recruitment for this treatment is unclear, however it has been shown that patients not showing DO at baseline seem to be more likely to respond to treatment [47]. Clinical and quality of life parameters are arguably the more relevant endpoints to study. Without urodynamic data, it is also harder therefore to describe the severity of the pathology and whether patients had high pressure DO or DSD, which may make their condition more refractory to treatment.

Since there was no sham treatment offered, positive results could be argued to be from placebo effect. The study design was a prospective clinical evaluation of PTNS for a series of patients with heterogenous pathology, all suffering from refractory OAB symptoms. Attempts were made to exclude the synchronous effects of other adjunct treatments such as physiotherapy and antimuscarinics, which were not used during the study due to potential confounding. In the placebo controlled studies, the response rate of the placebo arm of 20.9% [40] is much lower than this response rate of 66%. Also with the chronicity of the symptoms experienced by these patients, who had all failed previous therapies before trying PTNS, it is possibly less likely that they would have a profound placebo effect. However to validate this data properly with this group of patients a placebo controlled study is warranted.

One of the disadvantages of PTNS is the need for repeated insertion of the stimulation electrode and regular outpatient visits, which will have to involve long-term compliance for sustained clinical efficacy. This may affect patient suitability as some patients may find the weekly trips very difficult, especially if they have poor mobility and need to travel long distances.

Despite these limitations, these results are in line with previous studies examining the efficacy of PTNS in non-neuropathic patients. Although there were still relatively small numbers of patients in this study, the use of a validated questionnaire should allow comparison of these results with others. Compared to the SUmiT trial, from which 54.5% of patients self-reported moderate to markedly improved symtoms, this study has shown 45% of patients who felt that improvement was significant enough to warrant attendance for further continuation therapy.

Results from a systematic review evaluating PTNS for the OAB suggested that 54-79% of patients are 'successfully treated' [73]. Once again the definition of success varied among these studies from the use of urodynamic data to clinical and QOL measures.

Compared to the adherence to antimuscarinic therapy, which has been estimated to be at 40%-58%, and 17%-35% at 6 and 12 months respectively [74], this study shows that PTNS may perform favourably, with 45% of patients willing to continue with therapy at 3 months. The efficacy of PTNS compared with antimuscarinic therapy has already been demonstrated to be equivalent from the OrBIT Study [42].

There was a minimal adverse effect profile in this study with five patients complaining of mild discomfort at the needle insertion site, however this did not affect their compliance to

therapy. This correlates with the previous literature where no major complications have been reported [73].

These results re-confirm the safety of PTNS as a treatment option, and this may represent a reliable form of 2nd line management for patients who fail to respond to OAB medications. There were no serious adverse events with this treatment, and only mild discomfort with the treatment, with most patients regarding this as a highly comfortable and convenient treatment. With regards to cost-effectiveness, a report by Martinson et al disclosed that the projected three-year costs for the following treatments are $7,565 for PTNS, $11,748 for onabotulinumtoxinA, $16,830 for augmentation cystoplasty and $24,681 for SNS [29]. This is another reason why PTNS should be studied further to judge its efficacy, as it may perform more favourably in a National Health Service model for care provision. There is evidence to suggest that combination treatment of the use of anticholinergics concomitantly with PTNS offers even further efficacy in patients with idiopathic DO [45]. This also needs to be studied in a neuropathic population.

As with other medical therapies, it is recognised that for sustained symptom benefit, the treatment needs to be continued. There is no one-off definitive treatment or cure for the OAB. Behavioural modifications, antimuscarinic therapies, SNM, onabotulinumtoxinA all need require repeated treatments, and PTNS is no exception. It seems appropriate to allow patients to recognize any deterioration in their symptoms to guide them when to plan the next top-up treatment. No standardized long-term protocol has been established as yet, and currently it is being personalized and tailored to the needs of each individual patient.

Future studies

There is clearly a place for PTNS in the management ladder for OAB, although investigation does need to take such as how to best define a responder or a treatment success with this treatment.

Is there a role for combination therapies alongside antimuscarinic agents or the emerging beta-agonist medications? It could be argued that PTNS could be offered to patients before antimuscarinic use due to the unwanted side effects of these medications. Head to head studies of PTNS versus SNS could be conducted, as PTNS is emerging to be the more economical and least invasive form of therapy.

Other forms of Tibial Nerve Stimulation are also being developed, such as transcutaneous tibial nerve stimulation. The evidence for this is in its infancy and the European Urology Guidelines on incontinence report the evidence for this is lacking and is required. This would address the few disadvantages that PTNS entails, such as the need for repeated visits, and the discomfort with the needle placement, and if its efficacy is proven, it could offer an even more convenient management option [31, 75].

The role of a prognostic marker would be of use to identify patients who are most likely to do well from this treatment before starting a 12-week course of therapy. PTNS may be more suited to those patients with mild to moderate symptoms, compared to those with a moderate to severe burden. This requires further investigation, along with a prolonged period of follow up of patients to assess the long-term clinical efficacy.

CONCLUSION

Objective and subjective findings suggest that PTNS was effective for patients with neuropathic OAB and comaparable to idiopatic OAB. Thirty-five out of fifty-three patients, after 12-weeks of therapy, were keen to pursue further top up treatments. The patients with neuropathic OAB secondary to MS were significantly more likely to attend for top-up therapy. This confirms the efficacy of this form of treatment in a diverse group of patients with a heterogenous mix of disease aetiology.

PTNS is a promising treatment alternative for neuropathic patients with OAB, especially MS, and as time goes on, efforts could be to optimise the efficiency of electrical delivery to further improve efficacy.

Further multicentre study is required in a larger volume of patients to also investigate prognostic predictors of success, and the role of combination therapies.

PTNS is a well-tolerated, minimally invasive, readily applicable therapy with a minimal adverse profile compared to the alternatives of antimuscarinic therapy, BTX-A and SNM. This prospective study supports the data that was reported in the SUmiT study. This would therefore make it a much more acceptable treatment to patients.

CHAPTER TWO

Chapter Synopsis

Chapter two explores further the challenge raised in Chapter one regarding the need for an objective assessment tool for the OAB, especially for patients with neurological conditions who may have significant lower urinary tract dysfunction, with altered bladder sensation and motor function. Patient reporting outcomes (PRO) are an important part of the assessment, however there are inherent inaccuracies using this form of evaluation alone. Patient symptoms may not reflect the true severity of the disease process, and may also be influenced by placebo effect. An accurate urinary biomarker for the OAB would be easy to collect and greatly beneficial for patients:

- to assist diagnosis, to guide treatment choice
- to objectively monitor and quantify responses to treatment (to help define a treatment responder)
- to research and evaluate the mechanism of action of various treatments (especially new treatments such as PTNS)
- to identify any placebo effect with new treatments
- to predict response to treatment
- to inform clinician and patient regarding the disease prognosis
- to highlight the patients at higher risk of poor bladder compliance and unsafe detrusor pressures.

The urinary biomarkers that are currently being evaluated in practice are the neurotrophins. These have been explored by numerous studies recently with growing interest, and the aim of

this chapter is to perform a literature review of the two main urinary neurotrophins, known as Nerve Growth Factor and Brain Derived Neurotrophic Factor (NGF and BDNF) as biomarkers for the OAB.

A review of Urinary Neurotrophins as a biomarker for Overactive Bladder

INTRODUCTION

Subjective and Objective markers for assessment of the Overactive Bladder

The European Association of Urology guidelines recommend that accurate assessment of patient's symptoms require subjective and objective quantification of LUTS [31]. As discussed in the previous chapters, objective assessment tools can also be of use to evaluate the efficacy of a particular treatment, and to compare therapies with each other.

The well known phrase 'the bladder is an unreliable witness' still rings true today [76, 77]. Whilst the range of urodynamic dysfunction is large, the types of bladder symptom that can manifest are limited, to either storage or voiding LUTS. Therefore it remains difficult to assign symptoms to a particular disease process. For example, poor detrusor contractility may manifest with the same symptoms as bladder outlet obstruction such as hesitancy, urinary frequency, reduced urinary flow and sensations of incomplete bladder emptying. Despite a careful history, patients may interpret their own experiences differently and clinician's may be biased with interpreting their answers [78].

Questionnaires – Patient Reported Outcomes

The questionnaires used to assess symptoms and the impact on QOL, are a form of patient reported outcome (PRO), as the outcome originates directly from the patient, without interpretation by clinicians or anyone else. The aim of the PRO is to accurately assess how a

patient feels in relation to a disease or treatment. It was considered that subjective assessments can provide valuable information on aspects of disease that impact most on the patient's life and are most bothersome. Health related QOL outcomes area particularly valuable for chronic conditions, where the bother and distress can affect daily function [79].

PRO measures are therefore promoted for clinical trials and clinical practice. In addition, the more objective measures such as bladder diary variables and urodynamic parameters may not be so strongly associated with improvements in subjective measures such as treatment benefit, symptom bother and patient satisfaction [80]. If a treatment provides reductions in frequency, urgency and urge incontinence, yet these do not improve the health related QOL, whether the treatment can be considered clinically effective remains questioned. Patients must be able to perceive an improvement in well-being and daily functioning using a valid PRO measure.

Up until the development of the ICIQ modular questionnaires, there was a lack of standardisation of the multiple PRO assessments available [81]. Many of the PRO instruments were for patients with urinary incontinence, and few designed for those with OAB [82]. The decision to construct the modular ICIQ questionnaire took place after the first ICI meeting in 1998.

Questionnaires have been used for some time to attempt quantification of disease severity, assess symptoms, document response to therapy, and to compare effectiveness of various interventions. However these are not perfect tools, and LUTS have been notoriously difficult to accurately capture with them.

Symptoms including urinary incontinence, may be interpreted as severe by a questionnaire, however the tool may not clearly extract the extent to which it can impair an individuals quality of life. For example the QOL implications for a groups of patients scoring the sample

symptom questionnaire score may widely differ. Patient understanding may also affect the way they interpret questions, each in an individual manner, and their answers can also be affected by recall bias. Perceptions of urinary urgency especially can be difficult to standardize, and yet this is a pivotal symptom that underpins the OAB [82].

A questionnaire that is comprehensive, yet not lengthy is an issue that developers are often faced with [83]. However on the other hand, simplicity and brevity can risk lack of sensitivity and loss of qualitative description.

Likewise questionnaires such as the International Prostate Symptom Score, designed for a particular group of patients may not necessarily transfer for a similar situation in a different population, such as assessing men and women, or evaluating patients with idiopathic OAB and neurogenic OAB [84]. The former group of patients being distinct from the latter who may have a deficit of afferent nerve function and hence altered bladder sensations or may even be self-catheterising.

Responses to various treatments should be captured with a PRO, and represent not only a measurable change, but also one that is meaningful to the patient. This is known as the minimal important difference. Changes can be statistically significant, but this may not imply a meaningful difference to the patient [82].

Patient perceptions are another consideration as these are shaped by a number of factors such as age, sex, ethnicity, their family or caregiver's perceptions and these can in turn affect treatment expectations and consequently impact treatment outcomes [79]. Their experience with previous illnesses such as multiple sclerosis can influence their perception of symptom impact on QOL and treatment expectations. Expectation can affect treatment outcomes, and are therefore thought to be the basis for the placebo effect. This can motivate and

psychologically condition a patient to observe certain symptoms and ignore others [85]. Little is known about the expectation of OAB treatment and PRO measures, but evidence suggests realistic expectations are important for treatment satisfaction.

Bladder diaries

Bladder diaries (BD) are an integral aspect of the assessment with OAB, and their use recommended by the EAU [31]. They can record the day and night frequency of urinary episodes, the volumes voided, note the frequency and severity of urgency and incontinence episodes, and provide information about fluid intake.

However BDs are not as yet validated for use in the clinical or research setting, as with PRO symptom questionnaires. Only most recently has a validated format has been reported [86]. BDs can have inaccuracies and although valuable, do not represent a tool that is flawless.

Bladder diaries can miss the more infrequent symptoms such as urge incontinence if it did not occur within that particular three-day period. During the three-day diary collection patients are aware that their behaviour is being monitored by a medical team, and may adopt their behaviour and habits to prevent the occurrence of incontinence, such as fluid modification, and voiding more frequently to prevent the bladder reaching higher capacities.

Patients may complete these diaries at the end of the day, as it is inconvenient to keep a real-time record due to work commitments and the practicality of carrying around a measuring jug, from which the record may be fraught with inaccuracies due to poor patient recall.

Measures such as pad usage can be difficult to interpret as a marker of severity of incontinence, which is a very subjective symptom. Some patients may change pads when only slightly damp, whilst others tolerate a higher volume of leakage.

Patients may misinterpret nocturia, and wake up at night due to leg cramps or pain or a plethora of other reasons and take that opportunity to void and record it, rather than it being

the sensation of urgency urinary that wakes them from sleep.

Due to the inaccuracies of these assessment tools, effort has been invested to develop and validate the bladder diary instrument [86] .

Urodynamic Study

Traditionally it has been recommended that pressure flow studies, are a more objective measure, and are important in the evaluation of LUTS if any surgical interventions are being considered. The physiological assessment of the lower urinary tract to assess function and dysfunction has been used for many years. The different types of urodynamic studies are defined in the ICS terminology report [87]. The earlier descriptions of simultaneous pressure flow studies with a specific reference to urge incontinence was made in 1971 [88], however the earliest descriptions of this technique being simulated leads back to 1882 using a smoked-drum and a water manometer [89].

This study is an essential adjunct for patient assessment and especially for patients with neurological disease where this can be a part of ongoing urological evaluation. It is the only way to objectively assess lower urinary tract function in patients with incontinence. There are well-documented methods to standardize how this test is performed to ensure reliability and reproducibility [90].

However questions remain regarding its role, predictive value, and effect on treatment outcomes [90]. Studies have shown that urodynamic studies do not perfectly predict the outcome of relevant treatment in all patients such as those with DO. The ICS recommends that large multicentre prospective research is performed to also evaluate its cost effectiveness. It has been shown in a study that urodynamic evaluation of symptom free adults showed a wide range of all urodynamic parameters, some of which could be interpreted to be pathological signs. It was concluded that urodynamics are not physiological, but they are the

best tests available thus far, and that interpretations should be made with caution [91].

The American Urological Association recommend that prior to proceeding with an invasive urodynamic study, the clinician must have a clear question and indication for performing the test [92]. The invasive nature of urodynamic testing can subject the patient to risks of urinary catheterization such as urethral trauma, pain and infection.

The standard urodynamic study does not replicate real life for the patient. The bladder filling is not at a physiological rate, provoking inaccuracies, and the inhibited patient may become anxious by being exposed and with the task of voiding amongst medical staff. As a result the urodynamic trace is unable to represent real life for the patient, and it may fail to reproduce symptoms such as incontinence if the patient is trying to inhibit voiding. The process of urethral catherisation can be uncomfortable and the catheter itself can trigger off urgency that is not usually experienced by the patient.

Its invasiveness is not without risk of infection and bleeding for the patient and the cost of the study is also a limitation. Furthermore the lack of its availability means not all patients or clinicians can access this test.

The need for a biomarker

Because the OAB is a symptom-defined condition, patients are directly required to evaluate treatment response. This should be assessed using validated and reliable PRO instruments. However patient's responses can be influenced by their treatment expectations. Assessing the true efficacy of a treatment, does require PRO measures, but accuracy may be even stronger if combined with an objective, biological marker that can demonstrate the pathophysiology of disease activity.

Considering the limitations of the above mentioned assessment tools currently used to evaluate the LUT, it can be argued there is a clear need for a biomarker. A molecule that is

physıologıcally relevant to the urınary tract, and that upregulates ın pathologıcal states could be used as a marker for disease activity. This could offer an objective assessment for clinicians and patients with diagnosis, prognosis and monitoring response to treatment and assist with research into pathophysiology. Such a marker that is easy and non-invasive to collect, such as a sample of urine, is highly desirable.

Study Aim

To review the evidence, and discuss the prospect of the urinary neurotrophins Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) as potential urinary biomarkers for the overactive bladder (OAB).

METHODS

A pubmed literature search was carried out of published peer-reviewed studies that measured urinary Nerve Growth Factor or Brain Derived Neurotrophic Factor in patients.

Background

Biomarkers for OAB – Nerve Growth Factor and Brain Derived Neurotrophic Factor

In 1950, Nobel prize winners Stanley Cohen and Rita Levi-Montalcini, first discovered and explored the role of nerve growth factor (NGF), in the regulation of the developing neurological system [93]. Their early work showed that the transfer of mice tumour cells into chick embryos, promoted the development of the nervous system, particularly the sensory and sympathetic neurones. It was concluded that a growth-promoting substance was being released from the tumour cells, which had a selective influence on certain nerves. This substance was identified as NGF, which is a small signalling protein, which in the bladder, is produced by detrusor smooth muscle cells and by urothelial cells. NGF is now known to be necessary to promote the growth and survival of sympathetic fibres and sensory nerves, which are both vital for normal bladder functioning [94].

Overactive Bladder pathophysiology

The overactive bladder (OAB) is a clinical syndrome consisting of urinary urgency, frequency with or without urinary incontinence (UI) [95]. The diagnosis of OAB is a clinical one, which can be assessed, somewhat subjectively by patient reported symptoms. The

symptoms can be severely bothersome and negatively impact health-related quality of life. Symptoms of OAB, and in particular those with urgency UI, are also associated with DO, a urodynamic diagnosis, which may also be related to raised NGF levels in the lower urinary tract [96]. In the healthy bladder, sensory C-fibre activity maintains a quiescent role in the sensation of physiological bladder filling, except nearing functional bladder capacity, only being activated by distension that is greater than that required to stimulate Aδ fibres [97]. However, in an OAB, neuronal and non-neuronal release of neurotransmitter substances, from the urothelium and suburothelium, have a stimulatory effect on C-fibre activity.

The pathological mechanisms that lead to the OAB and DO are yet to be fully elucidated, although increasing evidence supports a central role for the increased excitability and activity of the sensory as well as motor bladder pathways [98]. The role of the urothelium is also thought to be crucial in the development of OAB [2]. The urothelium and suburothelium are innervated by a complex network of sensory afferent nerve endings which serve to initiate the micturition reflex and to reinforce the drive that maintains bladder contractions [1]. The afferent nerves consist of myelinated Aδ fibres and unmyelinated C-fibres (see figure one) [1]. The location of Aδ fibres is largely within the detrusor smooth muscle, which responds to detrusor stretch during bladder filling, and provides the sensation of fullness [97]. Unmyelinated sensory C-fibres are more widespread, and are found in the suburothelium and urothelial mucosa. These have a higher mechanical threshold, and are activated by and sensitive to heat, chemical and noxious stimuli [1]. C-fibres are less important in the sensation of physiological bladder filling, and may become primarily involved in pathological situations [97]. This makes them a stronger candidate to be involved in the pathological sensation of urgency [97]. A variety of neural receptors expressed within the superficial bladder layers have been identified such as the capsaicin receptor TRPV1, purinergic receptor

P2X3, and TrKA [2]. It is thought that the stimulation of urothelium by stretch, inflammation, acidity and temperature causes local release of a variety of neuropeptides, including NGF, BDNF ATP, substance P (SP), neurokinin A (NKA) and calcitonin gene related peptide (CGRP). Some of these have paracrine actions through these receptors, and modulate the expression of these receptors and afferent signaling [2]. The proposed urothelial complex of sensory signaling pathways are illustrated in figure seven [2]. After a neurological or inflammatory insult, it is thought that the C-fibre activity becomes the predominant route through which afferent impulses leave the bladder to the spinal tract to initiate the micturition reflex [1]. The increased amount of neurotransmitter substance being released by the urothelium, results in increased bladder excitability, hypersensitivity and ultimately OAB symptoms [1].

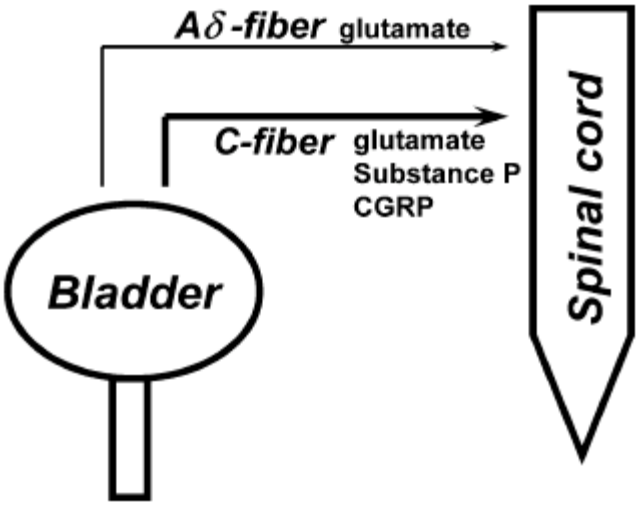


Figure six [1]: The afferent fibres leave the bladder and travel to the spinal cord with the neurotransmitter substances.

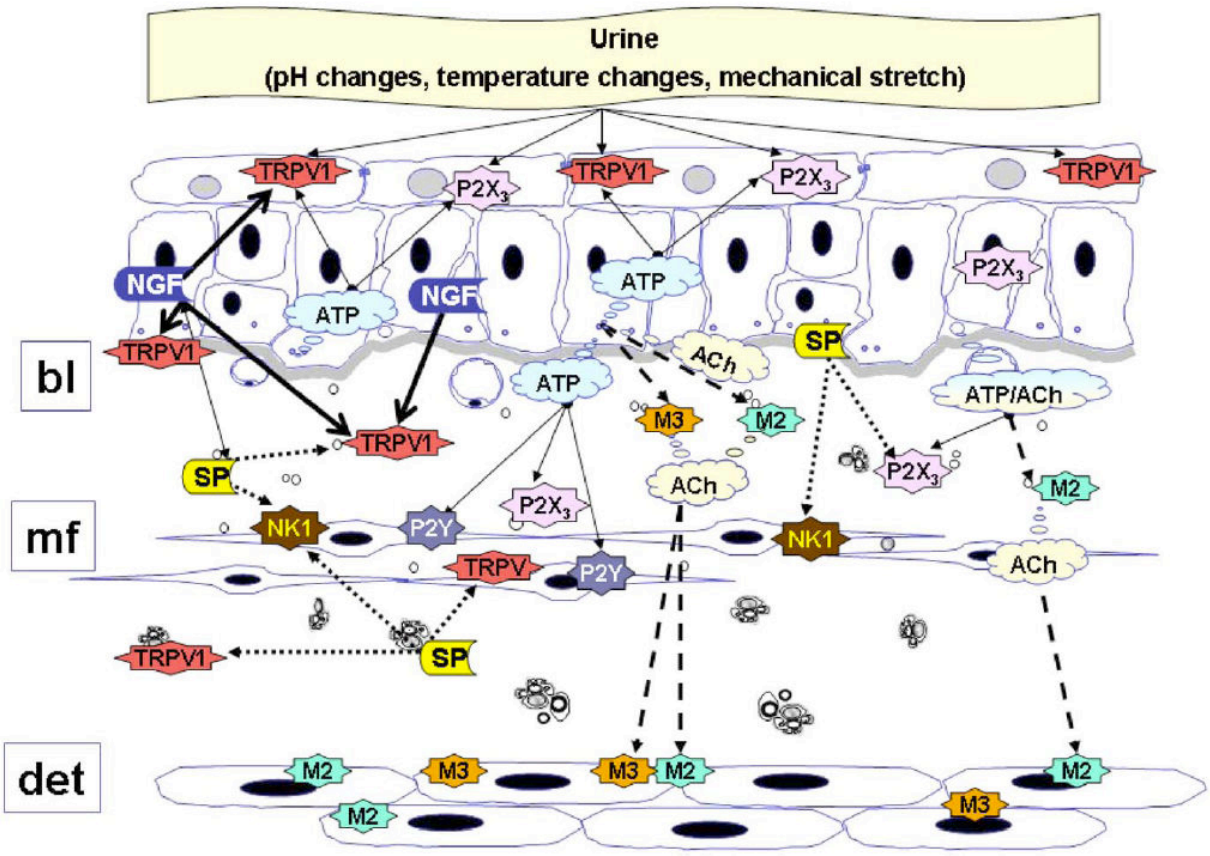


Figure seven [2]: The proposed complex interaction of afferent receptors and the different acting neurotransmitter substances. P2X3/P2Y (purinergic receptors) activated by ATP; Suburothelially released SP acts on NK1 (neurokinin receptors) on myofibroblasts; Acetylcholine (ACh) acts on M2/M3 (muscarinic receptor on smooth detrusor fibres); NGF is thought to affect the expression of urothelial and/or suburothelial TRPV1 (transient vanilloid receptor potential 1).

Figure eight below shows the close proximity and intimate relationship between the urothelial cells, myofibroblasts, and the afferent and efferent nerves [3]. This forms the basic mechanosensory unit, which may become overactive with up regulated neural receptors and neurotransmitter substances with OAB, NDO, IDO and conditions such as interstitial cystitis.

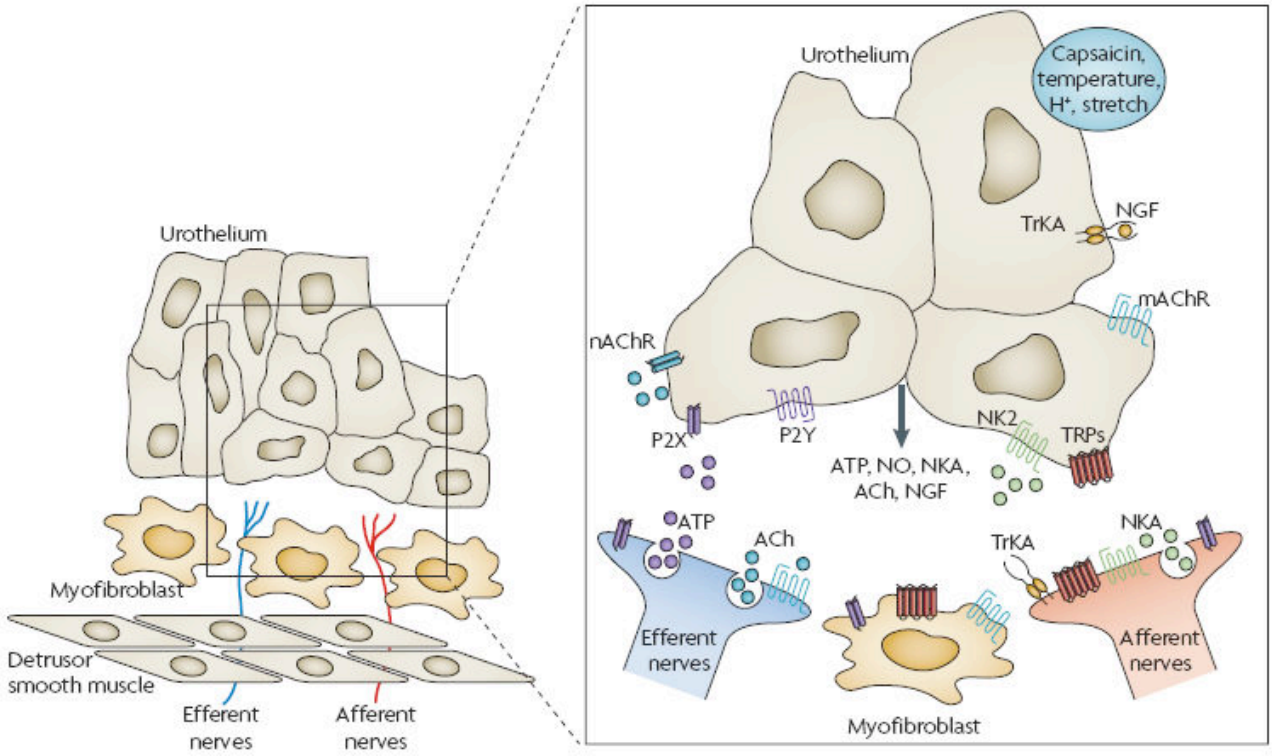


Figure eight [3]: A model illustrating possible chemical interactions between urothelial cells, afferent nerves, efferent nerves and myofibroblasts in the urinary bladder. Activation of receptors and ion channels in urothelial cells by bladder distension or chemical stimuli can release mediators, such as ATP, nitric oxide (NO), neurokinin A (NKA), acetylcholine (ACh) and nerve growth factor (NGF), that target adjacent nerves or myofibroblasts and might also act in an autocrine or paracrine manner on urothelial cells. NGF released from muscle or the urothelium can exert an acute and chronic influence on the excitability of sensory nerves through an action on tyrosine kinase A (TrkA) receptors.

Role of NGF in OAB pathophysiology

It is thought that NGF, which is expressed in excess in pathological bladder states, is one of these stimulatory substances, and has a knock on affect to sensitise afferent C-fibres, leading to sensory urgency, and ultimately reflex bladder hyperactivity, causing urinary frequency and urgency UI. The overexpression of NGF has led to the interest in this secretory protein as a potential urinary biomarker for the OAB, which represents a considerable health and economic burden.

The proposed role of NGF in the development of LUTD is illustrated in figure nine [4].

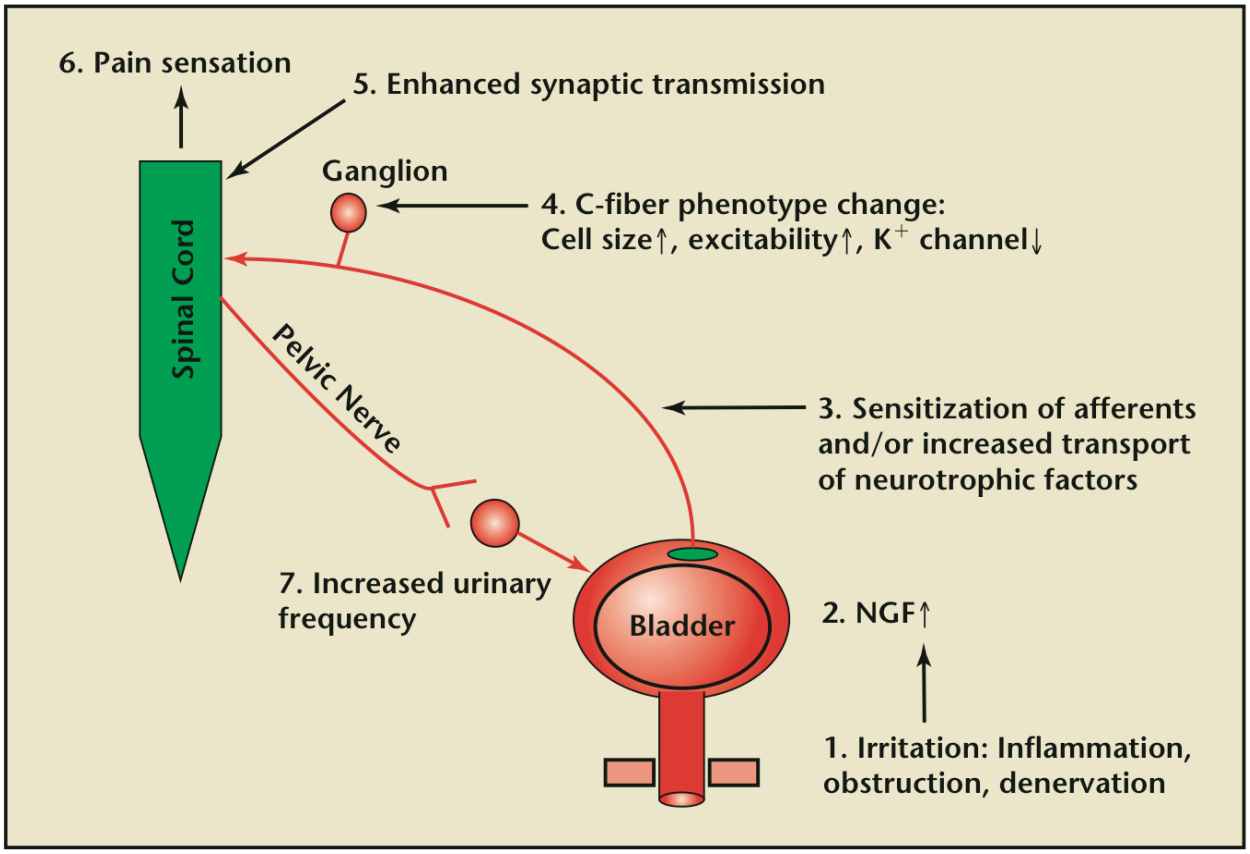


Figure nine [4]: Provoked NGF release from target cells occurs under certain circumstances, which sensitizes afferent nerves, enhances synaptic transmission and produces OAB symptoms.

Tissue NGF: Animal and Human Studies

Evidence has been accumulated from numerous animals studies, discussed below, to show the importance of NGF in the pathological bladder [99]. A study in cats examining a naturally occurring model of feline interstitial cystitis (IC), found an increase in the levels of NGF and Substance P in the urothelium, along with an increase in the cell body size of the dorsal root ganglia (DRG) when compared with controls. This suggests that the inflamed urothelium may be an important source of increased NGF, and also act as a transducer of physical and chemical stimuli, by undergoing retrograde transport to DRGs [100]. In rat models of spinal cord injury causing neurogenic DO (NDO) or cyclophosphamide-induced cystitis and overactivity, levels of NGF have also been found to be increased in the bladder, the DRG and the lumbosacral spinal cord [101]. Systemic treatment of rats with this experimental cystitis model, with an NGF sequestering molecule, has been shown to reduce the development of bladder overactivity [102]. Another study that reported a significant increase in NGF production, after cyclophosphamide -induced cystitis in female rats, showed this was also associated with a decreased intercontraction interval [103]. Once hyaluronic acid was administered intravesically, this significantly reduced the urinary NGF (uNGF) levels and increased the intercontraction interval. Furthermore, the application of hyaluronidase, which counteracts the action of hyaluronic acid, eliminated the suppressive effect of hyaluronic acid on NGF levels [103].

Chronic administration of NGF into the bladder or spinal cord of rats has been shown to induce bladder hyperactivity [104]. One study explored the role of NGF by generating transgenic mice, and inducing chronic overexpression of NGF in the urothelium. Certain characteristics were examined in comparison to wild-type littermate controls [105]. In these mice, the overexpression of NGF led to a reduced bladder capacity that exhibited an increase

in the number and amplitude of non-voiding reflex bladder contractions, when compared with the controls. There was also marked nerve fibre hyperplasia in the lamina propria and detrusor smooth muscle, along with causing elevated numbers of inflammatory cells, e.g. tissue mast cells.

Sensory afferent nerves also showed increased density of calcitonin gene-related peptide and Substance P containing nerve fibres [105]. These neuropeptides are released in inflammatory states, and through their release, NGF may also be implicated in visceral nociception by producing hyperalgesia. It is thought that NGF can also modulate the expression of membrane ion channels, e.g. P2X3 (purine-receptor), TRPV1 (transient receptor potential vanilloid-1), and voltage-gated sodium channels in the bladder, which are thought to play a major role in inflammation, tissue-induced pain and hypersensitivity [105].

Another study showed that raised NGF levels can also be related to DO secondary to Bladder outflow obstruction (BOO). In this study, 40 rats had a BOO created by partial ligation of the urethral outlet, and were shown to develop DO by cystometry. Urine was collected before ligation, during the obstructed period and after relief of the obstruction. NGF levels were reported to be significantly raised from baseline levels during the period with DO, which then decreased back to baseline levels after the DO disappeared after the relief of the BOO.
In the rats with persistent DO, despite removal of the urethral obstruction, the uNGF levels remained significantly raised from baseline and compared with the control rats [106]. Another study reported increased mRNA expression of NGF in the bladder in BOO rat models associated with DO, and in continued DO after BOO relief [107]. More recently, a study reported raised tissue NGF levels, quantified by immunofluorescence staining and Western blotting in an unstable BOO rat model, which were then significantly reduced after

detrusor onabotulinumtoxinA (BTX-A) injection [108]. It has been shown that once this excess quantity of expressed NGF is sequestered, it can reduce the observed bladder dysfunction. After spinal cord injury in rats, the resulting DO and detrusor-sphincter dyssynergia can be suppressed by intrathecal application of NGF antibodies, which neutralises the NGF in the spinal cord [109, 110]. This sequestration of NGF produces effects similar to treatments, e.g. resiniferatoxin or capsaicin, which desensitise C-fibre afferents.

Similarly to the studies in animals, which prove that NGF plays an important role in bladder dysfunction, human studies have corroborated this. One study that quantified human bladder tissue levels of NGF using tissue enzyme-linked immunosorbent assay (ELISA) techniques, collected bladder biopsies before and at one and three months after detrusor BTX-A injection in patients with NDO [111]. As the urodynamic parameters improved over this time frame, the tissue NGF levels were also noted to decrease significantly. This suggests that BTX-A may decrease the levels of neurotransmitters, which may otherwise stimulate NGF production and release. In bladder biopsies from patients with IC/painful bladder syndrome (IC/PBS), an increased expression of NGF mRNA has been reported, which falls 2 weeks after successful intravesical injection with BTX-A. This corresponded with a decrease in pain scores [112, 113]. However, NGF mRNA levels were not reduced in non-responders with persistent pain after BTX-A. Interestingly, decreased NGF mRNA levels correlated with decreases in pain scores, but not with changes in maximum bladder capacity, which suggests NGF may contribute more to pain perception, and increased afferent excitability. This is supported by the action of NGF on TrkA (neurotrophic tyrosine kinase receptor type A) receptors, via which it further mediates the release of Substance P, which is implicated in inflammatory responses and nociception [114].

Urinary NGF a Potential Biomarker for OAB/DO?

The accumulation of the above findings have led to the evaluation of urinary levels of NGF as a potential biomarker and as a simple, cost-effective method to objectively diagnose, and assess therapeutic outcomes in patients with OAB [4]. Such a tool may also be of use as a prognostic indicator and provide information about which patients may respond better to certain treatments.

A biomarker should be 'a characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention'. A suitable biomarker needs to be easily accessible, reliable, and as a test repeatable, whilst offering high specificity and sensitivity to diagnose and monitor OAB. There should be a relationship with the severity of OAB. Furthermore it must provide information that is not already available from the clinical assessment, improve the outcome of the disease, and predict responses to specific therapies. The cost and time aspect of the test should be acceptable, and ultimately save costs by avoiding unnecessary therapies [115].

The levels of NGF in the urine can be quantified using an ELISA system, with most studies reporting results from the same kit (Emax, Promega, Madison, WI, USA) for reproducibility. The antibodies that come within this kit have not specifically been developed or marketed for measurement in the urine, and are only being used within a research domain. The technique uses a sandwich ELISA assay, using a polyclonal antibody to NGF to initially coat a well, and after incubation with the patient sample, a second monoclonal antibody is then used to sandwich the molecule, between the two antibodies. A tertiary species-specific antibody is then used, which is conjugated with horseradish peroxidase, which is then incubated with a chromogen, to induce a colour change, the intensity of which is proportional to the concentration of the substrate within the well. The total uNGF level is normalised to the concentration of urinary creatinine (Cr), to overcome the differing dilutions of urine between

patients. Other NGF ELISA kits are available and a validation and standardisation between these different kits needs to be performed to check reproducibility.

Table ten: Summary of all the published studies that have measured urinary NGF (uNGF) levels in patients with various bladder dysfunctions.

Author and Year	Conditions investigated and sample sizes	Conclusions
Chan et al, 2014 [116]	10 females with bladder outflow obstruction (BOO), with a peak flow of ≤12ml/s, and a history of pelvic organ prolapse or previous incontinence surgery. Control group of 10 asymptomatic women.	uNGF/Cr levels were significantly higher in the patients with BOO (mean [SE] 20.8 [4.31] pg/mg) compared to age-matched control group (5.6 [0.65] pg/mg). After treatment, the urinary NGF/Cr level significantly decreased to 6.50 (0.57) pg/mg (P = 0.01).
Liss et al, 2014 [117]	Urine collected from 115 men before radical prostatectomy.	Increasing Log10 uNGF/Cr was associated with a higher gleason score ≥8, p=0.003. Perineural invasion was more prevalent in this higher grade disease group. This study concluded that uNGF could be a marker for higher grade prostate cancers.
Aydogmus et al, 2014 [118]	90 female patients with OAB-dry, randomized into either acupuncture, solifenacin or placebo arms. The number of patients that completed each arm of therapy were	Significant clinical QOL improvement with medical and acupuncture arms compared with placebo. Mean uNGF/Cr levels significantly improved with acupuncture and solifenacin arms after treatment compared with placebo.

	28, 30 and 24 respectively.	
Shalom et al (2014) [119]	23 patients with refractory OAB and DO before and 5 days after a trial of peripheral nerve evaluation (PNE) for sacral nerve stimulation and 22 controls.	Subjects with DO had significantly higher baseline uNGF/Cr levels compared with controls (19.8pg/mg vs 7.88 pg/mg, $p<0.002$). 17 patients had PNE and significant improvement in symptom questionnaires were seen with this and uNGF/Cr levels were also demonstrated to significantly decrease (17.23 pg/mg to 9.2 pg/mg, $p<0.02$).
Antunes-Lopes et al (2013) [120]	Females with OAB (37) and healthy controls (40)	uNGF/Cr and uBDNF/Cr (Brain-derived Neurotrophic Factor) were significantly higher in OAB than healthy controls. These levels decreased after anti-muscarinic treatment. No significantly raised urinary glial cell-line derived neurotrophic factor/Cr in OAB compared with controls. Reduction in number of urgency episodes per week correlated with BDNF/Cr but not with NGF/Cr. There was no circadian variation to uNGF/Cr levels.
Liu et al (2012) [121]	Interstitial cystitis (IC) (30) and healthy controls (28)	Increased mean uNGF/Cr and serum NGF levels in patients with IC compared with controls.
Liu et al (2011) [122]	Serum and uNGF levels in OAB (34), wet (17) and dry (17)	Serum NGF and uNGF/Cr levels were significantly raised in patients with OAB when compared with healthy asymptomatic controls. Serum NGF

		correlated with uNGF levels. No significant differences in serum NGF were detected between the OAB dry and wet groups. Serum and urinary levels remain unchanged before and after solifenacin treatment.
Liu et al (2011) [123]	OAB dry (113), OAB wet (104), controls (84)	uNGF levels were significantly higher in OAB dry and even higher in OAB wet patients. Urinary NGF levels were not associated with menopause, ageing or higher body mass index.
Pinto et al (2010) [124]	26 women with painful bladder syndrome who were treated with trigonal injection of 100U of BTX-A. Urinary neurotrophin levels were measured at baseline, and month 1, 3 and 6 post injection.	uNGF and uBDNF (brain derived neurotrophic factor) levels were measured at baseline, 1, 3 and 6 months after injection. uNGF and BDNF levels were significantly reduced after the injection at one month, which corresponded with the improvement in pain intensity on a visual analogue scale. At three months, levels remain reduced however not as low as at one month. At six months levels have risen back up to baseline levels.
Liu et al (2009) [125]	Interstitial cystitis/painful bladder syndrome (IC/PBS) (122) and normal controls (28).	uNGF /Cr levels were significantly greater in patients with IC/PBS than in controls. uNGF/Cr levels were very significantly higher when the bladder was distended in patients with IC/PBS. Patients who responded to treatment with an improvement in visual analogue scores of >2 had significantly lower uNGF/Cr levels than non-responders who had improvements of <2.

Kuo et al (2010) [126]	Urinary NGF versus Bladder wall thickness (DWT) as a biomarker for OAB (81)	DWT was not significantly greater in OAB patients compared to controls. uNGF/Cr levels were significantly increased in patients with incontinence and OAB and those with DO.
Liu et al (2010) [127]	Interstitial cystitis IC/bladder pain syndrome (40), OAB (54) mixture of DO or increased bladder sensation (IBS), controls (27)	uNGF/Cr levels were raised in the 40 patients with IC, and 23 with DO, but not in the 31 with IBS or controls. uNGF/Cr levels were not significantly different between the IC or DO groups.
Liu et al (2009) [128]	OAB (70) all treated with antimuscarinics, 50 responders, 20 non-responders, controls (38)	uNGF/Cr levels were significantly higher in patients with OAB taking and responding to antimuscarinics compared with non-responders and controls.
Liu et al (2009) [129]	Cerebrovascular accident (CVA) (93)	uNGF/Cr levels correlated with severity of neurological impairment after CVA.
Liu et al (2009) [130]	Urine collection at OAB (39) at first sensation of bladder filling (FSF), urge sensation (US) and controls at FSF and US (35)	uNGF/Cr levels were significantly higher in OAB patients than controls. In controls uNGF/Cr levels were very low at first sensation of bladder filling (FSF), but significantly higher at urge sensation (US). In OAB patients there was no significant difference in uNGF/Cr levels at FSF and US.
Liu et al (2008) [131]	Mixed urinary incontinence (urinary	uNGF/Cr levels were significantly higher in women with mixed urinary stress incontinence and DO than

	stress incontinence (USI) and OAB) (38), DO alone (26), persistent USI after anti-incontinence surgery (21), de novo DO (15), control (31).	those with pure stress incontinence or controls. uNGF/Cr levels were undetectable in women with persistent USI, but significantly higher in those with de novo DO after anti-incontinence surgery.
Liu et al (2009) [96]	IDO (143), NDO (100). Mixture of untreated, well treated and failed treatment with antimuscarinics. BoNT/A was given to IDO (24) and NDO (19), Controls (38).	uNGF/Cr levels were significantly higher in 66 patients with IDO and 59 with NDO compared to controls. Patients with well-treated IDO or NDO had reduced NGF/Cr levels, whereas those with failed-treated IDO or NDO did not. Patients who responded to BoNT-A treatment had significantly reduced uNGF/Cr levels in both the IDO and NDO groups compared to baseline levels. However, the NGF levels remained significantly higher at 3 months in 7 IDO and 5 NDO patients who failed BoNT-A treatment.
Yokoyama et al (2008) [132]	OAB total (51) due to IDO (13), sensory urgency (6), BOO (16), and NDO (16) due to spinal cord injury (SCI) or CVA.	The uNGF levels were significantly raised in patients with NDO due to SCI, BOO and sensory urgency when compared with controls. There was no significant difference in urinary levels between those with IDO, NDO due to CVA and controls.
Jacobs et al (2010) [133]	Neurogenic overactive bladder (NOAB) (13), idiopathic overactive bladder (17), interstitial	uNGF/Cr levels were significantly raised in NOAB and IC/PBS groups when compared with controls. Raised levels approached significance in the patients with nephrolithiasis.

	cystitis/painful bladder syndrome (IC/PBS) (8), prostate cancer (7), active bladder cancer (4), and nephrolithiasis (4).	
Liu et al (2008) [134]	BOO (153): BOO non OAB (21), BOO and OAB (25), BOO and DO (47), BOO on successful medical treatment (60), Controls (38).	The uNGF/Cr levels were very low in the control group and in patients with BOO/non-OAB and significantly greater in patients with BOO/OAB and BOO/DO. The uNGF/Cr levels returned to normal levels after successful relief of OAB symptoms with medical treatment.
Liu et al (2008) [135]	Normal controls (40), patients with increased bladder sensation (23), OAB dry (54), OAB wet (80).	uNGF/Cr levels were low in controls and patients with increased bladder sensation. Levels in patients with OAB dry were significantly higher than controls. Patients with OAB wet had significantly higher levels than those with OAB dry.
Kim et al (2006) [136]	OAB (65) controls (20).	uNGF levels were significantly higher in OAB patients than controls.
Kim et al (2005) [137]	OAB (75) and controls (20).	Urinary NGF levels and PGE2 (prostaglandin E2) were significantly higher in OAB patients than controls.

Baseline uNGF/Cr levels have been repeatedly demonstrated to be highly expressed in patients with OAB when compared with controls. This increase is even more significant in patients with OAB and urgency UI (termed OAB-wet), compared with those without (OAB-dry) [4]. It has been postulated that the difference between the OAB-dry and OAB-wet groups, may be due to the higher percentage of DO in the OAB-wet patients, which may suggest that higher levels of uNGF may predispose the occurrence of DO [99]. However, uNGF levels were not raised in patients with bladder oversensitivity alone [126]. Patients with mixed UI, with co-existing DO, were also shown to have higher NGF levels than patients with pure stress UI alone. However, in the latter group, urinary levels were raised in those patients who developed de novo DO after anti-UI surgery, which resulted from BOO [131]. In antimuscarinic-refractory patients with OAB, serum levels of NGF were also assayed, and correlated with urinary findings, by being raised compared with controls.

These patients were treated with 3 months solifenacin, after which serum and urinary levels remained raised. The complex pathophysiology of OAB, may therefore be multimodal, involving several neurotransmitter and inflammatory pathways [122]. NGF urine levels also improve in those patients who have responded to medical therapy, with antimuscarinics and BTX-A [4, 128, 129]. However, these levels do not fall in patients who have not responded to treatment with either of these methods. It was also evident that once patients discontinue antimuscarinic treatment for 1 month, the levels re-elevated in almost half of the responders. This suggests that uNGF levels could have potential use as an objective tool for assessing the response of DO to therapy, as the levels appear to fall as DO is treated. As both of these treatment methods either inhibit the release or action of acetylcholine, it may be inferred that acetylcholine, which also acts at muscarinic receptors on urothelial and suburothelial cells [138], is a required substance for the release of NGF.

Most recently a study has examined the effects after 5 days of peripheral nerve evaluation (PNE) in 17 patients, on uNGF levels [119]. All subjects showed a >50% improvement in voiding diary parameters such as number of daily voids, and/or leakage episodes, and significant improvements in urinary distress inventory scores and QOL scores. The mean post void residual also significantly decreased in this time, and concordantly uNGF/Cr levels were seen to significantly decrease from 17.23 pg/mg to 9.24 pg/mg after PNE. The authors conclude that since NGF induces OAB and DO through sensitization of afferent C-fibres, somehow through increasing the expression of sodium or potassium channels in the afferent nerves, that SNM may modulate afferent nerve activity from the bladder. They raise the prospect that uNGF/Cr could be an evaluation tool for patients undergoing SNM, as it correlated with symptoms and PVR. There was a lack of non-responders in this study however to comment on the behaviour of uNGF/Cr levels for patients with no clinical response as an internal control.

Another interesting study evaluates the use of acupuncture for the treatment of OAB-dry patients [118]. Acupuncture patients received treatment twice weekly for eight weeks and were placed by an accredited specialist. These needles were placed at four acupuncture points (including the SP6 point which is commonly used for PTNS), and also placed bilaterally. The placebo group received a sham acupuncture treatment using needles of the same size, however these were not skin penetrating but the patient still experiences a pricking sensation. Significant reductions in mean uNGF/Cr were also seen in both the treatment arms at end of therapy. It was reported that eight out of twenty eight patients receiving acupuncture did not respond to therapy and no decrease in uNGF/Cr level was noted for these patients. There was no reduction of these levels in the sham arm.

Some investigations have focused on the relationship between bladder stretch and NGF

release, as it is thought that the secretion of NGF from the bladder depends on stretch and duration of distension [126]. Detrusor smooth muscle stretch stimulates increased NGF production and hyperactive voiding in a rat model [139]. Urinary urgency that occurs with fast filling and distension during a cystometric investigation can also induce sensory urgency or provoke DO. Urinary NGF/Cr levels in OAB-wet patients are significantly raised, and less so with OAB-dry, with natural filling (by drinking water), when compared with levels from artificial stretch obtained with catheter filling [126]. This could be because natural filling allows more time for NGF to be released from the bladder. In control patients it has been shown that NGF levels are higher when volunteers wait to void at strong urge sensation, rather than sooner at the first sensation of bladder filling. However, as NGF levels are pathologically raised in OAB at small bladder volumes, it does not significantly elevate at sensory urgency sensation [130].

OAB is also associated with BOO, and in men with BOO and OAB, and BOO and DO, uNGF/Cr levels are significantly higher than in those with BOO and no OAB [134]. These levels are lower in treated patients after relief of the OAB associated with BOO. Chronic stretching of the urothelium, as with BPH, can stimulate NGF production, among other transmitters, and chronically sensitise afferent pathways and DRG constituency, which causes increased excitability. In rat BOO models, immunity to NGF prevented obstruction-induced hypertrophy of DRG neurones, less retrograde sacral afferent activity, and eliminated the spinal micturition reflex [140].

BDNF: Animal and human studies

NGF is most studied and better understood neurotrophin in LUTD. More recently BDNF has also received some attention as a potential biomarker in studies with results showing promise. BDNF is an abundant neurotrophin, and like NGF is vital for the survival and normal function of sensory nerves and has a role in nociception. BDNF, produced by the urothelium and spinal cord, has its expression regulated by NGF. It has been shown in animal models that after NGF instillation, either into the intrathecal or intraperitoneal compartment, both BDNF mRNA and protein are upregulated in the cells of sensory afferents [141].

Other animal models of cystitis or BOO, have shown an increase in the levels of BDNF and its receptor TrkB expressed in the urothelium and bladder sensory afferents. In spinal cord injured rats with resultant NDO, BDNF levels also appear to upregulate in the bladder and in the spinal cord segments. A recent study has shown that in CYP-induced cystitis in rats, BDNF sequestration resulted in a significant decrease in the frequency of bladder reflex activity and improved bladder function [142]. However BDNF did not have any impact on bladder reflex activity in intact animals that suggests that its role is more relevant to pathological conditions.

Urinary BDNF studies

There are a handful of studies that have measured the urinary BDNF levels in patients with LUT pathophysiology. These are summarized in Table eleven below:

Table eleven: Summary of studies evaluating urinary BDNF in patients.

Author and Year	**Conditions investigated and sample sizes**	**Conclusions**
Pinto et al, 2010 [124]	See table above	
Antunes-Lopes et al, 2011 [143]	Urine samples were collected from healthy controls, 20 men and 20 women in the morning, afternoon and evening. Seventeen female patients with naïve OAB were also included. This was repeated after three months.	Urinary BDNF levels were very low in the healthy volunteers, despite time of collection. There were no significant differences between male or female. BDNF/Cr was significantly higher in OAB patients compared to healthy volunteers. After three months of non-pharmacological interventions, OAB scores improved and correlated with a decrease in uBDNF/Cr levels. A significant correlation between uBDNF/Cr level

		and OAB symptom score was found (r=0.684, p<0.01).
Wang et al, 2014 [144]	90 women with OAB and 45 normal controls.	uBDNF levels were elevated in OAB groups compared to controls. ROC curves suggest a sensitivity of 93.33% and specificity of 88.9% for assessing uBDNF/Cr in OAB groups.
Koven et al, 2014 [145]	Urine samples were collected from 52 healthy adults undergoing executive functioning tests, and levels of uBDNF were measured.	BDNF concentration was positively related with cognitive flexibility but not with memory or reasoning.
Collins et al, 2014 [146]	52 young adults were assessed for aerobic fitness and urinary samples collected.	BDNF/Cr levels correlated with enhanced level of fitness reflecting exercise mediated changes in peripheral BDNF. Circulating levels of BDNF were affected by aerobic exercise.

Raised urinary BDNF levels have clearly been shown in patients with OAB [144] compared with controls with promising sensitivity. However as with NGF a similar concern is the specificity. Urinary BDNF levels have also been demonstrated in patients with interstitial cystitis and shown to reduce after successful treatment with BTX-A, following a reduction in bladder pain levels [124]. In OAB, uBDNF levels were also found to be raised compared to healthy controls, and reduced after behavioural modifications such as bladder training, limited fluid intake, and avoidance of caffeine [147]. BDNF is much less explored than NGF, however emerging studies suggest that this may be more sensitive and specific.

Pitfalls with NGF and BDNF as a Biomarker

Many proteins are involved in the complex pathophysiology of the OAB, of which NGF is only one. It seems to play a role in LUT dysfunction (LUTD), and is closely associated with inflammation in the bladder. However, due to the numerous sensory neurotransmitters and inflammatory substances that are likely to also play an important role, e.g. Substance P, ATP, calcitonin gene-related peptide, other neurotrophins and inflammatory markers, NGF alone may be insufficient to act as a urinary marker for OAB.

Not all patients with OAB have raised uNGF values at baseline, which was up to 30% in some cohorts [125]. In a cross-sectional study in 143 patients with IDO, uNGF levels were seen to be raised in 66 of these patients [128]. These findings suggest that there may be other causes of OAB pathophysiology, some which do not involve inflammatory or NGF pathways. Thus, as a potential biomarker, there will therefore be significant proportions of patients with OAB or DO, who do not have raised uNGF levels, which lowers its sensitivity.

Also, it was noticed that in responders to antimuscarinics, who had significantly improved urinary symptom scores, and a corresponding fall in uNGF levels after 3 months of therapy, that these levels although reduced were still significantly higher than those of controls. This may be due to incomplete resolution of the underlying OAB pathophysiology, and that overactivity of the cholinergic system is not the sole cause of OAB.

An issue that presents itself for the potential use of this protein as an OAB marker is its specificity, as raised levels are not limited to OAB. Links have also been reported for raised uNGF levels in patients with painful inflammatory conditions of the urinary tract, e.g. IC/PBS, BOO (in men and women), chronic prostatitis, renal stone disease, UTI, and most recently prostate and bladder cancer (see table above) [133, 148]. A common trend for patients with raised uNGF levels appears to be the presence of inflammation or neural injury,

which is an increasingly recognised process in the development of OAB. It has been shown that at least half of biopsy samples from patients with NDO and idiopathic DO (IDO) have histological evidence of chronic inflammation [149]. This would be consistent with the finding that uNGF levels are raised with bacterial cystitis, when compared with controls, and that once the infection has been treated with antibiotics for a week, these levels significantly decrease [99]. Furthermore, in patients with concurrent UTI and OAB, despite treatment with antibiotics, uNGF levels remain significantly raised. Patients with IC/PBS, who responded to treatment with hydrodistension with an improved visual analogue scale pain score, also had a corresponding reduction in uNGF/Cr level. This reduction in levels was not seen in those who did not respond to treatment, which is a further suggestion of the potential use of NGF in monitoring the response to treatment.

The presence of NGF in the urine has been affiliated with the raised NGF level expression in the urothelium. However, it is not entirely clear if this is the sole source of uNGF. The circulating serum NGF levels may also represent some of the sourcing of uNGF, via renal excretion, the proportion to which is unknown. Urinary NGF levels have also been identified to correlate with the severity of neurological impairment after cerebrovascular accident [129]. It is thought that after the brain injury in an acute phase of a stroke, levels of circulating neurotrophins are raised, which may have a knock-on effect on urinary levels. Furthermore increased serum NGF levels have been identified in numerous other medical conditions, e.g. asthma, allergy, Alzheimer's [150, 151]. Therefore, as a potential biomarker for OAB, this raises the possibility that in certain neurological conditions, or other medical conditions, when patients do not have any lower urinary tract (LUT) complaints, uNGF levels could be silently raised, causing false positives.

Neuropsychology and emotional state also may have a role in urinary levels. As these neurotrophins are so ubiquitous, it is unlikely that there presence in the urine can be attributed to LUT pathology alone. BDNF has a high gene expression in the prefrontal cortex and is though to be important with regulation of cognitive reserves and frontal lobe function, and urinary levels have been shown to be correlated with cognitive ability [145]. Furthermore it has been shown that circulating and uBDNF levels are also related with aerobic fitness, and that higher urinary levels indicate a level of higher fitness reflecting the exercise-mediated changes in peripheral BDNF [146]. Therefore its role as an indicator of the OAB, is limited by the numerous other physiological and pathological states that can influence its levels. The molecular weight of neurotrophins are low at approximately 13kDa. The molecular weight cut-off for kidney glomerular filtration is classically assumed to be around 30-50kDa, posing the possibility that excessive circulating neurotrophins could be removed by kidney filtration and hence urinary levels may subsequently rise.

Unresolved Questions

Diagnostic accuracy has been the subject of much attention, and applies to all medical tests. This refers to the ability of a test to identify the condition of interest. This involves comparing a test with the reference or 'gold' standard in a group of patients suspected of having the condition of interest. Accuracy refers to the degree of agreement between the new test and the gold standard [152]. Recent development of Standards for Reporting Diagnostic Accuracy (STARD) guidelines, which was a multidisciplinary effort of clinicians and scientists, have been created to provide a checklist for reporting the accuracy of a diagnostic test [153]. This checklist addresses several quality related issues, and an official assessment of this proposed biomarker would need to be performed in accordance with STARD guidance. To date neither of these potential biomarkers have been validated through this

process.

As a potential biomarker it is a non-invasive investigation, as urine is relatively easy to collect from the patient. The expression of this abundant protein can change with underlying pathological processes. As patients subjectively respond to successful pharmacological intervention, NGF levels also reduce, which may be of use as an objective tool to monitor the response to a treatment. However, a biomarker should also have a high level of specificity and sensitivity, add new information to the clinical assessment, and have a clear association with the severity of OAB and improve the outcome of the disease, which uNGF does not do. For the latter, not all of patients with symptomatic OAB will have significantly raised uNGF levels, and this suggests the possible variation in pathophysiology that exists in this condition. As a result, the consideration of NGF as a sole biomarker for this condition may be limited.

Clearly, as NGF levels may be raised in numerous conditions, an exclusion criteria needs to be established to highlight patient suitability, and avoid performing measurements in patients who may have false positive results for OAB. Other groups that may also require exclusion are those with active or recent UTIs, and those with indwelling catheters or performing clean intermittent self-catheterisation, which may be a significant proportion of the neurogenic population, as the minor trauma may theoretically exacerbate urothelial release; however, this is yet to be investigated. The exact source of the NGF and BDNF in the urine is also unclear, with regards to the proportion that is attributed to the urothelium, and the proportion that may be from systemically circulating neurotrophins, which may undergo renal excretion.

More study is also required within a control population, to assess within-person biological

variation; levels may change throughout the day. Variables such as the time of sample collection, whether the first morning void, or a later sample and bladder volume or degree of bladder stretch when providing a sample, may also influence urinary levels. These variables may have an impact on the end result and also need to be standardised for collection. Current recommendation is to collect samples and immediately placing on ice, followed by rapid transfer to the laboratory for processing. If processing is delayed this may risk NGF degradation, and hence levels may be affected. Further studies are required on assessing the stability of this protein in urine, to help standardise methods of collection in an outpatient setting. The costs of the detection techniques may also need to be considered, to gather information for cost-effectiveness calculations.

CONCLUSION

Whilst the evidence for an increased uNGF and uBDNF in OAB appears convincing, many questions about its validity remain including specificity, sensitivity, cost- and time-effectiveness. As a monitor for the response to treatment, it shows promise for certain patient groups who clinically respond to BTX-A, antimuscarinic therapy, PNE for SNS, and even acupuncture, and this response needs to be evaluated for other treatment options including Tibial nerve stimulation. If a role as a biomarker is not proven, it may still have some ability to indicate possible response to a particular treatment or to predict the natural history or aetiology of the disease.

Many criteria for what constitutes a biomarker still need to be evaluated and met before this molecule can be considered for this role. However, exploring the role of NGF in these conditions has elucidated more information about the complex urothelial signalling mechanisms that exist, the pathophysiology of OAB, and the role of the neurotrophins in particular. Other promising biomarkers are also being explored, both within the neurotrophin family, and in the inflammatory cascade, which may identify some more reliable biomarker candidates. It may well be that the ideal urinary biomarker for any particular type of LUTD may be the presence of a combination of proteins, the levels of which may differ between conditions and patients. Used together, their sensitivities and specificities may be combined, to make it a stronger investigation.

CHAPTER THREE

Chapter Synopsis

Chapter two reviewed the literature of urinary neurotrophins as biomarkers for the OAB. The vast majority of these studies have explored urine from patients with idiopathic OAB. Only a handful of studies of examined patients with neuropathic bladder dysfunction, and extremely few have focused on patients with Multiple Sclerosis (MS). At the National Hospital for Neurology and Neurosurgery, there are large numbers of patients with MS, and OAB. The aim of this study was to measure the urinary neurotrophin levels from patients with MS and healthy controls. As an internal control group, MS patients with no OAB symptoms were recruited to examine whether any raised urinary neurotrophin levels specifically correlate with OAB symptoms or the underlying neurological disease process. Ethical approval was granted for the study.

Measurement of Urinary Nerve Growth Factor and Brain Derived Neurotrophic Factor in patients with Multiple Sclerosis and Overactive Bladder

Abstract

Introduction

Studies examining the role of urinary neurotrophins as a biomarker for the OAB are emerging. Only a few have investigated this in patients with neurological disease and even fewer in patients specifically with Multiple Sclerosis (MS). OAB symptoms are prevalent in patients with MS and a urinary biomarker would be highly desirable to monitor disease activity, and monitor response to treatment. MS is also a condition known to have higher baseline circulating neurotrophin levels. It is unknown whether urinary neurotrophin levels will be related to OAB symptom score only, or whether they will also be influenced by neurological disease activity.

Materials and Methods

Patients with MS attending the outpatients at the National Hospital for Neurology and Neurosurgery were recruited. Three main groups were invited into the study, those with MS and OAB, MS and no OAB as an internal control, and healthy volunteers. Patients completed the Urinary Symptom Profile to objectively assess symptom severity, and urine was collected and neurotrophin levels were measured by enzyme-linked immunosorbent assay (ELISA) method and normalised by urinary creatinine levels (NGF/Cr).

Results

Sixty-seven patients with MS-OAB, ten with no OAB and twenty healthy controls were recruited. There are differences between groups in NGF levels with the highest levels seen in

MS-OAB patients (median 10.8 (IQR: 3.0, 27.0)) and the lowest levels seen in Controls (median 4.8 (IQR: 1.0, 9.5)), Kruskal Wallis p-value=0.04. Similarly larger differences are observed between groups in BDNF with the highest levels seen in MS-OAB patients (median 53.3 (IQR: 13.6, 127.5)) and the lowest levels seen in Controls (median 9.0 (IQR: 6.1, 38.5)), Kruskal Wallis p-value=0.002.

There is no significant difference in NGF/Cr or BDNF/Cr level between the MS and no OAB and the control group. MS-OAB patients have a significantly higher NGF/Cr and BDNF/Cr level compared to controls. Confidence intervals overlap between the MS no OAB and the MS-OAB, and hence they are unlikely to be significantly different, however the sample size is insufficiently powered to do pairwise comparisons.

Conclusions

This study is the first to analyse the levels of urinary neurotrophins NGF and BDNF in MS patients with mild to moderate OAB symptoms and without OAB symptoms compared to healthy controls. The results obtained confirm that urinary neurotrophin levels are significantly raised in patients with MS compared to healthy controls. This was more marked for patients with MS and OAB than for those with no OAB symptoms. Since the group with no OAB symptoms was small in number, no comment can be made with statistical significance, however there is a trend for the levels to be higher than healthy controls. This may reflect the pathophysiology of MS, patients of which have observed higher baseline circulating neurotrophin levels. This may be influencing urinary levels and therefore render these potential OAB markers less reliable in patients with MS.

INTRODUCTION

Multiple Sclerosis is an inflammatory condition affecting the white matter of the central nervous system (CNS) resulting in demyelination and, in advanced stages, axonal degeneration. The presence of plaques is the hallmark characteristic of this condition, which may be located in the white matter subcortically, brainstem and cerebellum and spinal cord and visible in MR (magnetic resonance) imaging. The aetiology of the condition is thought to be autoimmune, and involves T cell activation and cell mediated inflammation with targeted destruction of myelin.

In the developed world, MS is the most common progressive neurological condition of young adults, with a median estimated global prevalence of 112 per 100,000 person years [154], with a predominance for females of 3:1 [155]. Relapsing remitting MS (RRMS) is the most common type (85 % of patients), but nearly half of these patients convert to secondary progressive MS (SPMS) over a median time period of 11 years [156]. About 10% of patients may have progressive symptoms from the onset (primary progressive MS) and a minority have progressive relapsing MS [156].

The Expanded Disability Status Scale (EDSS) is a method of quantifying disability in MS. This scale ranges from 0 to 10 in 0.5 increments that represent higher levels of disability. An EDSS level of 4 (at which patients can walk some 500 m without aid or rest), is thought to the herald the onset of secondary progressive MS. The mean time from disability level EDSS 4 to 6 (when there is intermittent or constant assistance required to walk 100 m) has been estimated to be 6 to 8.4 years. It is during this period of progression that management of the lower urinary tract dysfunction (LUTD) may become particularly difficult.

Lower Urinary tract dysfunction in MS

LUTD is common in MS, with up to ninety percent of patients complaining of lower urinary tract symptoms (LUTS) at some point [62]. The severity of LUTS is related to the degree of pyramidal impairment in the lower limbs [157]. Urinary incontinence is considered to be one of the worst aspects of the disease, with 70 % of a group of patients with MS responding to a questionnaire classifying the impact bladder symptoms had on their life as "high" or "moderate" [158]. Whether patients suffer from an overactive or a poorly emptying bladder or a combination of both disorders, the bladder dysfunction experienced can greatly reduce the overall QoL due to the functional and psychosocial burden [63]. Effective treatments significantly enhance measures of QoL [159]. However, the severity of symptoms may differ in the degree of distress and bother they cause, as threatened urinary leakage in someone still ambulant may cause greater or less distress than regular episodes of incontinence in a permanent wheelchair user. As the disease progresses, patients may also become prone to developing urinary tract infections (UTIs) that can lead to progressive neurological deterioration [160]. However, as the disease worsens, with pyramidal symptoms affecting the lower limbs causing a decline in mobility and increased disability, the bladder dysfunction becomes more difficult to manage. It is thought that almost 100% of patients have LUTS once they experience walking difficulties [161].

The pattern of LUTS experienced can be either storage or voiding symptoms, or a combination of both. Voiding symptoms due to insufficient detrusor contractility include hesitancy, poor urinary stream, dribbling of urine, and incomplete bladder emptying. This itself can provoke involuntary contractions and result in urinary stasis with subsequent risk of infection. Both of these sets of OAB and voiding symptoms need to be tackled for effective management.

Objective evaluation of LUTD in MS

The current method of assessing patients complaining of this OAB symptom complex is through questionnaire scores, which rely on subjecting patient reporting of urgency. The assessment of symptoms is in itself fraught with limitations considering the vague nature of LUTS and difficulties patients experience in understanding the term "urgency" and providing information about the different severities of the condition. It can be difficult for patients to differentiate between the sensation of urge, a normal bladder sensation, and urgency. One way to overcome this would be with the availability of an objective test for the diagnosis of OAB and to judge its severity.

The measurement of the postvoid residual (PVR) is a critical part of the investigation in the management of LUTS in a patient with MS. One of the cornerstone investigations of patients with OAB symptoms is the use of a frequency/volume chart [162]. However the only objective evaluation tool to assess the storage function of the bladder whilst filling is to perform a urodynamic study. However this test can only identify whether DO is present and not itself diagnose OAB [87].

A urodynamic study of 100 patients with MS with LUTS showed that 78 % had dynamic evidence of LUTD [163]. A regression analysis showed that the likelihood of a urodynamic abnormality in MS was higher in patients who had an EDSS greater than 6.5, who were using more than one continence pad daily, and if the MS was not of the relapsing-remitting type [163].

DO is the commonest pattern of LUTD seen in MS reporting LUTS and in a review of 22 studies on MS populations combining a total of 1882 patients, DO was reported in 62 % of patients undergoing urodynamic investigations [164]. Other abnormalities responsible for bladder symptoms include detrusor underactivity (occurring in 0%-40%, mean 25% of patients) and dyssynergia between the detrusor and external urethral sphincter (detrusor

sphincter dyssynergia, DSD) occurring in 5%-83% (mean 35%) patients with MS [165]. LUTS can be related to the duration of the disease, however 10% of patients can have them at initial manifestation.

It is possible that this functionally disordered co-ordination can change over time, and progress in severity. It is also well known that the bladder symptoms that occur with the various forms of dysfunction are non-specific, coined in the well known phrase 'the bladder is an unreliable witness' [76, 77]. Whilst the range of urodynamic dysfunction is large, the types of bladder symptoms that can manifest are limited, to either storage or voiding LUTS. Therefore it remains difficult to assign symptoms to a particular disease process. For example, poor detrusor contractility may manifest with the same symptoms as bladder outlet obstruction such as hesitancy, urinary frequency, reduced urinary flow and sensations of incomplete bladder emptying. Despite a careful history, patients may interpret their own experiences differently and clinicians may be biased with interpreting their answers [78].

This lack of correlation can be yet more unclear in patients with MS who may have altered bladder sensations, and less able to predict poor or incomplete bladder emptying [157]. The role of urodynamics for the assessment of LUTS in this group of patients remains under some debate with suggested use limited to situations when invasive treatments are being planned, although guidelines from a French expert group recommend its use in all MS patients with symptomatic LUTS [63, 165]. The benefits and limitations of invasive urodynamics are enlisted in table twelve below [166].

Table twelve: Advantages and limitations of urodynamic testing

Advantages	Limitations
Objective evaluation of bladder function during filling and voiding	Invasive test • Discomfort during the procedure • Transient bleeding following procedure • Theoretical risk of infection: 2-4% risk of UTI • Important to exclude UTI as this may falsely result in increased bladder sensation and poor compliance • Prophylactic antibiotics may play a role in patients with recurrent UTIs
To identify or to rule out factors and mechanisms contributing to incontinence and their relative importance in complex cases	Failure to always provide a diagnosis
To obtain information about other aspects of LUTD such as urethral closing pressures and competence	Unable to reproduce patient's symptoms
To predict consequences of dysfunction for the upper urinary tract	Inaccuracies can occur due to • Poor subtraction which may lead to errors • Air bubbles in the system or leaks may lead to errors of measurement • Rectal contractions may be misinterpreted as DO (These artifacts should be identified and eliminated when possible)
To predict the outcome and side effects of a proposed treatment and understand	Interchangeability of study results

its mechanism of action	
To understand reasons for failure of previous treatments for incontinence	

Urinary neurotrophins: An objective marker for OAB in patients with neurological disease?

The role for a biomarker for OAB in patients with MS

The previous chapter has discussed the need for an accurate evaluation tool for patients with MS that could be used to assess physiological function, disease severity, reveal the underlying pathology, monitor the response to treatment, and predict prognosis [167]. Validated questionnaire tools are vital for patient assessment yet remain completely subjective, whilst urodynamics, the most objective tool available, is not without its limitations as mentioned above in table Twelve. A urinary biomarker would therefore be of potential use as sample collection is easy and non-invasive. In this way urinary neurotrophins are being evaluated as a biomarker for overactive bladder symptoms and more specifically DO.

Most of the literature examines urinary neurotrophin levels in patients with idiopathic OAB, with very few studies investigating neurological patients [96, 111, 129, 132, 133]. As for patients with idiopathic OAB, neurological patients would also benefit from such a biomarker. These patients may have more severe bladder dysfunction and symptoms with potentially progressive disease pathology, may not have the mobility to sustain the nature of the OAB symptoms of urinary frequency and urgency, and hence a significant detriment to QoL. Furthermore these patients are at risk of upper urinary tract complications and a biomarker that could identify those particularly at high risk would be highly desirable.

A recent meta-analysis of 8 studies measuring urinary NGF in patients with idiopathic OAB, showed that patients with OAB symptoms had a higher level of uNGF than healthy people [168]. Whether this applies to neurological patients is far less explored and needs further evaluation.

Most of the reports measuring uNGF in patients with neurological disease emanate from a single centre studying limited patient groups. These authors have shown that uNGF levels were significantly higher in 93 patients who have had a cerebrovascular accident(CVA) compared to healthy controls [129]. This increase in level correlated with the severity of neurological impairment. It was suggested that the elevated levels may be directly due to the neurological injury rather than secondary to any LUTD that followed. Levels did not correlate however with the site of the lesion, duration of the stroke, or urinary symptoms in these patients, suggesting that in these patients the uNGF levels were a marker for the severity of brain injury after CVA. It was also observed, albeit in the small number of patients that had urodynamic evaluation, that uNGF levels were comparable in the patients with DO and detrusor underactivity (DUA), also suggesting that in patients with profound neurological impairment, uNGF levels can still be raised despite low detrusor contractility. Serum BDNF levels have also been suggested to increase after stroke [169]. Although the link between NGF and BDNF is unclear, it may be that after brain injury, there is some neurotrophin leakage through the blood brain barrier causing systemic levels of circulating neurotrophins to be raised and therefore more detectable in the urine, rather than a response confined to the bladder. Alternatively there may be a systemic response to neural injury that leads to higher circulating levels. The potential origin of neurotrophins detected in the urine cannot however be distinguished between that released from an injured CNS or those from nerve endings within an overactive bladder.

In addition to this, the evolution of uBDNF as an additional proposed OAB biomarker [144], alongside NGF, also needs to be assessed in patients with MS. BDNF is a ubiquitous neurotrophin in the human body and is produced in sensory neurons in dorsal root ganglia [170]. It is also synthesized by non-neuronal cells in the inflammed bladder, taken up by

bladder afferent nerves and undergoes retrograde migration to the spinal cord where it modulates neuronal function via its TrkB receptors, which are located in the afferent nerves themselves and dorsal root ganglia. It is not fully known how BDNF is involved in the pathogenesis of OAB, but it is recognised to be up-regulated in the dorsal root ganglion in a neuropathic pain model, by overproduction of prostaglandins (PGE2) by injured nerves. NGF is also thought to increase BDNF expression and activate nociceptors [171].

Urinary NGF levels in patients with MS in particular, or other demyelinating disorders, have not been fully evaluated. There is very little evidence and few studies examining the levels of uNGF in patients with MS. Since the prevalence of OAB in MS patients is high, it is pertinent to assess the performance of this increasingly popular potential biomarker in this patient population. Patients with MS have been shown to have a significantly higher basal level of serum NGF, due to the inflammatory CNS pathology compared to healthy controls [172]. Whether this can affect the urinary levels of NGF and whether uNGF can be considered as a biomarker for OAB alone in these patients is unknown. This could be investigated by examining urine from patients with MS and no OAB symptoms as a control population.

Study Aims

The aim of this study was to determine whether urinary NGF and BDNF levels were elevated in patients with MS and OAB symptoms compared to patients with MS and no OAB symptoms or healthy controls. The MS patients targeted were those with mild to moderate OAB symptoms. The results of this study may help to clarify whether urinary neurotrophin levels can be used to diagnose OAB in patients with MS and whether urinary baseline levels are raised in MS patients with no OAB symptoms. Furthermore any relationships between urinary neurotrophin levels and self-reported OAB scores and EDSS scores can be observed. The primary and secondary objectives of this study are stated below.

Primary objectives

To measure urinary NGF/Cr and BDNF/Cr levels in three patient groups:

- Group One: Healthy volunteers with no OAB symptoms (hospital staff).
- Group Two: Patients with MS who have no OAB symptoms.
- Group Three: Patients with MS with OAB symptoms.

Secondary end point

To evaluate any relation between uNGF and uBDNF levels and the severity of OAB assessed using a subjective patient reported OAB subset of the Urinary Symptom Profile questionnaire (USP) [173] (See appendix). Any relation with between urinary neurotrophin levels and EDSS scores can also be observed.

Study Hypothesis

The levels of urinary neurotrophins, specifically NGF and BDNF are raised in the urine of patients with MS who are suffering from OAB symptoms compared to both:

1) Patients with MS not reporting OAB symptoms

2) Healthy controls.

MATERIALS AND METHODS

Study design

Roughly 3000 patients with MS visit the National Hospital for Neurology and Neurosurgery (NHNN) annually. Therefore the hospital is well suited to recruit patients for this study, who were counselled, provided with the patient information leaflet, consented and recruited during their clinic visit.

The close proximity of the Neurometabolic Unit to the OP at the Department of Uro-Neurology allows for efficient sample collection and storage. Negative control samples were taken from members of staff representing the healthy volunteers group with no OAB symptoms.

Inclusion Criteria

Patients and healthy volunteers who were able to and willing to provide written consent to enrol into the study were included.

Participants had to demonstrate the ability to understand and complete the self-reported urinary symptom profile (USP) to assess symptom severity.

Exclusion Criteria

The conditions which excluded participants from being recruited, as these may influence the urinary NGF result, either causing a false positive (FP) or a false negative (FN):

- Evidence of a urinary tract infection on urine dipstick testing which may cause a FP
- Patients with a history of bladder outflow obstruction due to benign prostate enlargement as this may cause a raised urinary NGF level causing a FP [134].
- Patient having received intradetrusor injections of Botulinum toxin over the previous 6

months with continuing improvement in symptoms, which may reduce NGF levels causing a FN [112].

- Patients with any known history of urological disease such as malignancy or stones may cause a FP [174].
- Patients with long-term indwelling urinary catheters or carrying out CISC as the urothelial trauma induced may cause a FP in urinary NGF.
- Renal disease

Patients on antimuscarinics were not excluded, however, their use was noted. This is important as it has been shown in previous studies that patients with OAB, who are responders to antimuscarinic therapy have reduced levels of urinary NGF [128].

Urinary Symptom Profile (USP)

Patients completed the USP to assess their OAB symptoms (See Appendices). The USP is a questionnaire developed by the MAPI research trust. It has been standardised and validated and had its clinical validity demonstrated against bladder diary parameters in a large study that assessed data from OAB patients and healthy controls [173]. It is a straightforward and brief questionnaire that enables symptom screening and evaluation of symptom severity. The USP has been recommended by the International Consultation on Incontinence (ICI) that reports its development and validation has followed rigorous and thorough methodology.

Patients self-assess symptoms through a series of questions and total a final aggregate score. The final score ranges from 0-21, with 21 representing highest symptom severity and 0 representing absence of symptoms. MS patients with no OAB, and healthy controls were determined with a score of zero on the USP.

Collection and Urinary Enzyme-Linked Immunosorbent Assay (ELISA)

Once consented, participants were asked to provide a urine sample when they had a strong desire to void. Voided urine was immediately dipstick tested for traces of a UTI. If an infection was detected, patients were excluded from the study and the UTI was treated. Voided urine was then immediately placed on ice and transferred to the laboratory for preparation for NGF measurement. The urine samples were centrifuged at 3000g for 10 minutes at 4°C. The supernatant was separated into five 1.5ml aliquots in polypropylene eppendorf tubes and preserved in a freezer at -70°C. Because freeze-thaw cycles decrease protein stability, the samples for frozen storage at -70°C were dispensed and prepared in numerous single-use aliquots so that, once thawed, the urine sample would not be refrozen.

At the same time, 3mL of urine was taken to measure the urinary creatinine (Cr) level.

Urinary NGF concentration was determined using an immunoassay system (Emax®, Promega, Madison, WI, USA) with a specific and highly sensitive ELISA kit, which had a minimum sensitivity of 7.8 pg/mL. Assays were conducted according to the manufacturer's instructions. Generally, urine samples were not diluted in the ELISA assay. When the urinary NGF concentration was higher than the upper detection limit (250 pg/mL) the urine samples were diluted to fit the detection limit. All samples were run in duplicate and the values were averaged.

Security: Data Handling and Record Keeping

The investigators permit study-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Patient consent to this is specifically sought in the Consent Form. Participants enrolled into the study were given a study number, and completed questionnaires were identifiable by the hospital number. These documents will be stored in a case report file (CRF). Results were stored in a secure password protected database, only accessible by a member of the research team.

Patient details were anonymised and study data could not be linked to individual patients other than by those authorised to have access and not in any publication of results. All records were maintained in accordance with the Data Protection Act 1998 and Caldicott committee.

Ethics

To acquire the necessary approvals, a study protocol, participant study information leaflet (PIL), participant consent form, and letter to the general practitioner was produced. An NHS research ethics committee (REC) form and site-specific (SSI) application forms were completed on the integrated research application systems (IRAS) website. These documents were compiled and reviewed by the relevant authorities.

Approvals (See Appedices)

This study was reviewed and a certificate of approval acquired from:

- UCLH Biomedical Research and Development department.
- East London Central REC1 Research NHS Ethics Committee at the Royal Free Hospital approved the study with REC reference number: 10/H0721/33. Informed consent was obtained from all participants.

Signed, informed consent was obtained from all participants before recruitment into the study. Participants were given time to read the study patient information leaflets, and the opportunity to ask questions about the study.

These approvals are provided in the appendices. Approval for the use of the Urinary Symptom Profile was also acquired from the MAPI research trust.

The study was conducted in compliance with the protocol, good clinical practice (GCP) and the applicable regulatory requirement(s).

The study represents the collaboration between the department of Uro-Neurology and the Neurometabolic unit where regular delivery of samples were being received from the outpatients department. Freezer space and use of centrifuge equipment, bench space was provided under their supervision and experience with running the laboratory experiments.

MATERIALS

Patient recruitment and sample collection

Urinary symptom profile

Urine dipsticks

Box of ice

Polypropylene 25ml urine collection tube

Refrigerated centrifuge (for spin at 300g at 4°C), and -70°C freezer

The urinary ELISA

96-well plate (falcon-polystyrene)

Anti-NGF pAb

Anti-NGF mAb

Anti-Rat IgG-HRP conjugate

NGF standard

Block and sample buffer 1X

TMB One solution

These items are provided by PROMEGA in the NGF E_{max} ImmunoAssay System Kit

The constituents of the necessary reagants for this assay are described below:

1. Carbonate coating buffer:

   100mls deionised water

   1.59g of Na_2CO_3 powder

   2.93g of Na HCO_3 powder

   Adjust pH to 9.7 using 1N HCL or 1N NaOH

2. TBST wash buffer

   500mls deionised water

1.2114g of Tris-HCL

4.383g of NaCl

0.05% Tween 20 (500µl for 1L)

3. Block and sample buffer 1X

5mls of block and sample 5X

20mls of deionised water

1N Hydrochloric acid

Multichannel pipettor

Pipettors capable of accurately delivering volumes of 1µl to 1ml

Plate shaker

Microplate reader capable of monitoring absorbance at 450nm

The ELISA

An ELISA is a basic application of antibodies to analyse soluble antigens by passive binding [175]. This allows the simultaneous processing of many small samples. It requires at least two antigen specific antibodies which will capture the antigen in a sandwich format [175]. The capture antibody is used to coat a solid substrate, in this case a plate well. This captures and immobilizes the antigen from the sample, which is then applied.

Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay by washing [176]. This ability to wash away non-specifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation [176].

An unlabelled primary antibody is then added, which also specifically binds the antigen. The capture and primary antibodies must be different isotypes, so that the secondary, when added only detects the presence of the primary antibody to correctly indicate that the antigen has been captured. A second antibody is then used which is usually conjugated with an enzyme, in this case, horse radish peroxidase for detection [175]. Figure ten illustrates the capture sandwich assay.

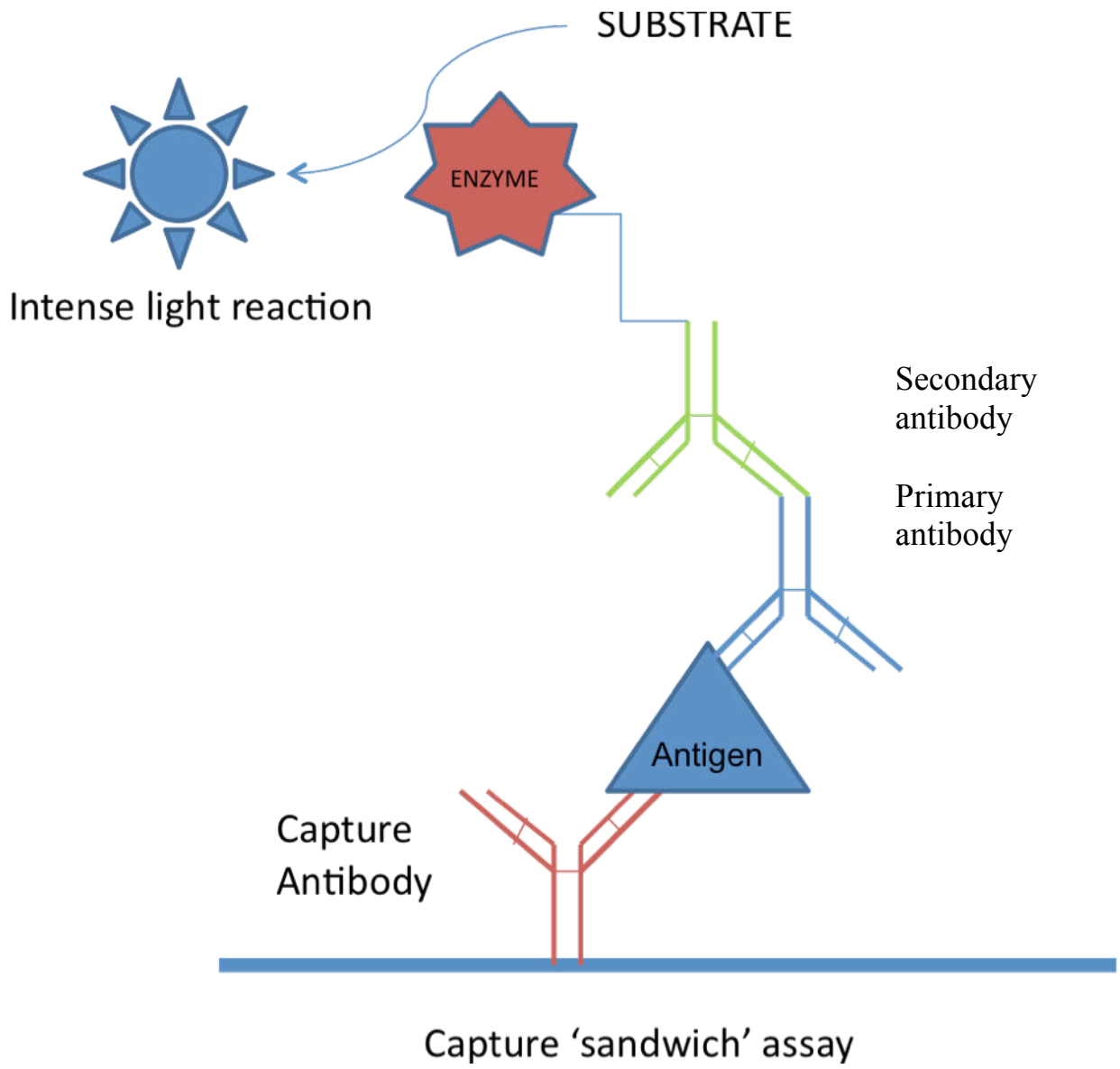


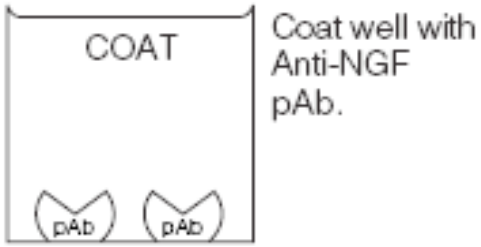
Figure ten: The sandwich assay. The capture antibody is coated on to the plate. The antigen is then applied. Non-specific binding is minimized by washing in between each step. Unlabelled primary followed by conjugated secondary antibodies are then used. After the addition of a chromogenic substrate, a direct light reaction can be directly visualized, and quantified using a plate reader.

The NGF ELISA is designed to sensitively and specifically detect NGF in an antibody sandwich format. The kit manufacturers state that this test demonstrates a less than 3% cross-reactivity with other neurotrophic factors at 10ng/ml and offers a sensitivity of detecting a minimum of 7.8pg/ml of NGF. This is a three-day ELISA with two overnight incubations using a flat-bottom 96-well plate. The anti-NGF pAb, captures the soluble NGF. The captured NGF is bound by a second specific monoclonal antibody (mAb). After washing this specifically bound mAb is detected using a species specific antibody conjugated to horseradish peroxidise (HRP) as a tertiary reactant. The unbound conjugate is removed by washing, and following incubation with a chromogenic substrate, a colour change is observed and measured. The amount of NGF in the solution is proportional to the intensity of the colour generated in the oxidation reaction. The absorbance of the light intensity is recorded at 450nm on a plate reader. The ELISA protocol is described below, as per the instructions for product use provided by Promega. The quantities of solutions described are those required for a full 96-well plate. The procedure is described below.

The Procedure

Day ONE

Step one: The wells are coated with the primary anti-NGF polyclonal antibody (pAb) and left to incubate overnight at 4°C. This involves creating a solution of 10mls of carbonate coating and 10µl of anti-NGF pAb. 100µl of this solution is added to each well (see figure below).



Taken from NGF Emax ImnunoAssay Systems Protocol

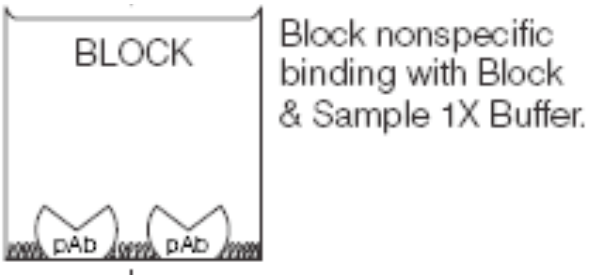
Day TWO

Step two: Wash once

The contents of the well need to be emptied and washed. This is achieved by turning the plate upside down, to discard the contents and the slap it down on to paper towels. Each well is then washed once, by applying 200µl of TBST wash buffer and then slapped out again numerous times on to paper towels.

Step three: Block nonspecific antibody binding

To prevent non-specific bind of NGF to the parts of the well which are not coated with pAb, 200µl of block and sample buffer 1X are added to each well. The plate is then incubated at room temperature for one hour. At the end of this hour, the plate contents should be emptied into a waste tray, and then slapped onto paper towels.



Taken from NGF Emax ImnunoAssay Systems Protocol

Step four: Wash once

The plate should then be washed once with TBST wash buffer (200µl/well) and slapped out onto paper towels.

Step five: Create the standard curve and load samples into plate

During the one hour incubation in step three, the standard curve needs to be prepared, and the aliquots of urine samples should be removed from the freezer and allowed to thaw.

Standard curve: The standard curve is a quantitative research tool. This is a method of plotting assay data, using a known set of concentrations of a protein, against the assay measure, in this case NGF and absorbance. Once these are plotted in a graph, the relationship between the two can be identified, and an equation for this derived. After this, absorbances from the patient samples can be used to calculate the level of NGF in the urine sample.

Nine eppendorfs should be lined in a rack. The standard curve created from the NGF standard provided with this kit, will generate a linear standard curve from 3.9-250pg/ml. Only values within this linear range can be used to determine the NGF concentration of the test samples. The NGF standard provided is at 1µg/ml. The standard is accurately diluted to a concentration of 1:4,000 in block and sample (B&S) 1X buffer. This dilution represents 250pg/ml of NGF standard.

For example:

1:50 dilution is created first	5µl of NGF standard is added to 245µl of B&S 1X
Then a 1:80 dilution is created	10µl of (1:50) is added to 790µl of B&S 1X. This results in a 1:4,000 dilution which represents 250pg/ml.

The first eppendorf contains the 1:50 dilution and the second the 1:4,000 which represents 250pg/ml. At this stage 250µl of B&S 1X should be pipetted into all of the other eppendorfs. Once the 1:4,000 dilution is created, then a serial doubling dilution is performed down the eppendorfs in the rack, creating the following concentrations 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, 15.6pg/ml, 7.8pg/ml, 3.9pg/ml, & 0pg/ml. Figure eight illustrates how these 1:2 dilutions are performed to produce the standard column.

The standard curve is run in duplicate, in case of contamination out of or into one of the wells within the standard column. The average value will then be taken from the two results. The two standard columns can be arranged together or at either end of the plate. Figure nine is an example of a plate format with two standard columns. The curve can then be transferred from the eppendorfs into the wells. Each well should contain 100µl.

Once the standard curve has been created, the participant urine samples should have thawed and can now be arranged. These will be added in duplicate and the average result taken. Participant samples will also be added as a neat concentration and a 1:2 dilution. In case the signal strength of the neat sample is too high for the assay, above the range of measureable NGF of 250pg/ml, then the 1:2 dilution may then be within this range. The NGF value can be extrapolated from the standard curve acquired and multiplied by two for the true NGF concentration.

Taken from NGF Emax ImnunoAssay Systems Protocol

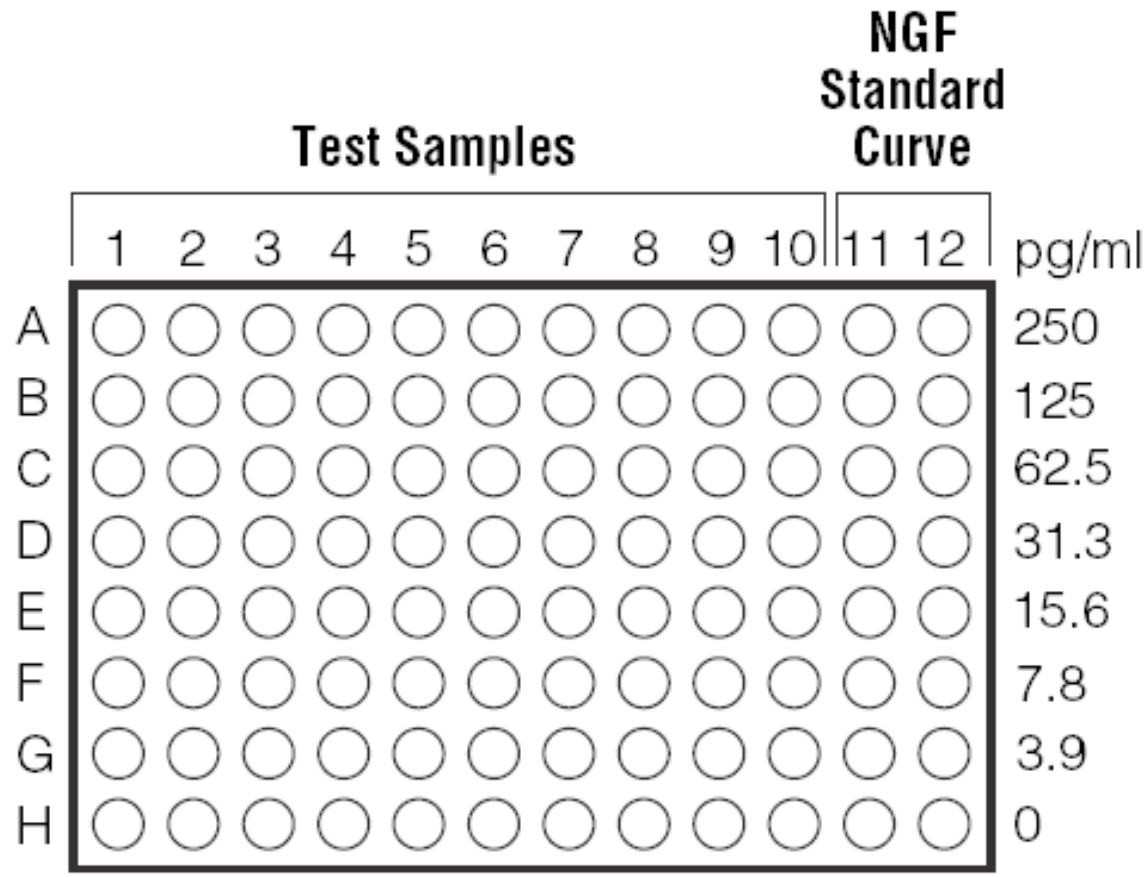


Figure eleven: An example of a plate format with two standard columns.

Throughout the ELISA, care needs to be taken not to handle or touch the bottom of the plate, as any finger prints or dirt that may be imprinted on the under surface can reduce the absorbance value for those wells when being read in the plate reader.

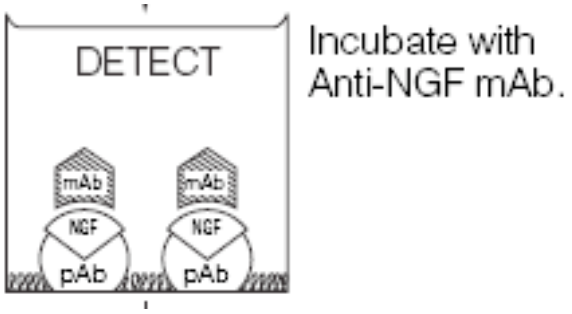
Once the standard columns and samples are all added to the wells, 100µl in each well, then this is left to incubate for 6 hours with the plate is left shaking on a plate shaker.

Step six: Wash five times

Stop the 6 hour incubation, and wash the plate 5 times using TBST wash buffer, as performed previously.

Step seven: Overnight incubation with secondary antibody (monoclonal anti-NGF mAb)

The secondary antibody is prepared, with 2.5µl of anti-NGF mAb and 10mls of B&S 1X. 100µl of this mixture is then pipette into each well. The plate is then stored at 4°C without shaking, to allow effective incubation.



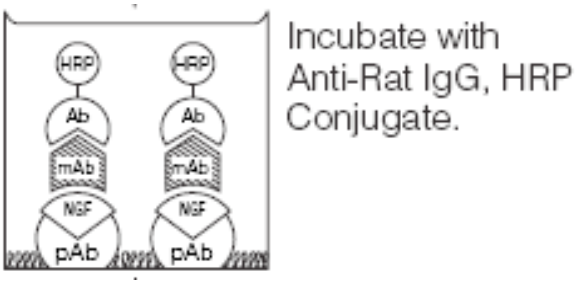
Taken from NGF Emax ImnunoAssay Systems Protocol

Step eight: Wash five times

The plate is washed five times as previously described.

Step nine: Incubation with tertiary antibody

The tertiary antibody is prepared. This is 100 µl of anti-Rat IgG-HRP conjugate mixed with 9.9ml of B&S 1X. 100µl total of this mixture is added to each well. The plate is then incubated for 2.5 hours at room temperature on a plate shaker.

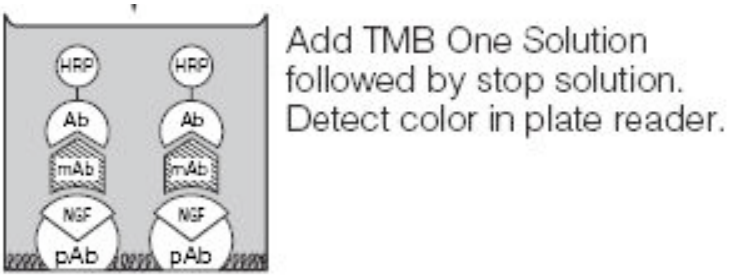


Taken from NGF Emax ImnunoAssay Systems Protocol

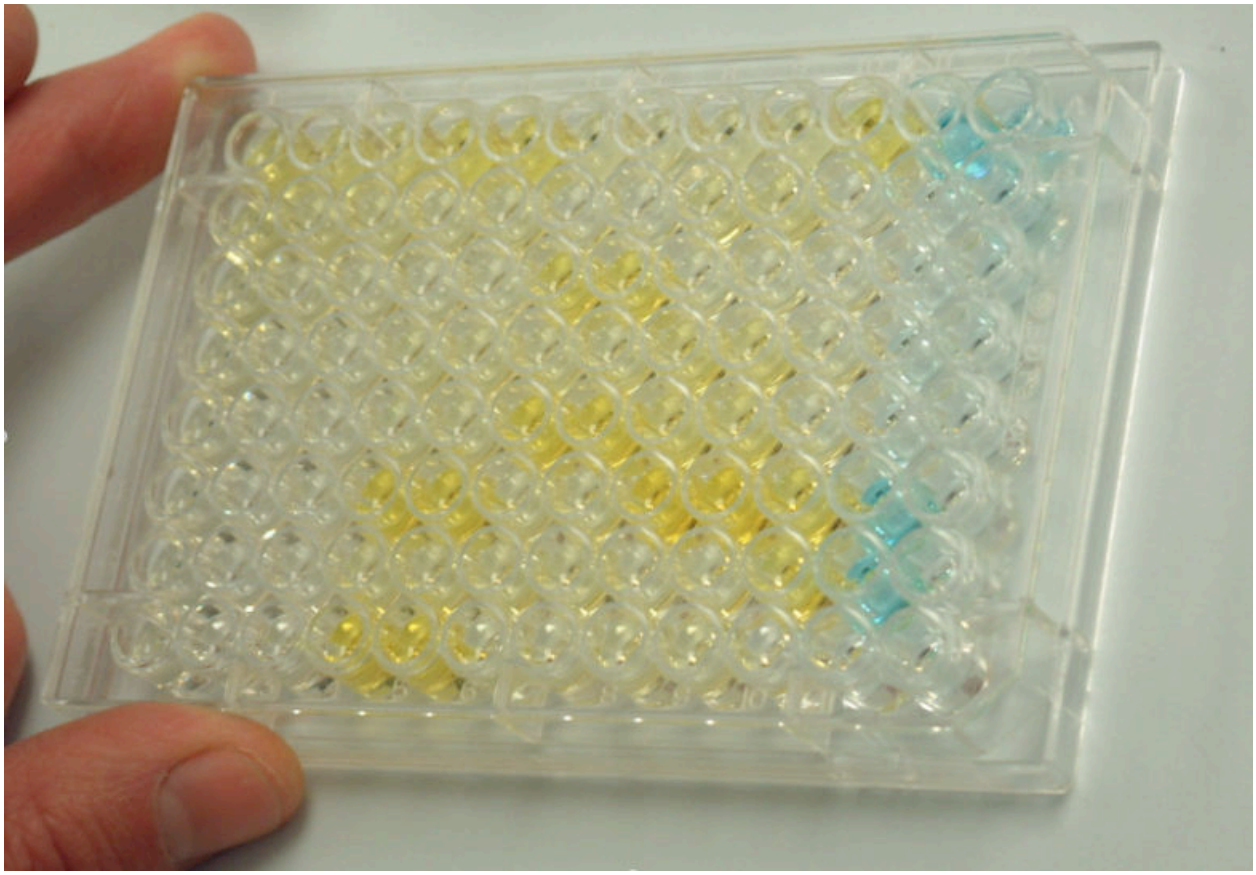
Step ten: Wash plate five times

Step eleven: Colour development with TMB one solution

100µl of TMB one solution is then added to each well. The plate is then incubated with shaking for 10 minutes. An intense blue light is produced with this reaction. After ten minutes 100µl of 1N hydrochloric acid (HCl) is added to the wells to stop the reaction. The blue colour becomes yellow upon acidification. The absorbance is then read at 450nm on a plate reader, within 30 minutes of stopping the reaction.



A picture of the plate and wells can be seen in photo one below. The blue colours of the peroxidase reaction turn yellow once this is stopped with HCl. The intensity of the yellow colour is proportional to the concentration of NGF within each well. This is analysed by the plate reader at 450nm.



Photograph One. The loaded plate with ELISA. (Photo taken by author JS from the neurometabolic unit laboratory at Queen Square).

METHOD DEVELOPMENT

The method development was full of challenges and several steps had to be taken to optimise this. During the course of this study, it took many attempts, by changing one parameter at a time to perfect the ELISA. Some examples of the challenges faced are shown below from individual plates.

Plate One

The results for the first plate:

Table thirteen below. Plate plan for plate one. Only four columns of this plate were used.

Table thirteen	1	2	3	4
A	250	P1 1:1 (neat)	P3 1:1 (neat)	250
B	125	P1 1:1 (neat)	P3 1:1 (neat)	125
C	62.5	P1 1:2	P3 1:2	62.5
D	31.3	P1 1:2	P3 1:2	31.3
E	15.6	P2 1:1 (neat)	P4 1:1 (neat)	15.6
F	7.8	P2 1:1 (neat)	P4 1:1 (neat)	7.8
G	3.9	P2 1:2	P4 1:2	3.9
H	0	P2 1:2	P4 1:2	0

The placing of the standard columns: Each standard is loaded twice, and the average absorbance reading will be taken, in case of any well to well contamination. The wells loaded with the standard curves have been placed on either end of the plate. This can be seen from the absorbances acquired in table three below. The plate is loaded manually, well by well, from the left side to the right.

Four participant samples, number P1 to P4, have been loaded in the middle two columns. Participant samples were loaded as neat and as a one in two dilution. The absorbances for this plate are tabulated below in table fourteen:

Table fourteen: The absorbances from the plate reader for plate one.

ABSORBANCES	1	2	3	4
A	0.919	0.075	0.094	0.917
B	0.592	0.076	0.094	0.57
C	0.363	0.077	0.121	0.358
D	0.235	0.071	0.099	0.228
E	0.159	0.084	0.073	0.155
F	0.117	0.084	0.077	0.119
G	0.18	0.079	0.075	0.096
H	0.083	0.08	0.075	0.075

Inference from plate: Insufficient participant sample signal

From this table it can be seen that the average absorbance values for 0pg/ml in wells H1 and H4 is 0.079. This absorbance represents the average background absorbance value in the blank wells, and needs to be subtracted from the rest of the values in the remaining wells to provide the true absorbance being produced from the enzyme-substrate reaction.

The absorbances from the standard columns have replicated the doubling dilutions of the NGF standard appropriately. This can be verified as the values from well B1 and B4 and approximately half of A1 and A4 respectively, and the values from C1 and C4 are approximately half of B1 and B4, and this pattern is observed down to G1 and G4. However there is an anomaly for the disproportionally high absorbance of 0.18 in well G1, which was loaded with 3.9pg/ml of NGF, when compared to the duplicate in G4 which is 0.096. This could represent contamination from another well during one of the numerous washing steps of the assay.

From table fourteen, it can be seen that the absorbance readings for the participant sample one to four are small and not significantly different from that observed from the 0pg/ml loaded wells from the standard column.

The absorbance values of the standard column can be plotted against the concentrations of standard NGF loaded into each well, to produce a line which represents a linear relationship. This can be seen in figure twelve below.

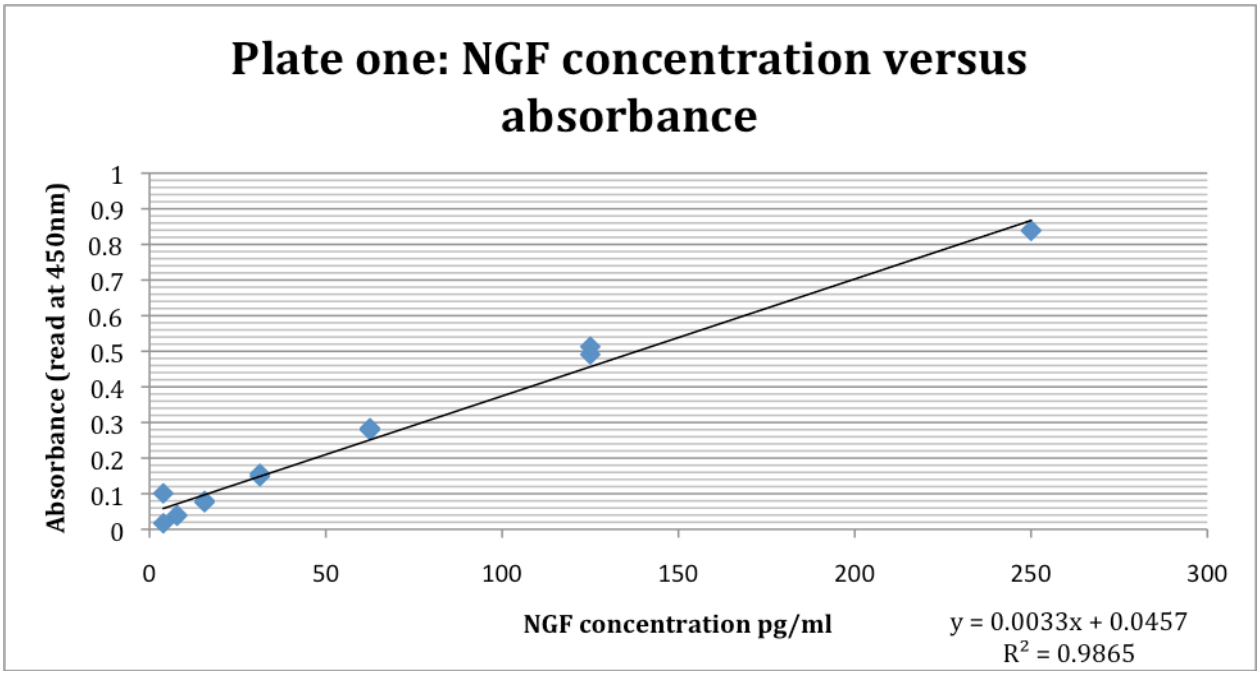


Figure twelve: The linear relationship between the standard dilutions of NGF concentrations and absorbances are demonstrated. The equation for the relationship is provided, and this can be used to detect NGF concentrations from the absorbances acquired from the patient samples. The R-squared value of 0.987 reflects a truly linear relationship between the NGF concentration and the absorbance. This value for r-squared represents a strong correlation between NGF concentration and absorbance.

Troubleshooting:

The absorbances from the sample wells are very low, and not significantly different from the wells containing 0pg/ml. This particular plate therefore detected 0pg/ml in each sample P1 to P4. A low sample absorbance could be due to:

- Instability of NGF (it has been previously reported that NGF is unstable, and levels deteriorate rapidly, and the time from sample collection to storage at -70°C to be minimised)
- A collection error (whereby the method of collection is leading to the loss of NGF protein)
- The urine samples being too dilute, which may need concentrating to increase the NGF signal
- There is no measurable NGF in any of the samples

It was concluded that the plate had not been successful, as there was insufficient signal strength from the absorbances of the wells with patient samples. To tackle this, based upon these possibilities, the protocol was modified to try and maximise the signal strength from these wells.

To correct this numerous steps were taken, changing one variable at a time. Each step taken and the rationale for its change is summarised below. After each single modification, a new ELISA plate was processed to assess the impact of that change. For the purposes of brevity, not all of the individual plate plans and absorbances are shown, but just some to illustrate how the challenges were overcome.

Modification step one: Place urine samples on ice immediately after collection

Previous reported protocols on the collection of urine for NGF measurement suggest, placing urine samples on ice immediately [96]. Studies examining other urinary proteins have

demonstrated that these proteins are often unstable when not in their native environments, and short term storage at room temperature can lead to degradation [177]. Up to this stage collected urine samples were collected and transferred to the lab, and within 60 minutes were then centrifuged at 4°C, before storage at -70°C. This measure should minimise the risk of NGF protein deteriorating, during this time.

Therefore the sample collection protocol was changed and samples placed on ice immediately after collection and then immediately transferred to the laboratory to reduce any time delay to freezer storage.

Plate Two

The results from plate two were very similar to plate one. The absorbances of the standard columns were reproducible, with accurate doubling dilutions. However there was still negligible signal from the sample wells.

Modification step two: Using an internal quality control

It is well known that urine contains proteases and proteinases, which could theoretically break down any measurable NGF [178]. Therefore any NGF protein within the urine samples could be degraded and broken down by other urinary constituents, and hence by the time of analysis there is no measurable quantity remaining. To ensure that NGF is stable with the natural matrix of the urine samples provided, urine samples were spiked with a known quantity of standard NGF.

Table fifteen below shows the plan of plate three with a standard column of spiked control urine from an asymptomatic, healthy volunteer. These control samples were freshly collected and once provided, were spiked with standard NGF and immediately loaded into the wells.

Patient samples (P1 and 3) were also loaded into wells in a neat or a 1:2 dilution. Control urine was spiked (SC) with doubling dilutions of a known concentration of standard NGF and loaded as a standard column in wells 2E to 2H and 3E to 3H.

Again only four columns in total were loaded in this plate seen in table fifteen.

Table fifteen	1	2	3	4
A	250	P1 1:1 (neat)	P3 1:1 (neat)	250
B	125	P1 1:1 (neat)	P3 1:1 (neat)	125
C	62.5	P1 1:2	P3 1:2	62.5
D	31.3	P1 1:2	P3 1:2	31.3
E	15.6	SC 250	SC 15.6	15.6
F	7.8	SC 125	SC 7.8	7.8
G	3.9	SC 62.5	SC 3.9	3.9
H	0	SC 31.3	SC 0	0

Table fifteen shows the plan of plate three

SC: Control urine spiked with a known standard quantity of NGF

The absorbances of the ELISA for plate three are illustrated below in table sixteen below:

ABSORBANCES	1	2	3	4
A	0.578	0.068	0.072	0.498
B	0.404	0.068	0.067	0.31
C	0.255	0.064	0.067	0.198
D	0.167	0.062	0.066	0.133
E	0.115	0.527	0.081	0.098
F	0.09	0.342	0.079	0.078
G	0.078	0.2	0.066	0.074
H	0.063	0.106	0.064	0.067

Inference from plate: Reproducible standard curve for spiked control urine (wells E2-H2, E3-H3). Spiked NGF is therefore stable within the urine matrix. There remains to be negligible signal in participant samples.

The standard columns on either end of the plate are reproducible and the doubling dilutions are clear and appropriate from the absorbances. The absorbances from the spiked control urine, also corresponds well to the absorbances from the standard curve. The absorbances demonstrate the effective doubling dilutions that have been performed in the SC urine. There was still no signal acquired from the patient samples.

However it was noted that the signal strength from the absorbances acquired for the standard columns (A1 to G1 and A4 to G4) were becoming reduced, compared to plate one. The wells loaded with 250pg/ml of NGF standard in plate one demonstrated absorbances of above 0.9, whereas the absorbances for plate three are just above 0.5. The plot of the dilutions of NGF concentrations versus the absorbances also provides a linear relationship that was previously

demonstrated, although the intensity of the absorbances is lower. This is demonstrated in figure thirteen below.

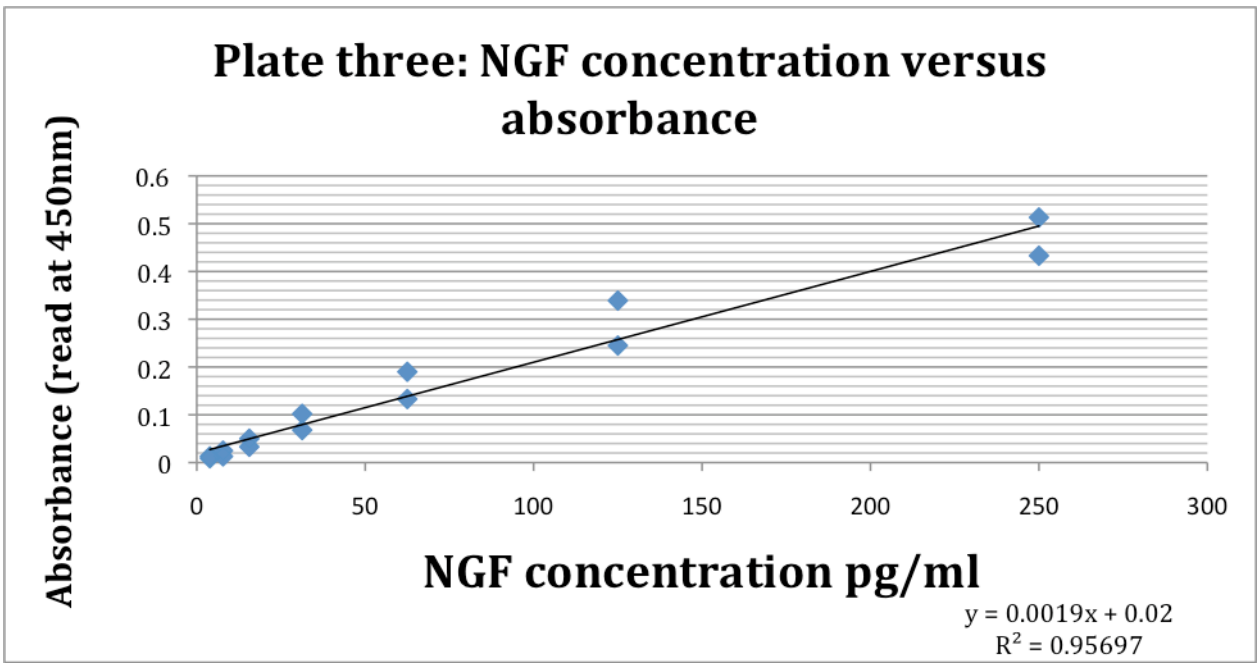


Figure thirteen: The linear relationship between the standard NGF concentrations and absorbances are demonstrated. The equation for the linear relationship is also described, with a strong correlation as demonstrated by the R-squared value.

Troubleshooting:

There was a lack of significant signal from the patient samples. The difference in signal strength of the standards from the previous plates, was thought to be from premature expiry of the standard. Instructions from Promega (see Appendices), suppliers of the standard, which is frozen on delivery at -20°C, is to store the standard NGF, after first use, once opened and thawed at 4°C, which should be stable for three months. At this stage the standard used had been stored at 4°C for three weeks, which is within this recommended time frame. However since the standard columns were reproducible and produced a similar linear relationship when plotted, this stock of standard was still used to develop the assay.

Modification step three: Change the collecting tubes from polystyrene to polypropylene.

The different types of sample collecting tubes available can have an effect on an ELISA. It has been demonstrated to have an effect on the concentration of particular proteins. An experiment examining concentrations of protein biomarkers for neurodegenerative diseases, in cerebrospinal fluid (CSF) showed that there is significantly less detectable protein when polystyrene collection tubes are used [179]. Samples collected with polypropylene collection tubes, on the other hand had significantly higher levels of these proteins or biomarkers [179]. This is suspected to be due to adherence of these molecules to the polystyrene surface [179]. It has also been reported that many proteins can adhere to a solid polystyrene surface, and affect the outcome of immunoassays [180].

Previously, all patient samples had been collected in a 25ml polystyrene collection tube. The protocol was then changed to using polypropylene tubes for collecting patient urine samples, which caused a slight delay as it took some time to recruit a sufficient number of new samples. Once enough samples were collected using polypropylene tubes, another ELISA was performed. Participant samples 1 to 6 (P1 to 6) were loaded into the wells.

Internal quality control (IQC): numerous aliquots of control urine had previously been spiked with 125pg/ml of NGF, and were stored at -70°C. The NGF concentration within this IQC would represent the total of 125pg/ml and the background NGF level in the control sample. This was loaded into the wells in G2, G3 respectively. The use of an internal quality control is a well recognised way to monitor the performance of an ELISA [176]. This is part of good laboratory practice, along with regular charting of all data, and monitoring the assays between different plates to identify any trends such as poorly performing kits or differences between operators [176]. As the specified range of sensitivity for the assay, advised by the suppliers is from 3.9 to 250pg/ml of NGF, a concentration of 125pg/ml was used to spike the IQC as this is comfortably within this range for sample detection.

Photograph two: Left a polystyrene tube, which is more clear in appearance. Right, a polypropylene tube which appears more cloudy. Photos taken from www.djblabcare.co.uk

Table seventeen: The plate plan for plate four.

Table six	1	2	3	4
A	250	P1 1:1	P1 1:2	250
B	125	P2 1:1	P2 1:2	125
C	62.5	P3 1:1	P3 1:2	62.5
D	31.3	P4 1:1	P4 1:2	31.3
E	15.6	P5 1:1	P5 1:2	15.6
F	7.8	P6 1:1	P6 1:2	7.8
G	3.9	IQC	IQC	3.9
H	0	0	0	0

Table eighteen: The absorbances of plate four.

ABSORBANCES	1	2	3	4
A	0.445	0.291	0.09	0.435
B	0.241	0.121	0.063	0.243
C	0.157	0.074	0.07	0.153
D	0.118	0.148	0.059	0.107
E	0.088	0.125	0.063	0.088
F	0.078	1.171	0.282	0.079
G	0.074	0.692	0.68	0.074
H	0.057	0.058	0.058	0.081

Inference from plate: The ELISA has worked, as there is now a stronger signal detected from the patient samples. The importance of the types of tubes used to collect urine samples for measuring uNGF was therefore identified.

However, as with the previous plate, the signal strength from the standard curve is evidently decreasing further, and the signal from the IQC at 125 pg/ml from the standard stock remains high. This can be seen by comparing the absorbance from wells G2, G3 to B1 and B4. However by this stage the NGF standard had been stored at 4°C, for two months, since it was thawed, as advised by the supplier, whilst the IQC has been stored at -70°C. By using the IQC, it was identified that the NGF standard stock, once out of -70°C deteriorates rapidly. It can be seen that the signal acquired by IQC, corresponds well with the absorbances of 125pg/ml acquired from the first plate, when the fresh standard stock was first thawed, and can be compared from table three. The stock has evidently denatured during this time, and this was fedback to the supplier, who had suggested its stability for 3 months at 4°C.

Using the polypropylene tubes to collect and centrifuge the urine, appears to have provided an absorbance in the wells containing patient samples. From the graph of the standard curve, the concentration of NGF in the patient samples can be deduced. However as the absorbances from the standard column are deteriorating over time, this would progressively lead to falsely high sample absorbances and therefore values as a result.

Standard denaturisation would affect all absorbances, and can impact on the accuracy of the assay. As it can be seen in figure five below that it does not denature in a linear fashion. This graph demonstrates the deterioration of the absorbances from the stock at 125pg/ml, 250pg/ml, stored at 4°C and the slower deterioration of the IQC, aliquots of which was stored in the freezer at -70°C. This stock of NGF standard was first used in the plate on the 14th February and by the 14th March, absorbances from the same standard concentrations are considerably less.

As a result all of the study patient samples were analysed using standards from a fresh kit straight away once opened to limit the effect of freeze-thaw signal protein degradation and signal deterioration.

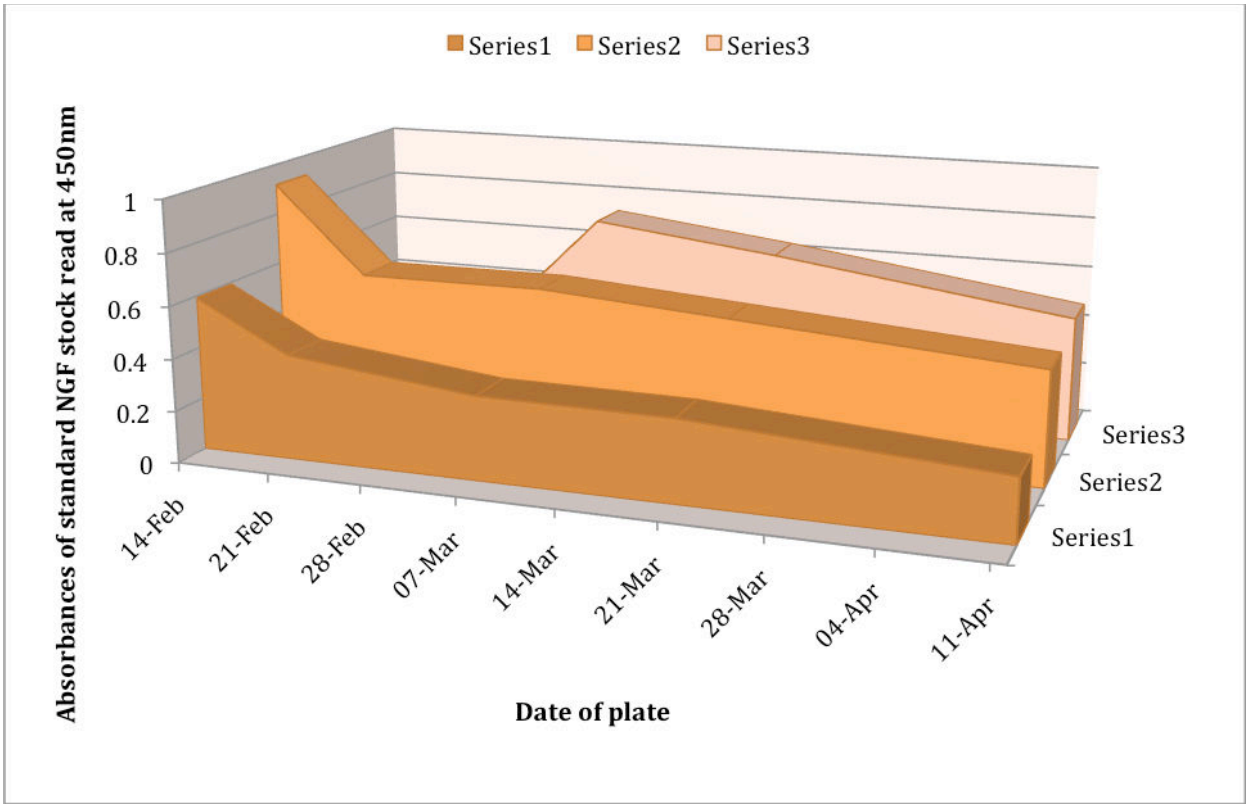
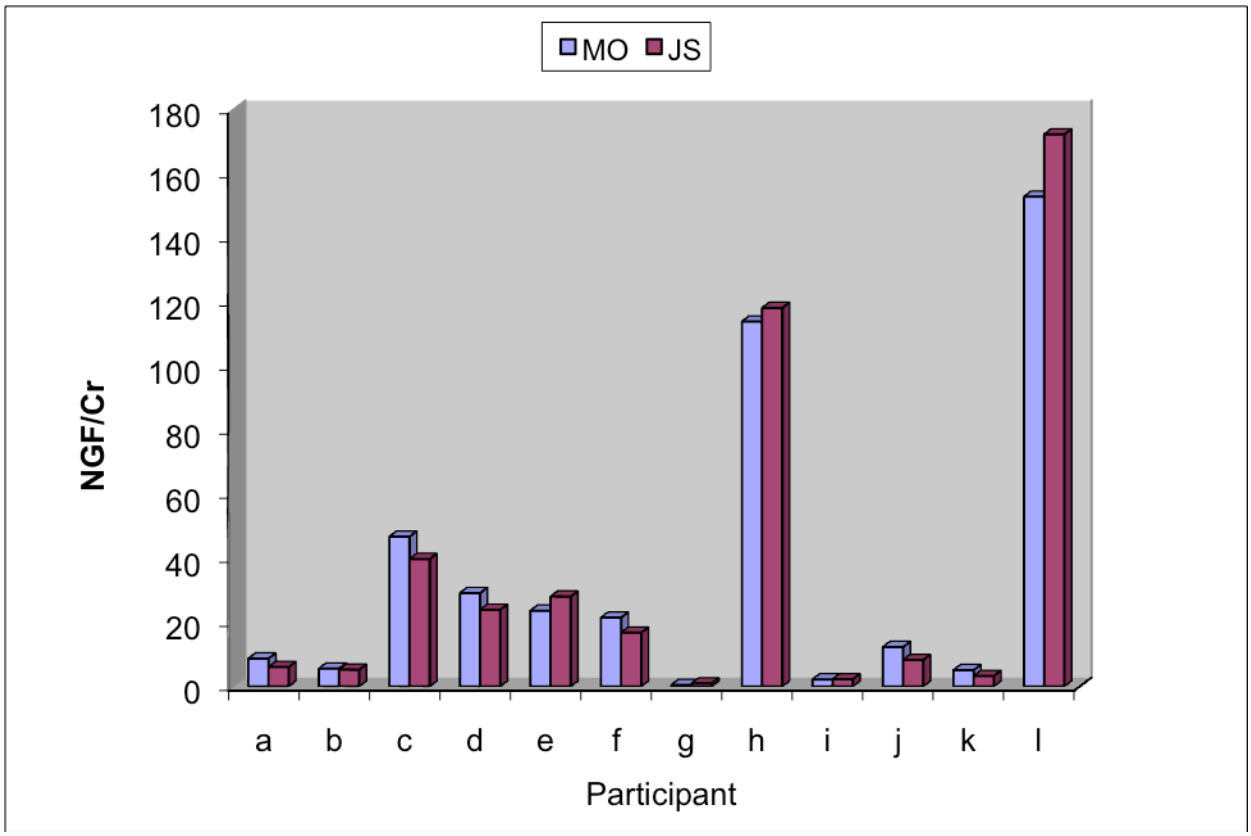


Figure fourteen above: This graph shows the deterioration of the absorbances from the 125pg/ml, 250pg/ml and IQC dilution of NGF standard over a two month period. Series 1: 125pg/ml. Series 2: 250pg/ml. Series 3: IQC (spiked urine from healthy control number 4 with 125pg/ml).

As another quality control measure, to ensure that the low absorbances from the standards were not due to operator error from lack of experience, a scientist more familiar with performing ELISA's also performed an independent plate for comparison. The results of two plates with identical plate plans, but with two different operators have been compared in figure fifteen below.

Figure fifteen: Bar chart showing the comparable ELISA results from two different operators.



This bar chart demonstrates the effect of using double operators to perform the ELISA. It is observes that the absorbances and NGF/Cr ratios acquired for 12 participant samples, from two operators on two separate plates are comparable. As these values are obtained from the standard curves loaded in each plate, this also demonstrates that the low absorbances from the NGF stock are not due to an error with technique, but more likely due to an expiry of the stock.

RESULTS

A total of 97 subjects were recruited into the study. Urine samples were collected from sixty seven patients with MS and OAB (mean age 45.5, SD: 8.6), 10 patients with MS and no OAB (mean age 43.3, SD 8.0) and twenty healthy controls (mean age 44, SD 9.9). There was no significant difference in the mean age between the control group and MS patients (p=0.62). Six out of the sixty-seven (9.0%) patients with MS and OAB were on antimuscarinic therapy. Results are presented in table nineteen.

	Healthy Controls (n=20)	MS no OAB (n=10)	MS with OAB (n=67)	Overall (n=97)	P-value
Mean Age yrs (SD) based on ANOVA as the data is normally distributed	43.9 (9.9)	43.3 (8.0)	45.5 (8.6)	44.9 (8.7)	0.62
Gender Number Female:Male Percentage Female:Male Based on chi-	12:8 60%:40%	7:3 70%:30%	46:21 69%:31%	65:32 67%:33%	0.75

squared test					
Median OAB subscore (IQR)	0 (0)	0 (0)	7 (5, 10.5)	5 (0, 8)	
Median EDSS score (IQR) from Mann-Whitney U test between the two MS groups	n/a	2 (1.5, 2.5)	3 (2.5, 3.5)	3 (2, 3.5)	0.02
Median uNGF/Creat (IQR) based on Kruskall Wallis test	4.8 (1.0, 9.5)	5.3 (2.3, 14.7)	10.8 (3.0, 27.0)	8.4 (2.4, 21.0)	0.04
Median uBDNF/Creat (IQR) based	9.0 (6.1, 38.5)	27.9 (4.2, 53.1)	53.3 (13.6, 127.5)	38.4 (10.2, 101.6)	0.002

on Kruskall Wallis test					

Table nineteen: Characteristics of the three groups.

EDSS: Expanded disability status scale

uNGF/Creat: urinary NGF to creatinine ratio

uBDNF/Creat: urinary BDNF to creatinine ratio

IQR: interquartile range

SD: Standard deviation

Subjects age followed a normal distribution between the three groups and was assessed using ANOVA. There are differences between groups in NGF levels with the highest levels seen in MS-OAB patients (median 10.8 (IQR: 3.0, 27.0)) and the lowest levels seen in controls (median 4.8 (IQR: 1.0, 9.5)), Kruskal Wallis p-value=0.04. Similarly larger differences are observed between groups in BDNF with the highest levels seen in MS-OAB patients (median 53.3 (IQR: 13.6, 127.5)) and the lowest levels seen in controls (median 9.0 (IQR: 6.1, 38.5)), Kruskal Wallis p-value=0.002.

When the urinary NGF/Cr was compared against the OAB symptom score of the USP questionnaire for the patients with MS and OAB, there was a slight correlation between symptom severity and urinary NGF/Cr levels with Pearsons correlation coefficient (r= 0.0558, p=0.65), as can be seen in figure sixteen below.

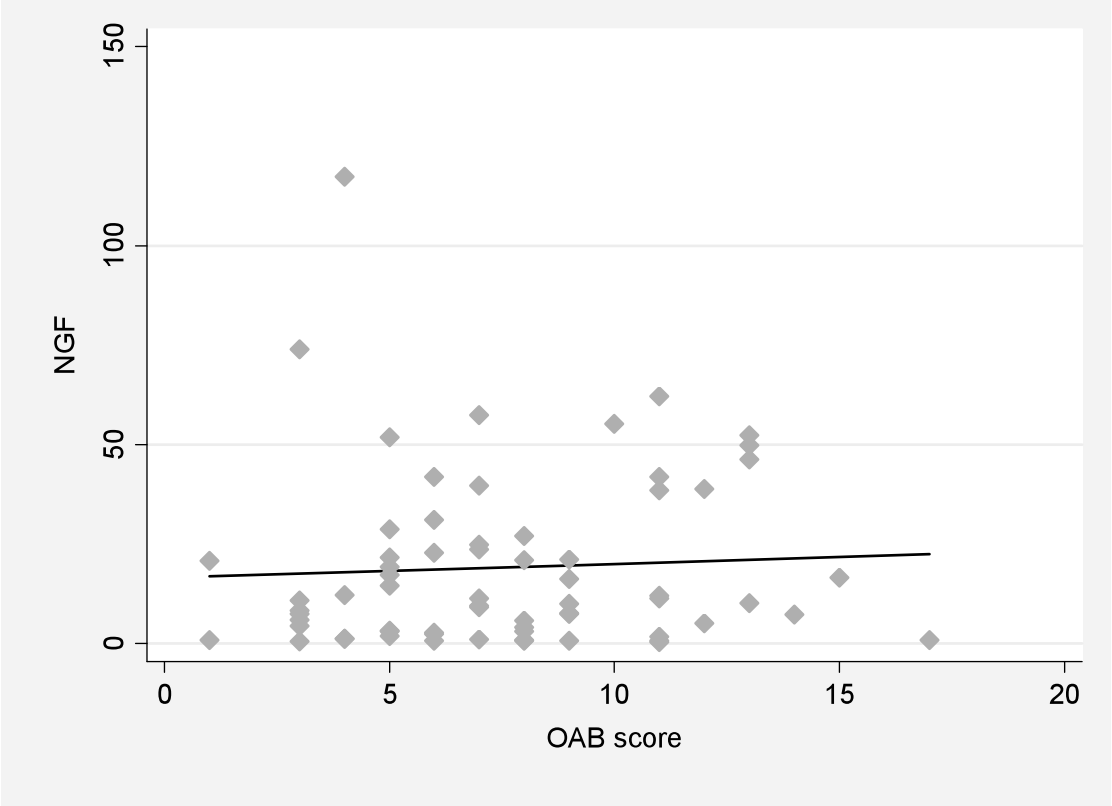


Figure sixteen: Correlation between y-axis: urinary NGF/Cr levels (pg/μmol) and x-axis: OAB score for the patients with MS reporting OAB symptoms (n=67). R value=0.06.

	Unadjusted analysis	
Variable	Estimate (95% CI)	P-value
Group: Control MS No-OAB MS-OAB	 Ref 0.73 (-0.47, 1.93) 1.07 (0.28, 1.86)	0.03
OAB score (per unit increase)	0.08 (0.01, 0.15)	0.03
Age (per year older)	-0.01 (-0.04, 0.03)	0.75
On Antimuscarinics (Yes versus No)	-0.56 (-0.78, 1.91)	0.41

Table twenty: Unadjusted linear regression analysis to explore factors related to the log NGF/Cr levels.

The log of the NGF/Cr values were calculated to follow a normal distribution. Table twenty suggests that no factor is independently related to NGF/Cr levels. In unadjusted analysis there is a 0.73 (95% CI: -0.47, 1.93) higher log NGF/Cr level among those with MS and no OAB and a 1.07 (95% CI: 0.28, 1.86) higher log NGF/Cr level among those with MS-OAB compared to controls, global p-value 0.03. There is no significant difference in NGF/Cr level between the MS and no OAB and the control group. MS-OAB patients have a significantly higher NGF/Cr level compared to controls. Confidence intervals overlap between the MS no OAB and the MS-OAB, and hence they are unlikely to be significantly different, however the sample size is insufficiently powered to do pairwise comparisons.

There is also a 0.08 (95% CI: 0.01, 0.15) higher log NGF/Cr level for each unit increase in OAB score. Neither age or antimuscarinic usage is significantly related to the log NGF levels.

There was a fair correlation between Urinary BDNF/Cr and OAB symptom score for the patients with MS and OAB, with Pearsons correlation coefficient (r=0.26, p=0.03) as seen in figure seventeen below.

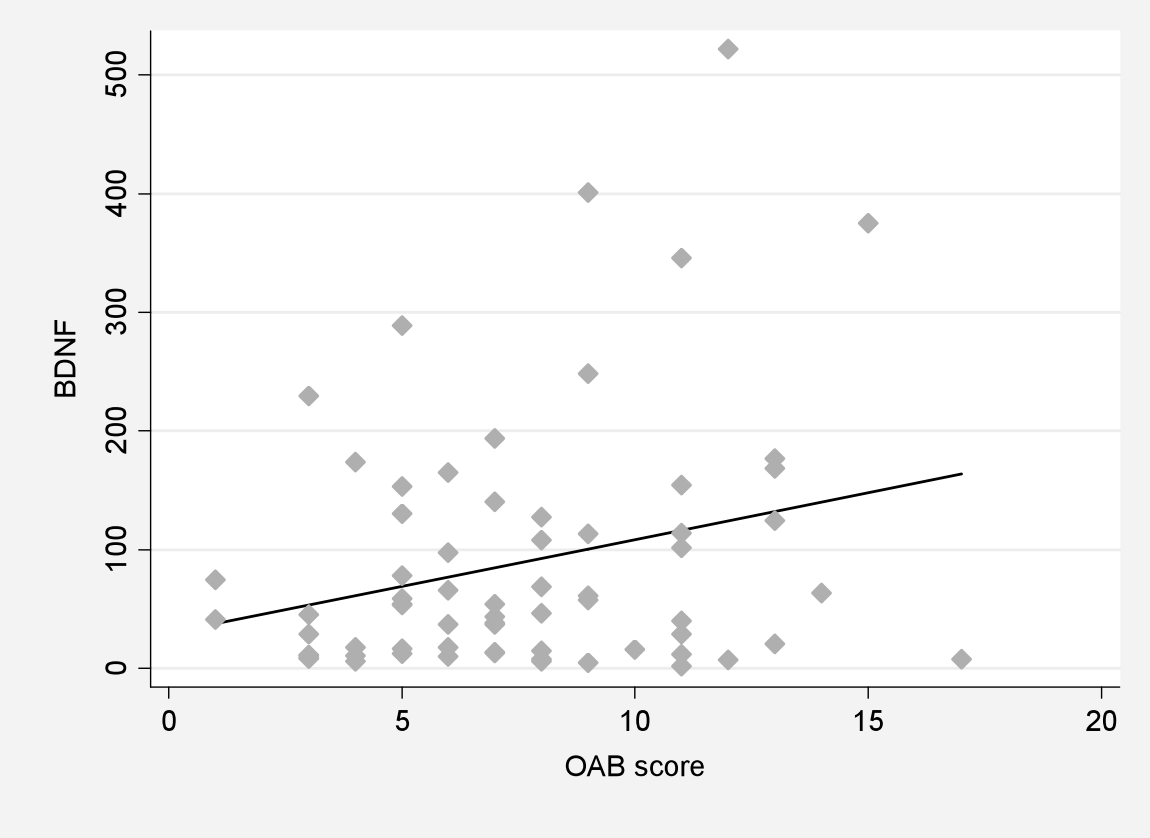


Figure seventeen: The plot of urinary BDNF/Cr levels (pg/μmol) (y-axis) versus OAB score (x-axis) for the patients with MS and OAB symptoms (n=67). R value=0.26.

Table twenty-one: Unadjusted linear regression analysis to explore factors related to the log BDNF/Cr levels

	Unadjusted analysis	
Variable	Estimate (95% CI)	P-value
Group: Control MS No-OAB MS-OAB	 Ref 0.23 (-0.79, 1.24) 1.19 (0.52, 1.85)	0.0009
OAB score (per unit increase)	0.11 (0.05, 0.17)	<0.0001
Age (per year older)	0.02 (-0.01, 0.06)	0.10
On AMS (Yes versus No)	-0.15 (-1.33, 1.02)	0.80

The BDNF/Cr values were skewed, therefore a log transformation of the BDNF/Cr values were calculated to follow a normal distribution prior to the analysis. The above table suggests that no factor is independently related to BDNF/Cr levels. In unadjusted analysis there is a 0.23 (95% CI: -0.79, 1.24) higher log BDNF/Cr level among those with MS and no OAB and a 1.19 (95% CI: 0.52, 1.85) higher log BDNF/Cr level among those with MS-OAB compared to controls, global p-value 0.0009. There is no significant difference in BDNF/Cr level between the MS and no OAB and the control group. MS-OAB patients have a significantly higher BDNF/Cr level compared to controls. Confidence intervals overlap between the MS no OAB and the MS-OAB, and hence they are unlikely to be significantly different, however again the sample size is insufficiently powered to do pairwise comparisons.

There is also a 0.11 (95% CI: 0.05, 0.17) higher log NGF/Cr level for each unit increase in OAB score.

Receiver operator curves for urinary NGF/Creat and OAB symptoms

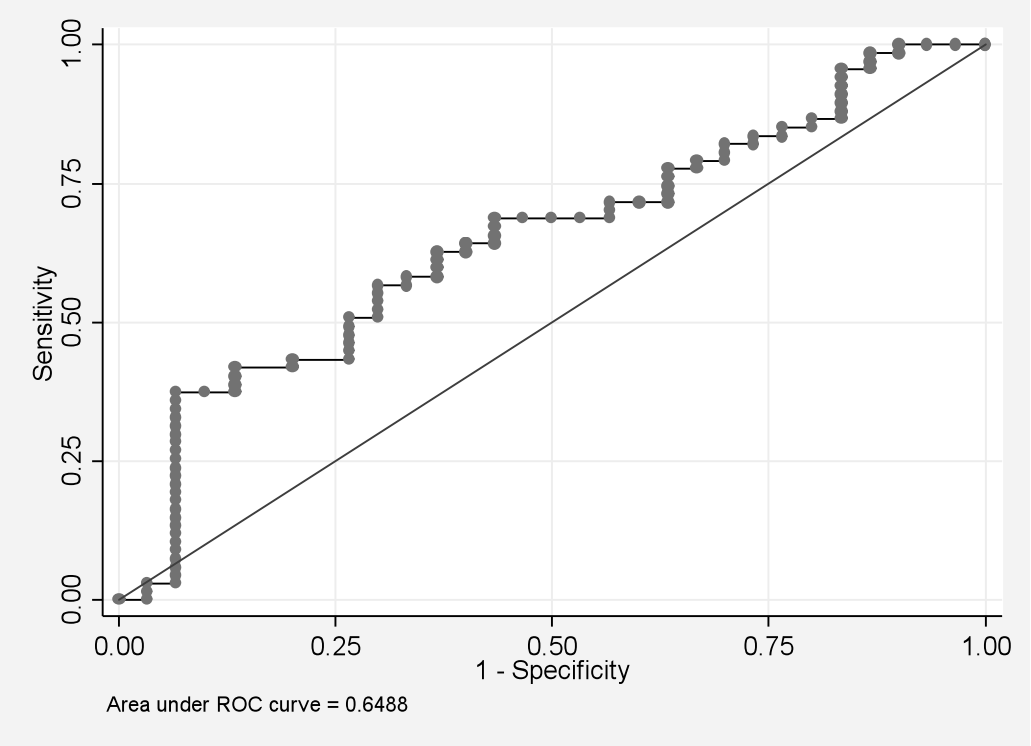


Figure eighteen: Receiver operator curves for urinary NGF/Creat and OAB score for the MS patients with and without OAB symptoms (n=77).

OAB score has been dichotomized into >0 versus 0 and then seen whether NGF/Cr levels can predict having an OAB score >0. Using the Youden Index, the cutpoint in NGF/Cr levels is 18.97. This gives a 37% sensitivity and a 93% specificity for predicting that the patient will have an OAB score of > 0 (using the USP OAB subscore). The AUC at this point is 0.65.

Receiver Operator curves for urinary BDNF/Creat and OAB symptoms

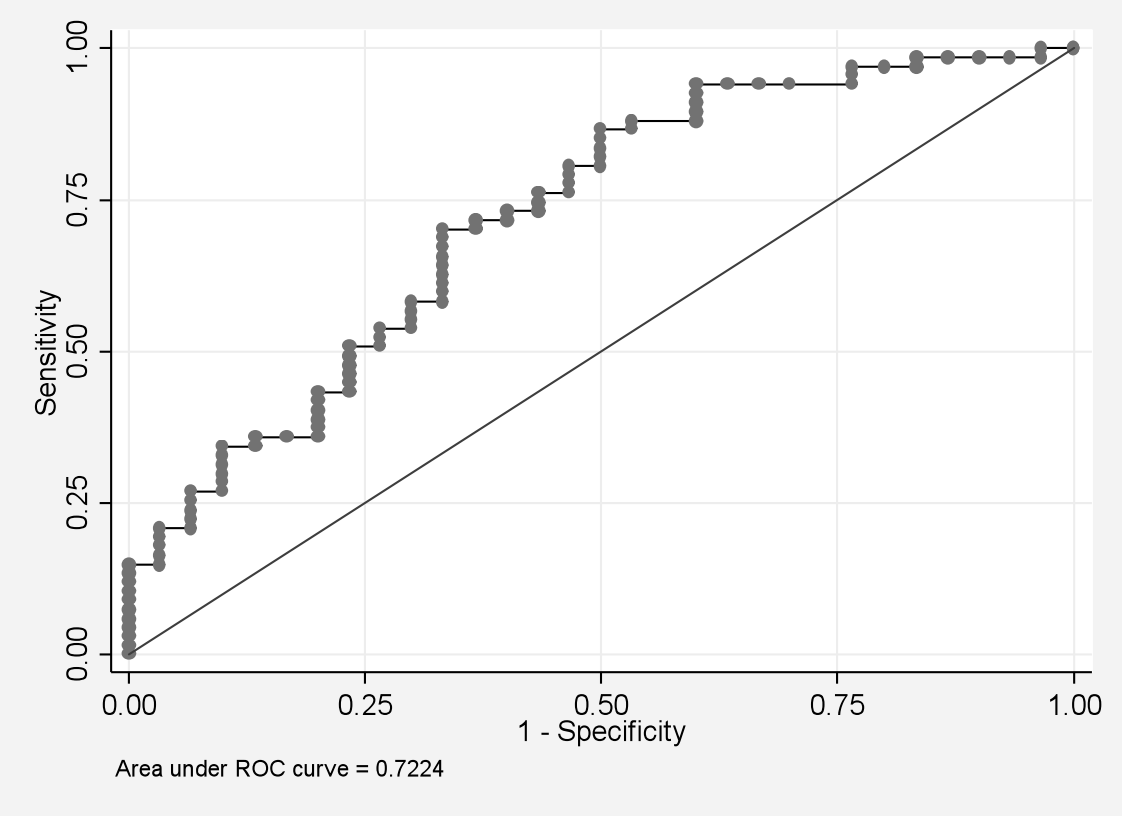
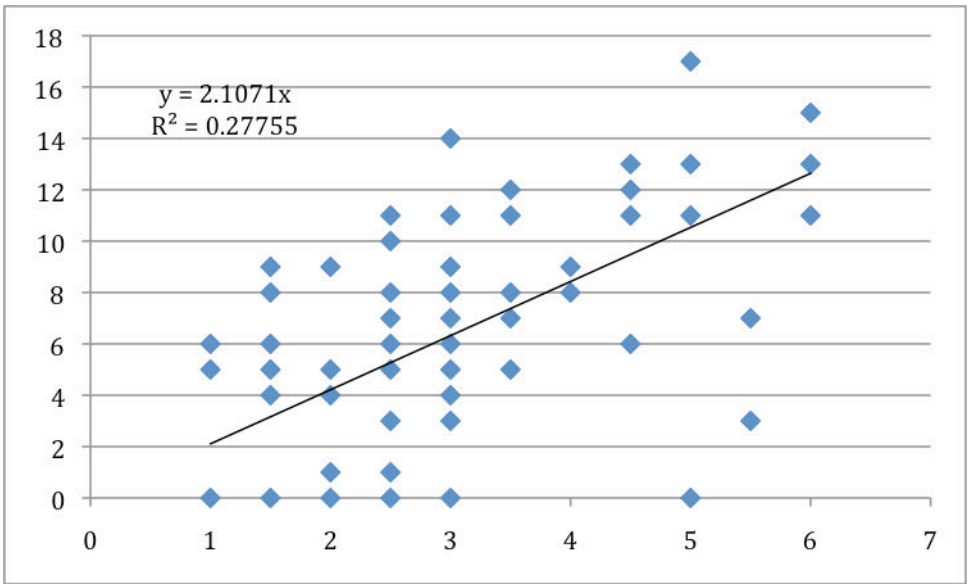


Figure nineteen: Receiver Operator Curves (ROC) for urinary BDNF/Creat and OAB score for the MS patients with and without OAB symptoms (n=77).

Using the Youden Index, the cutpoint in BDNF/Cr levels is 17.37. This gives a 70% sensitivity and a 67% specificity for predicting that the patient will have an OAB score of > 0 (using the USP OAB subscore). The AUC at this point is 0.68.

Figure twenty: The correlation between EDSS and OAB score in MS patients both with and without OAB symptoms.



X-axis: EDSS score and Y-axis: OAB subscore. This shows a positive relationship with R^2=0.28.

The correlation between EDSS and uNGF/Cr and uBDNF/Cr levels demonstrate a coefficient of R^2=0.06 and R^2=0.006 respectively.

DISCUSSION

This study is the first to analyse the levels of urinary neurotrophins NGF and BDNF in MS patients with mild to moderate OAB symptoms and without OAB symptoms compared to healthy controls. The results obtained confirm that urinary neurotrophin levels are significantly raised in patients with MS compared to healthy controls. This was more marked for patients with MS and OAB than for those with no OAB symptoms. Since the group with no OAB symptoms was small in number, no comment can be made with statistical significance, however there is a trend for the levels to be higher than healthy controls.

However, interestingly there was only a slight correlation between symptom severity of the USP OAB symptom subscore and urinary neurotrophin levels, though it was slightly better for BDNF (r=0.26) than for NGF (r=0.06). This relationship is not as strong as that shown in a group of patients with idiopathic OAB where a correlation coefficient of 0.65 and 0.51 was observed respectively [144].

The correlation with the MS patients and OAB symptoms in this study also compares poorly to the relationship demonstrated in a group of twenty-six women with painful bladder syndrome with an R^2=0.73 and R^2=0.61 for urinary BDNF and NGF respectively [124].

The ROC curves achieved for uBDNF/Cr and uNGF/Cr in this cohort showed an AUC of 0.72 and 0.65 respectively. Compared to a similar study with ninety women diagnosed with idiopathic OAB, the accuracy was stronger for idiopathic patients with an AUC of 0.95 and 0.78 respectively [144]. Similarly another study in patients with idiopathic OAB showed stronger receiver operator characteristics of 0.78 and 0.68 have been shown for BDNF/Cr and NGF/Cr respectively [120].

Using the Youden Index, the optimized cut-off point in NGF/Cr levels is 18.97. This gives a 37% sensitivity and a 93% specificity for predicting that the patient will have an OAB score of > 0 (using the USP OAB subscore). Whilst using the Youden Index, the optimized cut-off point in BDNF/Cr levels is 17.37. This gives a 70% sensitivity and a 67% specificity for predicting that the patient will have an OAB score of > 0 (using the USP OAB subscore). Both of these values compare poorly to that demonstated in a group of women with idiopathic OAB where the optimized cut off points for NGF/Cr was 0.14 for a 54% sensitivity and 96% specificity for predicting OAB, and a BDNF/Cr value of 4.6 for a 89% sensitivity and 100% specificity [144]. This again suggests that baseline urinary neurotrophin levels in this patient cohort with MS are higher than that expected for an age matched population with idiopathic OAB. The cut-off points are important to determine to provide an idea of the normal range of values to expect from a healthy control population before defining where the abnormal range would begin.

This suggests that the ability of urinary neurotrophins to act as a biomarker for severity of OAB symptoms in patients with MS, using the urinary symptom profile, is less reliable. This is further supported by the observed levels from the group of MS patients with no OAB symptoms. The mean urinary NGF/Cr and BDNF/Cr levels were higher in this group compare to the healthy controls, which suggests that this group may have a higher baseline level. Due to the small number of patients within this group, statistically significant comparisons cannot be made. This seems to suggest that urinary neurotrophins are derived from sources other than the lower urinary tract.

There was a positive correlation between EDSS and OAB subscore which demonstrates that patients with more severe disability from the MS are more likely to have a greater OAB

symptom score. The correlation between EDSS and uNGF/Cr and uBDNF/Cr levels demonstrate a coefficient of R^2=0.06 and R^2=0.006 respectively, which suggests there is no strong correlation between EDSS and urinary neurotrophin level.

Possible reasons for higher baseline levels of circulating neurotrophins in MS

Enhanced expression of NGF and its receptors in MS lesions and cerebrospinal fluid have been previously described [181, 182]. It is thought that NGF plays an important role in myelin repair, through promoting axonal regeneration, survival and protection, and differentiation of oligodendrocytes [183]. It also facilitates migration and proliferation of oligodendrocyte precursors to sites of myelin damage, and directly regulates key structural proteins that comprise myelin, and induce the production of BDNF, which is also integral for myelination. It has been suggested that NGF could even be a promising therapeutic candidate in white matter disorders such as MS [183].

It has been suggested that initially there is a relative neurotrophin deficiency in the damaged CNS tissue in MS, and that there may be a resultant increase in the expression of peripheral blood mononuclear cells (PBMCs), which also express neurotrophins [184]. It is thought that these PBMCs can provide some additional neurotrophin support for the CNS tissue, and failure to produce this may eventually lead to the atrophy that is seen and is the main determinant of the end-point disability for patients with MS. The expression of neurotrophins in circulating PBMCs is depicted in figure twenty one below. Whether this immunological mechanism can contribute to the raised serum and urinary neurotrophin levels is unknown.

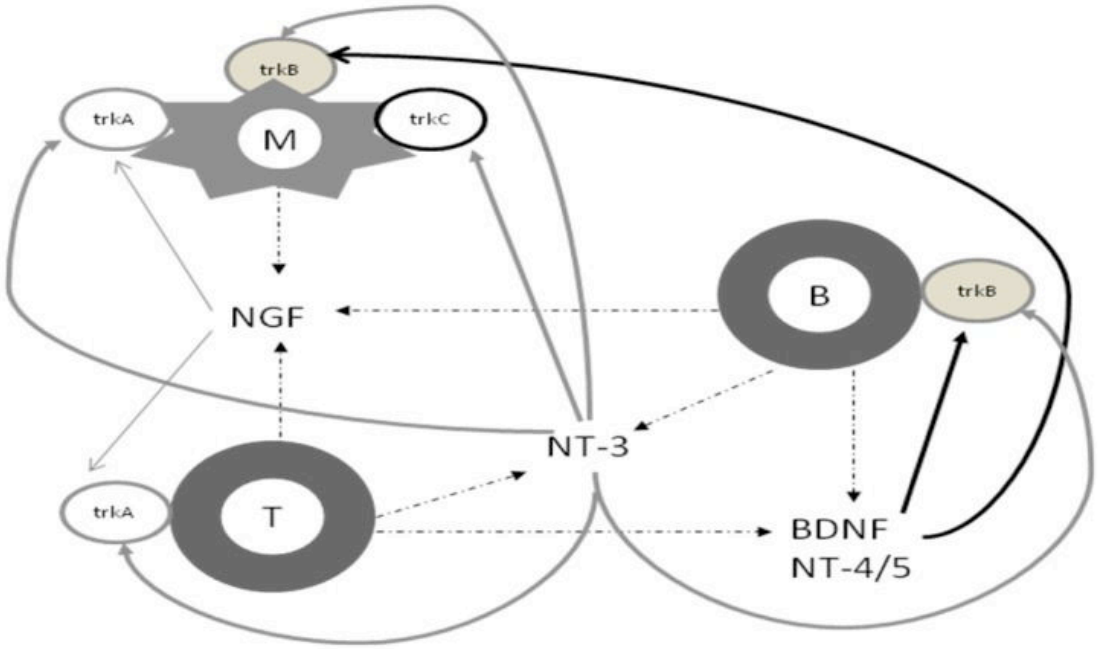


Figure twenty-one: Interactions between neurotrophins and the immune cells [184].

NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, NT-3: neurotrophin 3, NT-4/5: neurotrophin 4/5, trk: tropomyosin related kinase receptor, M: macrophage, T: T lymphocyte, B: B lymphocyte. Dotted lines indicate secretion. Straight lines indicate affinity towards receptors.

BDNF has been shown in animal models of MS, to mediate axon protection [185]. Mice deficient for BDNF in immune cells displayed an attenuated immune response in an experimental model of autoimmune encephalomyelitis. Once lentiviral BDNF overexpressing T-cells were injected, this lead to direct axonal protection and a less severe course of encephalomyelitis.

Therefore the possible source for the urinary levels, may be at the site of CNS inflammation, where there may be an upregulation of these neurotrophic factors to promote neuronal repair, resulting in a raised systemic level of circulating neurotrophins as a byproduct [183]. Therefore these patients may have higher urinary neurotrophin levels as a baseline, regardless of LUT symptoms, compared to healthy controls. As a result, urinary neurotrophin levels may be skewed and therefore less reliable as a sole biomarker for OAB and DO, but potentially more so as a generic marker of inflammation.

There may likely be a difference in the pathophysiology of the OAB between idiopathic and MS patients. The expression of neurotrophins in the lower urinary tract is complex and not fully understood. Several other factors are thought to be involved with the regulation of NGF expression including TNF-α, interleukin-1β, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), steroid hormones, β-adrenergic agonists, direct camp activators, and stretch [94]. The expression of these numerous growth promoting substances may differ in patients with MS, due to complex inflammatory processes that are ongoing, and therefore less of a strong correlation than with idiopathic OAB is seen. Other biological molecules that may have a role in the pathogenesis of OAB include prostaglandins [136], cytokines and CRP although, and the activity of these in the urinary tract in MS is also unknown. This could also possibly explain the poor correlation between uNGF/Cr and uBDNF/Cr levels and OAB symptoms in this group.

Neurotrophins and other conditions – lack of specificity for the OAB?

As mentioned in chapter two, the specificity of urinary neurotrophins for diagnosing OAB alone is also in question. A rise in urinary levels of NGF are not limited to OAB, but has also been shown to be elevated in other urological conditions such as interstitial cystitis, urinary tract stones, urothelial cancer, bladder outflow obstruction, prostate cancer with perineural invasion, asthma, Alzheimers disease, allergies, keratoconjunctivitis, and most recently in patients post renal transplantation where it has been proposed to have a role in chronic allograft nephropathy [117, 125, 134, 186, 187]. Raised serum NGF levels have been noted in several inflammatory and auto-immune diseases such as systemic lupus erythematosus (SLE), and juvenile arthritis [188] although whether this represents a causal or counter-regulatory mechanism is unknown.

NGF is also thought to have a neuroendocrine role, involved with homeostasis and coping with emotion and psychological stressors. Raised serum levels have been observed for example in soldiers experiencing their first parachute jump, thought to be triggered by the stress and the highly arousing experience [189]. Whether these factors can influence resultant levels in the urine is unknown. High serum levels have been significantly correlated with raised urinary levels in patients with idiopathic OAB, and these levels remained raised in patients who were refractory to antimuscarinic therapy suggesting a chronic inflammatory process in these patients [122]. In the same way it is possible that the raised baseline serum levels of NGF in patients with MS, as previously described can be related to raised baseline urinary levels [172].

These generic characteristics of NGF, which appears to have a ubiquitous presence and role, may explain the lack of specificity of uNGF as a marker for the OAB alone. Raised levels could therefore represent other urological or neurological pathologies that may be ongoing.

Urinary BDNF/Cr, however has demonstrated a stronger correlation with OAB symptoms than uNGF/Cr which is in agreement with other studies assessing idiopathic OAB and bladder pain syndrome [124]. The reason for this is unclear, and has not previously been examined in MS. However evidence is emerging suggesting that BDNF is a more sensitive marker of idiopathic OAB than NGF, although the latter has been more extensively investigated. Urinary BDNF levels have been shown to be raised in patients with OAB compared to healthy controls, and that this responds to behavioural changes such as bladder training, limiting fluid intake, avoiding caffeinated, acidic or carbonated drinks [120]. It does appear therefore that between these two neurotrophins investigated, BDNF shows more promise as a urinary biomarker.

Study challenges and limitations

There were numerous challenges to the ELISA technique employed as discussed in the method development section. The main ones included the fast deterioration of the reference standard NGF and BDNF protein provided by the kit manufacturers. To get around this the kit had to be used promptly once opened and first thawed from -70°C. The other issue that took numerous attempts before discovery, was the actual device used to collect the urine, as described in modification step three. The urinary NGF was evidently sensitive to the use of polystyrene collection tubes, where much of the protein was sequestered to the tube itself and causing apparent loss of signal and false negatives errors from the urine samples being analysed. This was immediately evident once polypropylene tubes were used and sample protein was not being lost. Once these hurdles were identified, the ELISA was more straightforward, although the considerations that face its integration into a clinical service remain its cost, it collection requires rapid placement of the sample onto ice and its immediate transfer to the laboratory for centrifugation at 4°C, the technique itself involves a lengthy and labour intensive, with numerous manual steps, that require two overnight incubations, therefore requiring three days to obtain a result. This is far from being a urine dipstick like analysis such as when assessing for β-HCG content, with an immediate result, which would be the gold standard.

Practical issues that also need to be considered concern patient selection to avoid false positives and negatives. There are many circumstances where false positives can be forseen such as patients with urinary tract infections, performing self-catheterisation, both of which are common circumstances for patients with MS. False negatives can be encountered from patients taking antimuscarinic medications and antibiotics again both of which are frequent occurrences [190].

One of the limitations of this study is the lack of urodynamic data to further define the patient groups. It is unknown whether those patients in this cohort with MS and no OAB had poor detrusor contractility, or whether DO was present alongside poor bladder sensation. This may therefore also influence the levels of urinary neurotrophins in some way. However the OAB itself is a symptom complex and not a urodynamic diagnosis, and the objectives of this study were to assess and compare neurotrophin levels in patients with and without the symptoms of OAB rather than with or without DO.

It proved difficult to recruit patients with MS and no OAB symptoms, with only ten of the patients in this group. This could reflect that the MS patients attending hospital outpatients, have a higher disease severity and therefore may have more urinary symptoms. Those patients with the more mild MS, who are community dwelling may be a more appropriate population of patients to source for this particular group.

As serum neurotrophin levels were not assayed, no comment can be made on whether urinary levels correlate with raised serum neurotrophin level in patients with MS, however this requires further study. It has been shown in other inflammatory conditions that the levels of circulating serum NGF is raised in certain systemic inflammatory conditions that may or may not involve or originate from the lower urinary tract [121, 122, 150, 151]. Patients with higher circulating serum neurotrophin levels may have higher urinary levels as a result, and therefore measurement of urinary levels alone cannot distinguish whether these proteins originate from a dysfunctional lower urinary tract, or from inflammatory CNS pathology.

Future study

These are relatively new markers in the field of functional urology, however it seems that for MS the reliability and validity is yet to be proven, and not superior to current methods of assessing bladder function. To complete a more comprehensive study of urinary biomarkers for the OAB, it would be valuable to conduct a multicentre study involving a larger population group, especially with more patients with MS and no OAB symptoms, to assess what is the normal baseline value for patients with MS. Within the same study there could be an arm consisting of patients with idiopathic OAB. To answer whether circulating levels are higher in patients with this condition and whether this causes a resultant increase in urinary levels, both urine and serum samples should be collected. Alongside measuring NGF and BDNF levels, the quantities of other suggested potential biomarkers such as ATP, neurotrophin 3, and 4/5 (NT3 and NT4/5), and prostaglandins can be collected to assess the characteristics of the group as a whole to have a greater chance of identifying the most accurate biomarker [191, 192]. It may be that the ideal biomarker will be an array of molecules, that when assessed in combination, can more accurately represent the pathological state of the bladder.

CONCLUSION

Translational clinical-science research has clearly shown that neurotrophins play a role in the plasticity of afferent nerves and spinal micturition pathways controlling bladder function. They seem to have a normal physiological role in the development of normal and strong desires to void, and an even more dominant role in the development of lower urinary tract dysfunction that accompanies numerous conditions such as inflammatory bladder states, bladder outflow obstruction and idiopathic and neurogenic OAB both in the animal and human models. Whether these molecules can be used as a biomarker for OAB still needs to be investigated more thoroughly in these conditions. However in patients with multiple sclerosis, it appears there is more to the pathophysiology of OAB than neurotrophins alone, and there must be other growth factors that also need to be considered before an ideal biomarker is found.

CHAPTER FOUR

Chapter Synopsis

Chapter four brings the first three chapters together. Chapter one introduced PTNS, an upcoming treatment for the OAB. The limitations of PTNS were also discussed such as the need for recurrent outpatient visits and the option of a transcutaneous device that would be highly desirable. Patients could self-treat at home, avoid numerous hospital visits, avoid the few adverse effects that come with PTNS and needle insertion, whilst having a portable treatment without the need for being connected to an external power source. Cost analyses studies will need to be performed, however on an initial glance, a transcutaneous treatment option would also be more cost-effective.

This chapter reports a prospective randomised study, evaluating the efficacy of a novel transcutaneous device, called the Geko^{TM} used to provide tibial nerve stimulation for treatment of the OAB over 12 weeks. Patients with idiopathic and neuropathic OAB were recruited.

Chapter three also examined the accuracy of urinary neurotrophins in evaluating patients with MS and OAB syndrome, and discussed the need for an objective assessment tool. The suggested reasons for a biomarker as mentioned in chapter two included:

- to objectively monitor and quantify responses to treatment (to help define a treatment responder),
- to research and evaluate the mechanism of action of various treatments (especially new treatments such as PTNS)
- to identify any placebo effect with new treatments and
- to predict response to treatment

Chapter four examine these requirements further, by measuring urinary neurotrophin levels to help evaluate this new treatment.

Single centre randomised pilot study of two regimens (30mins daily or 30mins weekly for 12 weeks) of Transcutaneous Tibial Nerve Stimulation for the treatment of patients with Overactive Bladder (OAB) Syndrome

Abstract

Hypothesis / aims of study

The aim of this study was to evaluate the clinical efficacy of a novel transcutaneous device for the treatment of patients with either multiple sclerosis or idiopathic OAB. A transcutaneous device, effective at treating OAB, with minimal side effects and simple enough for patients to self treat at home, would be very a convenient addition to the armentary of therapy available. The geko™ is a discrete, self-contained and portable, CE marked device that sticks to the skin.

Study design, materials and methods

A randomized, single centre, phase II pilot study, which enrolled 48 patients (24 with MS and 24 with idiopathic OAB), suffering from OAB. Patients were randomized into either daily or weekly treatment arms. Both arms involved 30 minutes of stimulation for 12-weeks. Patients complete a Global Response Assessment Score (GRA) at 12-weeks, and those that reported moderate or significant improvement in symptoms with this were defined as a responder. Objective outcome measures were used to evaluate symptoms at baseline, week 4, 8, and 12. This included the ICIQ-OAB, ICIQLUTS-QoL, bladder diary scores and urinary neurotrophin levels NGF and BDNF were also measured at baseline, week 4 and 12. Ethics committee approval was obtained prior for the study.

Results

Forty eight patients were recruited into the study, with thirty four patients completing the study (19 with MS and 15 idiopathic OAB). Significant improvements in the ICIQ-OAB by mean (SD) -9.9 (11.5) (p=0.001) and ICIQ-LUTSqol by -34.0 (60.1) (p=0.001) scores for both patient groups by week 12. Weekly treatment seemed equivalent to daily, and there were no significant adverse effects. Likert scales showed that patients rated the treatment as easy to use, comfortable, and were very satisfied wth this as a treatment modality.

Neurotrophins:

Idiopathic OAB and uNGF/Cr:

In patients who responded to therapy, mean NGF/Creat levels were 11.3 (2.7-18.9) and 13.6 (1.8-22.4) pg/mg at baseline and week 12 respectively (p=0.99). Non-responders had mean levels of 96.6 (40.6-97.8) to 48.4 (4.5-133.6) pg/mg at baseline and week 12 respectively (p=0.23). The NGF/Creat levels at baseline for the non-responders were significantly higher than the responders (p=0.05). This could suggest potential for uNGF as a predictor of treatment response.

Idiopathic OAB and uBDNF/Cr:

There was a trend for baseline BDNF/Cr levels to be higher in the non-responders than responders. In patients who responded to therapy, mean BDNF/Creat levels significantly reduced from 66.6 to 19.1pg/mg (p=0.03) by week 12. Non-responders had a change of mean levels from 117.7 (6.7-337.8) to 76.8 (0-182.5) pg/mg at week 12 (p=0.89). BDNF appeared more sensitive to response to treatment in those that have a successful response to treatment.

MS and OAB urinary neurotrophins:

There were no significant changes in the urinary NGF/Creat or BDNF/Creat scores from baseline to week 12 for the patients with MS and OAB, in either the responders or non-

responders.

Interpretation of results

Significant improvements were seen in subjective and objective symptom and QOL assessment parameters for patients using this novel form of tibial nerve stimulation. Adverse effects were minimal. Weekly treatments were equivalent to daily. Response from the MS patients was comparable to the idiopathic patients. Patients reported the treatment as easy to use, comfortable, very satisfactory. Urinary neurotrophin assessment in the idiopathic OAB group shows that perhaps higher levels of NGF/Creat at baseline may be a predictor of poor response, whilst urinary BDNF/Creat levels could be investigated further to monitor response to treatment as they significantly reduce in the patients who respond to therapy. Urinary Neurotrophin levels did not change significantly in the patients with MS during treatment.

Conclusion

This randomised pilot study shows that transcutaneous tibial nerve stimulation, using the geko™ device, appears to be an effective, safe and convenient method of management for patients with severe OAB symptoms. Additional work is required for a multicentre study to demonstrate the long-term efficacy of this treatment.

INTRODUCTION

The success of posterior tibial nerve stimulation (PTNS) in the management of the overactive bladder (OAB) against placebo has opened a novel modality of peripheral nerve stimulation for managing the overactive bladder [40]. Clinical efficacy has been demonstrated through clinical studies and its use is supported by NICE guidance in the United Kingdom.

However it is yet to be considered as a perfect solution for patients. Its non-inferiority compared to current treatments such as onabotulinumtoxinA and SNM is yet to be proven. There are non-serious adverse events to consider. These have been reported in up to 8.5% of patients and include pain, bruising, tingling or bleeding at the insertion site [193]. Patients have also reported leg cramps, and pain and numbness in the sole of the foot. Other rare side effects which have been reported include swelling, worsening of incontinence and vaso-vagal response to needle placement. The practicality of offering PTNS within the framework of an NHS clinical service needs to be considered. With current treatment regimes, patients must attend an outpatients department on a weekly basis for a total of 12 weeks. This may be a limitation for those travelling considerable distances, or may be too much of a time commitment for patients with busy schedules. This would take longer for patients with neurological disorders who may have problems with mobility. In the STEP study, out of those patients attending follow up sessions, two out of the fifty patients (4%) withdrew due to practical difficulties in attending regular follow-up visits [71]. A further 4% of the patients withdrew as they moved further away from the hospital. The requirement of regular attendances may negatively impact compliance and adherence to this treatment regime.

In addition the resources required for providing medical and nursing staff to deliver the treatment must also be considered when preparing a cost-effectiveness model.

Transcutaneous nerve stimulation

The option of transcutaneous tibial nerve stimulation (TTNS) is therefore an attractive option [194]. Transcutaneous electrical nerve stimulation (TENS) has been used as a medical remedy for musculoskeletal complaints such as pain management and spastic paraparesis. TENS is thought to be able to modify central CNS signaling, for example when applied to the palm for median nerve stimulation, it was observed that this suppressed some cortical amplification. Likewise when applied to somatovisceral complaints, it was observed that gastric electrical activity can also be modified by TENS. This has been studied by measuring gastric myoelectric activity and electrogastrography, and it has been shown that TENS can activate centrally mediated somato-visceral reflexes and be therefore used to treat gastric dysrhythmia [195].

Transcutaneous tibial nerve stimulation studies

A handful of TTNS studies have been conducted previously to explore the effects for the OAB, the early results of which show some promise with efficacy. However further study is clearly required to investigate this treatment further.

Amarenco et al in 2003, carried out a study using TENS for a heterogeneous group of patients with demonstrated DO [51]. A total of 44 patients were included with a background of neurogenic and idiopathic origin. The acute urodynamic affects of TTNS were assessed. Patients had a baseline cystometric study, which was recorded. A self-adhesive, disposable, stimulation patch was then placed over the posterior tibial nerve behind the medial malleolus with two contact electrodes (positive and negative), and stimulation was applied at a frequency of 10Hz with a pulse width of 200 milliseconds in a continuous mode, and toe flexion was confirmed. Second cystometry was performed during stimulation. It was observed that during stimulation there was an increase in the mean volume at first involuntary

detrusor contraction from 162.9mls to 232.1, and and the mean cystometric capacity from 221 mls to 277.4mls which was statistically significant.

Another small study assessed the affects of TTNS on a group of six female patients with OAB secondary to Parkinson's disease or Multiple System Atrophy [196]. This was performed daily for 20minutes for six weeks and patients were assessed at the end with the patient global impression of improvement scale and urodynamics. Five out of six patients considered this to be an effective treatment, and were still using the treatment six months after the end of the study. There was no significant improvement in urodynamic or symptom scores, however.

Schreiner et al in 2010 performed a placebo controlled, randomized trial of 12-weeks of TTNS in addition to standard therapy versus standard therapy alone, to treat urgency incontinence in a group of fifty-one older women (>60years of age). Standard therapy consisted of 12-weeks of bladder training and pelvic floor muscle exercises. Sixty eight percent of patients in the TTNS group reported cure or improvement versus 35% in the control group, with supported evidence from bladder diary parameters and quality of life questionnaires [197].

De Seze et al, performed a multi-centric study of 70 patients with MS and symptoms of OAB across five neurorehabilitation departments [75]. The mean duration of MS was 13.4 years and LUTS 8.2 years. Patients were excluded if they had an Expanded Disability Status Scale of >7, indicating that patients were able to walk at least 20m with or without assistance, were relapse free for the last three months and not wheelchair bound. All patients were refractory to antimuscarinic therapy either due to lack of efficacy or intolerable side effects. Clinical efficacy was assessed with questionnaire tools at baseline, 30 and 90 days following initiation of treatment, and cystometry was performed before and at the end of the 90-day study. The transcutaneous patches of a TENS machine were applied through two adhesive electrodes,

above and behind the medial malleolus. These were attached to an external stimulator through wires. Stimulations were delivered as 200μsec pulses with a pulse rate of 10Hz and a value of 19-30 mA. The intensity was aimed at a threshold above perception of stimulation but below that of pain being caused. Patients were taught the technique of self directed stimulation and performed this for 20 minutes daily. This study demonstrated significant efficacy and safety of this device. By day 30, significant improvements in urgency were seen in 51.3%, and frequency in 66.7% of patients with a reduction of 2.7 voiding episodes per day. Continence was improved in 62% of patients with a reduction of 2.7 leakage episodes per week. Psychological burden due to LUTS was also noted to decrease through quality of life assessment scores.

It was concluded that TTNS is safe, effective, with no local or systemic adverse effects noted from this cohort. There was no development of voiding dysfunction after this treatment, which is relevant in patients with MS where CISC may be difficult due to problems with co-ordination. Other advantages of this treatment option were related to feasibility as this device could easily be used at the home setting either by the patient or a carer. This provides a useful management option for patients with MS, where options can be limited. The effects of more intermediate to chronic term stimulation need to be evaluated.

The limitation of a TENS machine, where the patient is attached to an external power source, is the inherent restriction to activity and limited portability of such devices. The availability of a patch electrode would address these limitations, and provide a patient-delivered option. The aim of this study was to evaluate the safety and efficacy of TTNS using a novel portable device, which acts as a single stick-on patch electrode.

An introduction to - The geko™ device

Sky Medical Technologies Ltd (also trading as Firstkind Ltd) have developed a novel method and produced a CE marked medical device [geko™] for transcutaneous nerve stimulation. The geko™ is a discrete, self-contained and portable, CE marked device that sticks to the skin via an adhesive conductive hydrogel and weighing just 16g can be worn at home without impeding most normal activities (see www.gekodevices.com).

The geko™ device is an internally powered, that uses a new technology called OnPulse™ a neuromuscular stimulation technology enclosed in a disposable device, that is applied externally to the leg. It was developed by Firstkind Ltd as a portable, disposable, neuromuscular stimulation device of the popliteal fossa for the improvement of blood flow in the lower limb [198]. The device has received the CE Marking for increasing blood circulation and for the prevention of venous thrombosis (MDD Annex II certificate CE 558928 issued by BSI (Notified Body number 0086, expires 25 Oct 2015).

In its original application for prevention of deep vein thrombosis, geko™ triggers small electrical impulses that gently activate the common peroneal nerve within the popliteal fossa, behind the knee, in turn activating the venous muscle pumps of the calf and foot. Substantive increases in lower limb blood flow have been demonstrated to improve venous return and thereby reduce stasis with no reported morbidity [198]. NICE is currently developing guidance on this technology for this indication [199].

The device fits behind the medial malleolus of the ankle (positioned as figure 1) where it can readily stimulate the posterior tibial nerve. Upon activation, by pressing the on-off switch of the device, the abductor hallucis muscle in the foot contracts. Positioning the adhesive geko device for use in this way is straightforward and the application surface is flat making good contact via the conductive hydrogel. Paraesthesias are essentially confined to the foot. These sensations are not expected at the point of contact as the current density is spread across a

wide area. Devices should be set to the highest comfortable setting using one of the seven settings available which alter the pulse width whilst maintaining a constant current.

The device has default stimulation parameters at 27mA constant current, at a frequency of 1Hz. The pulse width (μsec) will vary between 70 and 560μs depending on the maximum tolerable sensory and best motor responses (movement of toes) as determined by the patient using one of the seven settings in the device selected using the single push button switch. Apart from antimuscarinic therapy patients could continue all other medications *ad libitum* during the trial, although these will be recorded.

With the device attached, the patient was able to continue with normal daily activities with no restriction to ambulation (although vigorous activity e.g. running and gym visits are not encouraged, driving is prohibited whilst wearing the device, swimming or bathing are not possible as the hydrogel adhesive is water soluble). It is fully insulated by the protective moulding and there is no risk of electric shock. (Refer to Appendices for Instructions for use of the device). The device has been assessed to current medical-electrical equipment safety standards.

The portable disposable device design is well suited for this application as:

- It is non-invasive. Adoption could offer a significant reduction in the number of surgical procedures undertaken per annum with associated reduction in risk.

- It will reduce the cost of treatment of mild to moderate OAB. The cost of a 12 week course of the geko™ device is estimated to be £300, as compared to £1,800 or

£16,000 for PTNS outpatient treatment or Sacral Nerve Stimulation, respectively, according to NHS tariffs.

- It does not require specialist skills or knowledge to apply. The device can be applied by the patient at home with minimal initial instruction. Many patients decline PTNS because of the associated time and travel inconvenience.

- The patient is fully mobile during therapy, and the device is invisible beneath clothing in contrast to the TENS approach which requires physical connection to a stimulator unit.

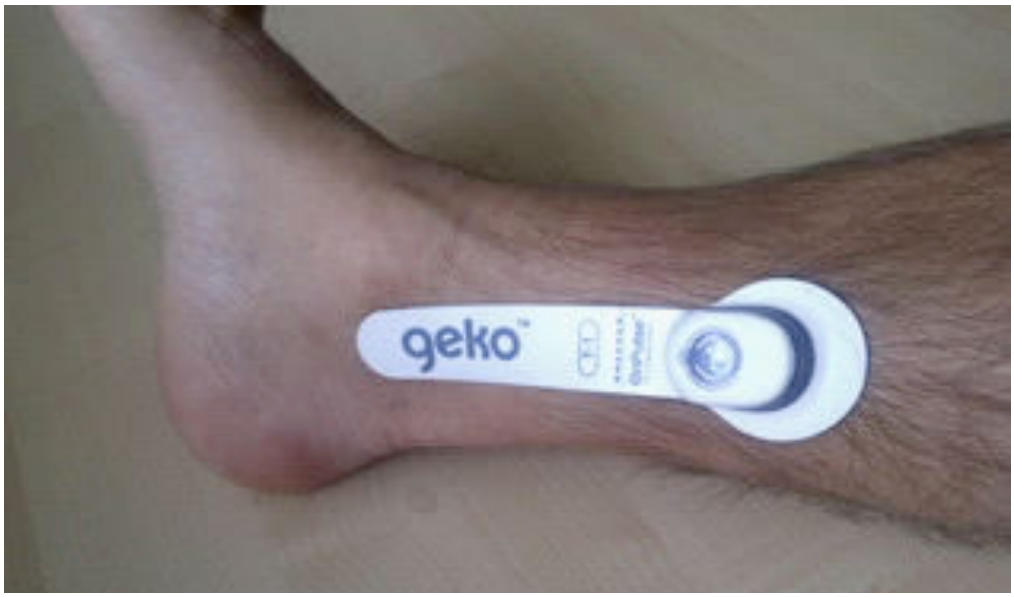
A study evaluating the Geko device would assess changes in OAB symptoms over the period of time of the intervention. However considering the subjective nature of OAB symptoms, evaluating a biomarker of OAB might provide more robust evidence of benefit. The urinary levels of two neurotrophic factors involved in the pathogenesis of OAB [135], known as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF), have been identified in the urine and are being explored currently as biomarkers for the overactive bladder (as discussed in chapters two and three). Studies have shown a reduction in the urinary levels of the neurotrophic factors after successful BTX-A, antimuscarinic therapy, SNM and acupunture therapy for OAB, and therefore can be used to assess response to therapy [118, 119] [112, 128]. Therefore, the measurement of urinary neurotrophins was also performed before, during and after treatment to provide an objective biological marker for treatment response.

Study Aims and objectives

The aim of our study was to Investigate the clinical efficacy and safety of transcutaneous tibial nerve stimulation using the geko™ on OAB symptoms in patients with MS and Idiopathic OAB. To determine both a subjective outcome using patient reported outcomes and an objective outcome by evaluating urinary neurotrophins for response to treatment.

A picture of this device can be seen in figure 1 below.

Figure 1: Proposed Ankle application of the geko™ device



MATERIALS AND METHODS

This study was performed with the approval of the London Surrey Borders Health Research Authority National Health Service Research Ethics Committee (REC) approval, with REC reference number 12/LO/1613 and was sponsored by the Research and Development department of University College London. These documents can be seen in the Appendices. All subjects gave written informed consent prior to enrollment into the study. The study was supported by external funding from FirstKind Ltd.

Study design

In this randomised open label investigator-led clinical study, forty-eight (48) patients with OAB symptoms attending the Department of Uro-Neurology, at the National Hospital for Neurology and Neurosurgery, Queen Square, for treatment were recruited. Patients enrolled were male and females, aged ≥18 years with symptoms of OAB (urgency, urinary frequency, and/or urge incontinence) for ≥3months. Patients had either ≥eight or more micturitions, and ≥one urgency episode (with or without incontinence) per 24hours based on the three-day bladder diary and the ICIQ-OAB symptom questionnaire. Prior to starting the study, subjects had a 2-week run-in washout period, where prior OAB medications (including antimuscarinic or B3-agonist mirabegron) were stopped. The intention to treat included twenty-four (24) patients with multiple sclerosis and twenty-four (24) patients with idiopathic OAB.

It is accepted that ideally this study would have a validated sham arm. However, an appropriate validated sham was not available at the time of study design. Therefore an internal control was incorporated into the study design, where patients were randomised to receive different doses of neurostimulation ie a ‘large and small dose’ by introducing weekly and daily arms. This has the advantage of giving basic information on dosing and

acceptability. Also in the literature, various studies have used a range of PTNS regimes from daily, twice weekly, three times and four times per week and weekly [40, 43, 44, 46, 52, 75]. Whilst the most widely recognised regime remains weekly, a daily arm was chosen to see if this would offer superior efficacy due to the higher dose of therapy. The previous study evaluating TPTNS in patients with MS, by de Seze et al in 2011, also used a daily stimulation regime [75].

The patients were provided with a full explanation of the nature, purpose and requirements of the study including Patient Information Leaflet (PIL) and signed an Informed Consent Forms (ICF). They were invited to participate in a screening assessment, which included collection of information about past medical history, drug history and a physical examination. Voiding diaries and questionnaires were used to determine severity of symptoms prior to treatment. No study related procedure was undertaken prior to the signing of the ICF.

Patients were randomised into two potential treatment arms of:

1. 30 minutes of TPTNS once a day
2. 30 minutes of TPTNS once a week

Randomisation was undertaken using a web-based programme 'Sealed Envelope' https://www.sealedenvelope.com/freerandomiser/v1/lists that generated the randomisation list for both patients with MS and idiopathic patients. Thus giving:

- 12 patients with MS randomised to 30mins/day
- 12 patients with MS randomised to 30mins/week
- 12 patients with idiopathic OAB randomised to 30mins/day
- 12 patients with idiopathic OAB randomised to 30mins/week

Assessments (See Appendices)

All of the documents mentioned as part of the patient's assessment can be found in the Appendices.

Assessments were made at inclusion, then at week 4, week 8 and week 12 following the start of treatment. ICIQ-OAB and ICIQ-LUTSqol questionnaires and 3-day bladder diaries were completed for each of these visits to assess the severity of symptoms and the social and psychological burden. These questionnaire tools have been thoroughly validated and have received a grade A rating from the fourth ICI [14]. ICIQ-OAB is divided into parts A and B. A assesses symptom severity and B questions the patient about the degree of bother for this symptom, giving a score of up to 16 for A, 40 for B, and 56 in total. The ICIQ-LUTSqol, also divided into part A and B, examines the impact of urinary symptoms on the restriction of activities of daily living and various psycho-social aspects of the patients life (A), and how much each restriction bothers the patient.

Patients were required to attend outpatient visits at 0, 4, and 12 weeks during the treatment period, and were telephoned on a weekly basis by a member of the research team to enquire about device use and any adverse events. At the end of the study patients also completed a Global Response Assessment (GRA), which assesses whether patients perceive their symptoms to have improved, deteriorated or remained unchanged after 12 weeks of therapy, on a visual analogue scale as well as indicating the degree of this change. Responders were defined as those whose bladder symptoms are moderately or markedly improved on a 7-level GRA, as used in the SUmiT study [40]. Patients also completed a Patient Satisfaction Questionnaire that assesses their treatment tolerability and satisfaction.

The electrical stimulus setting was dependent upon each patient and their stimulus response, regardless of which arm of the study the patient was in. Once the device was applied, patients

had to demonstrate a sensory-motor response in their toes. The device setting was adjusted chosen according to this response. At the initial education session regarding the device, the patient was shown the device, and demonstrated how it works. The device was then be applied by the research team member, and set to an appropriate stimulation to achieve the required motor and sensory response in the toes [59]. The device was applied unilaterally, and the patient was taught the technique of application to self-apply at home.

Inclusion and Exclusion Criteria

Inclusion criteria

In order to be eligible to enter the study, patients must have met the following criteria:

1. Age 18 – 70 years
2. Self-reported overactive bladder symptoms for >3 months
3. Average urinary frequency of 8 voids per day
4. Self-reported failed conservative management
5. Discontinued all anti-muscarinics for at least 2 weeks
6. In patients with Multiple Sclerosis: The Kurtzke Expanded Disability Status Scale (EDSS) score 6.5 and below and free of relapses in the previous 3 months
7. Intact cutaneous sensations to nociception in the lower limb, as determined by the investigator
8. Able to understand the Patient Information Sheet and capable and willing to give informed consent and follow the protocol requirements (including attending all follow-up visits)
9. Willing to delay treatment with botulinum toxin for 12 weeks
10. On effective contraception if sexually active – oral contraceptive pill (> 3 months use), condoms, intrauterine contraceptive device, depot injection

Exclusion criteria

Patients would be excluded from the study if they met any of the following exclusion criteria:

1. Pregnant or planning to become pregnant during study duration
2. Use of botulinum toxin for the bladder or pelvic floor within the past one year
3. Pacemakers or implantable defibrillators
4. Bladder scans consistently showing an elevated post void residual > 100 mL
5. Current urinary tract infection
6. Current vaginal infection
7. Use of any other neuro-modulation device such as Interstim®
8. Current use of TENS in pelvic region, back or legs
9. Recent PTNS treatment
10. Use of investigational drug/device therapy within past 4 weeks that may interfere with this study
11. Participation in any clinical investigation involving or impacting gynaecologic, urinary or renal functions within past 4 weeks
12. Clinical symptoms of peripheral arterial disease, significant varicose veins or lower limb ulceration
13. Recent surgery (such as abdominal, gynaecological, hip or knee replacement).
14. Recent trauma to lower limbs.
15. Chronic Obesity (BMI Index >34)
16. Any medication judged to be significant by the Principal Investigator
17. Any significant illness during the four (4) weeks preceding the screening period of the study
18. Recent diagnosis of Deep Vein Thrombosis

19. If a patient was deemed to clinically have a benign enlarged prostate by clinical examination, an elevated residual volume, and evidence of an obstructed flow on uroflowmetry

Urine sample collection and ELISA

Urine samples were collected for measurement of urinary NGF and BDNF (uNGF) and (uBDNF) in patients with MS and idiopathic OAB. Samples were collected at baseline, and at week 4 visits and at week 12 (end of therapy). Urine samples were collected at full bladder sensation, immediately placed on ice, transferred to the laboratory, and centrifuged (3000rpm; 10 min at 4°C). The supernatant was collected in 1ml eppendorf tubes and stored at -70°C. NGF and BDNF concentrations were measured using ELISA (Emax Immunoassay System, Promega, Madison, WI, USA), with a minimum sensitivity of 3.9pg/ml and 7.8 pg/ml, respectively, following the manufacturers instructions.

Outcomes

Primary endpoint:

To assess the patient response to 12 weeks of treatment with geko™ to manage OAB symptoms using the Global Response Assessment Score (GRA). (This score can be found in the Appendix section)

Secondary endpoints:

- To determine the efficacy of a 12 week course of tibial nerve stimulation by the geko™ device using two treatment frequency paradigms, as measured by the ICIQ-OAB, ICIQ-QoL and bladder diary.
- To assess the patient's satisfaction of the treatment using the Likert scale.
- To estimate dose response using two treatment duration paradigms by comparison of daily versus weekly efficacy.
- To evaluate the urinary NGF and BDNF levels in patients before, during and after 12 weeks of transcutaneous tibial nerve stimulation.

RESULTS

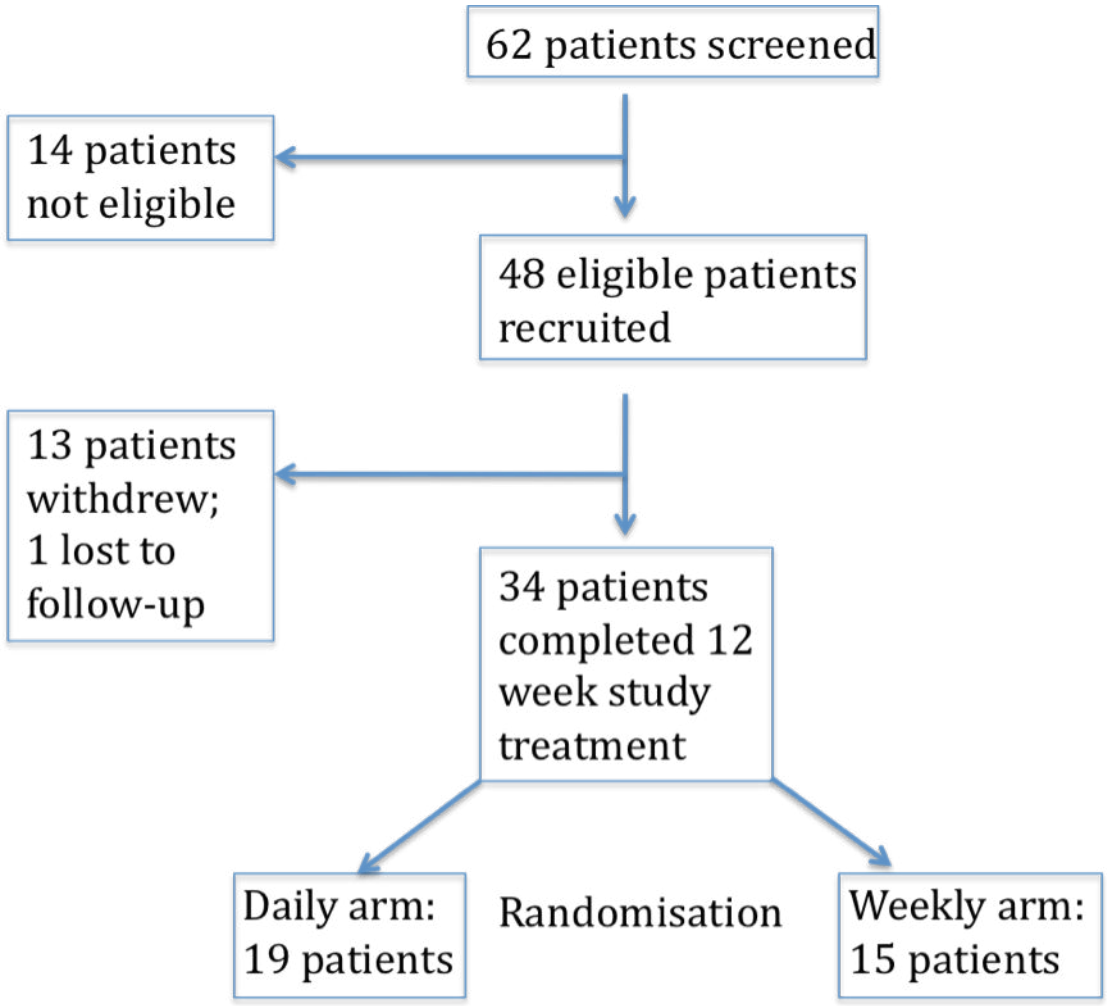
Population

Sixty-two patients were screened in total, to identify 48 eligible patients to begin the study. Forty-eight patients were recruited into the study, with nine males and thirty-nine females with a mean age of 46 years. Fourteen patients withdrew from the study after starting, leaving 34 patients (5 men and 25 women) completing 12 weeks of treatment. Of this group, 19 and 15 patients had MS and Idiopathic OAB respectively. The groups of those having daily versus weekly treatments is illustrated in the table twenty-two below.

	Daily	**Weekly**	**Total**
Multiple Sclerosis	**9**	**10**	**19**
Idiopathic OAB	**10**	**5**	**15**
Total	**19**	**15**	**34**

Table twenty-two: The distribution of diagnoses in the two treatment arms

The summary flow chart of patients enrolling into the study is shown below in figure twenty-two:



Failed screening

The reasons why 14 patients failed screening are listed in the table twenty-three below.

Reason for failed screening	Number
No sensory-motor response in feet once device applied	12
Raised post-void residual volume	2
Total	14

It is evident that the device was unable to elicit a sensory-motor response in the feet of 12 patients. For sufficient stimulation of the PTNS, as reported in the SuMIT study, there should be sensory and or motor responses in the feet of the subject. Evidence of effective stimulation should cause toe flexion of the great toe and/or of digits two to five. Patients should also notice some sensory paraesthesia in the sole of the foot. It has been shown in a previous study that patients with unsufficient sensory motor response in the feet do not respond to PTNS [59], and therefore these 12 patients were not included in the study. However before exclusion, the device position was altered and adjusted to try to evoke a response, considering the possibility of intersubject variability in local anatomy. More than one device was tried in case there was a manufacturing fault with that particular device. If this failed, an attempt was also made using the contralateral leg to establish a response. Notably these patients had intact sensation in their lower limbs on neurological examination to both touch and pin prick over the L5 and S1 dermatomes. Six out of these twelve patients had a degree of ankle oedema, which may have limited the efficiency of the stimulation.

All patients went on to have a trial sensory-motor stimulation using the percutaneous technique with a fine needle, and eleven out of these twelve patients were able to feel some sensory-motor response with this.

Study withdrawals

The reasons for withdrawal from the study are presented in table three below. To note thirteen patients withdrew from the study, and one was lost to follow up. Two out of the thirteen patients who withdrew from the study were due to lack of efficacy, and three due to discomfort from the device stimulations. In the table these are classified as device related and non-device related withdrawals of which the former comprises 8/14 of the cases.

Device related		**Unrelated to device**	
Reason	Number of patients	Reason	Number of patients
Skin reaction to device (redness)	1	Family bereavement	1
Discomfort from device stimulations	4	Lost to follow-up	1
Withdrew from study as ineffective	2	Became ill with MS prior to starting device	2
Daily stimulation was too intense for patient	1	MS related leg weakness	1
		Could not come off antimuscarinic medication	1
Total	8	Total	6

Table twenty-four: The device related and non-device related reasons for patient withdrawal from the study.

Adverse events

Adverse events from the study were 7 in total, which again can be divided into device related and unrelated adverse events. Five out of seven were device related whilst 2/7 were unrelated to the device. These are tabulated in table four below. Four of the device related adverse events were due to discomfort from the device stimulations, and one due to skin redness after a reaction to the adhesive on the device. The two non-device related adverse events include one UTI in a patient who performed clean intermittent self-catheterisation, and the other bilateral leg weakness in a patient who had a history of episodic leg weakness due to her MS. There were no systemic side effects and no patient reported voiding dysfunction or urinary retention.

Device Related		**Unrelated to device**	
Reason	Number	Reason	Number
Skin rash	1	UTI	1
Discomfort due to device stimulations	4	Bilateral leg weakness due to MS	1
Total	5	Total	2

Table twenty-five: Describes the adverse events – classified into either related or unrelated to the device.

The spread of EDSS scores for the patients with MS ranged from one to six, and there was no clear correlation between EDSS score and the response to treatment. The graph below shows that for the mean change from baseline to the end of study for ICIQ-OAB scores, improvements were seen in both patients with low or high EDSS. There were also 2 patients whose questionnaire score had worsened by 12 weeks. Improvements in symptoms after twelve weeks of treatment, were not affected by the severity of disability imposed by MS.

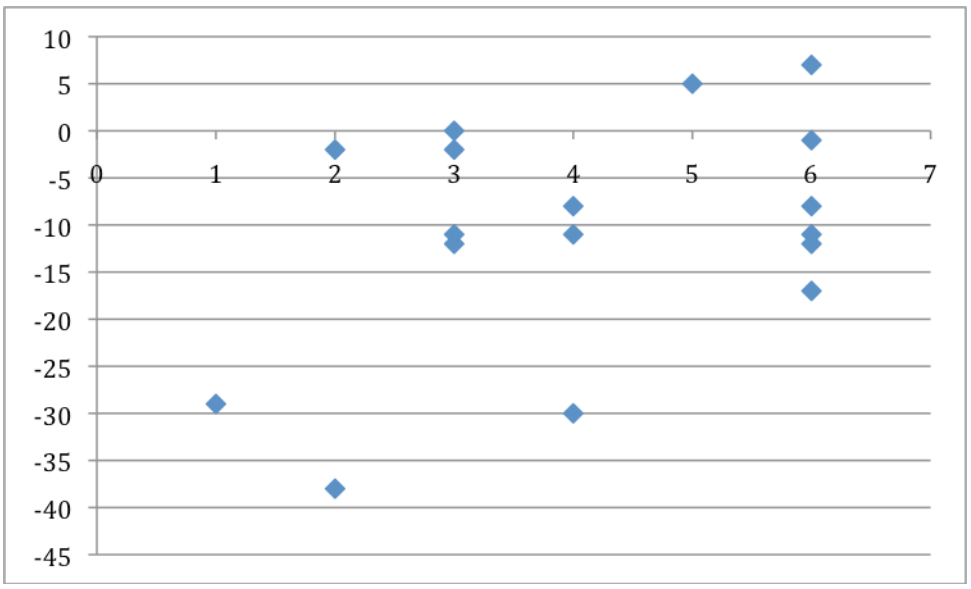


Figure twenty-three: A graph to show the change in ICIQ-OAB score among the MS patients with differing EDSS scores. X-axis EDSS score, Y-axis change in ICIQ-OAB score (the baseline score subtracted from the end of study score). A negative value denotes an improvement in symptoms, whilst a positive value suggests a worsening of score in the questionnaire.

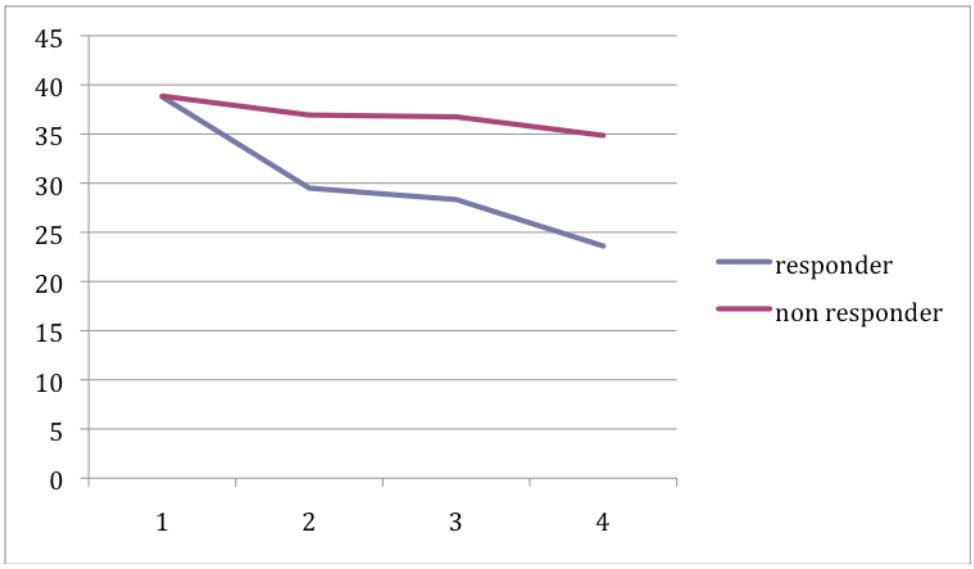
Primary endpoint:

Eighteen out of thirty four 18/34 (53%) patients self scored their response after 12 weeks of treatment as moderate or significant improvement on the GRA scale, and were termed as responders. The remaining sixteen out of thirty-four (47%) patients who were non-responders scored themselves as having no or only mild improvement on the GRA. No patients scored themselves to have worsened on the GRA visual analogue scale.

Secondary endpoints:

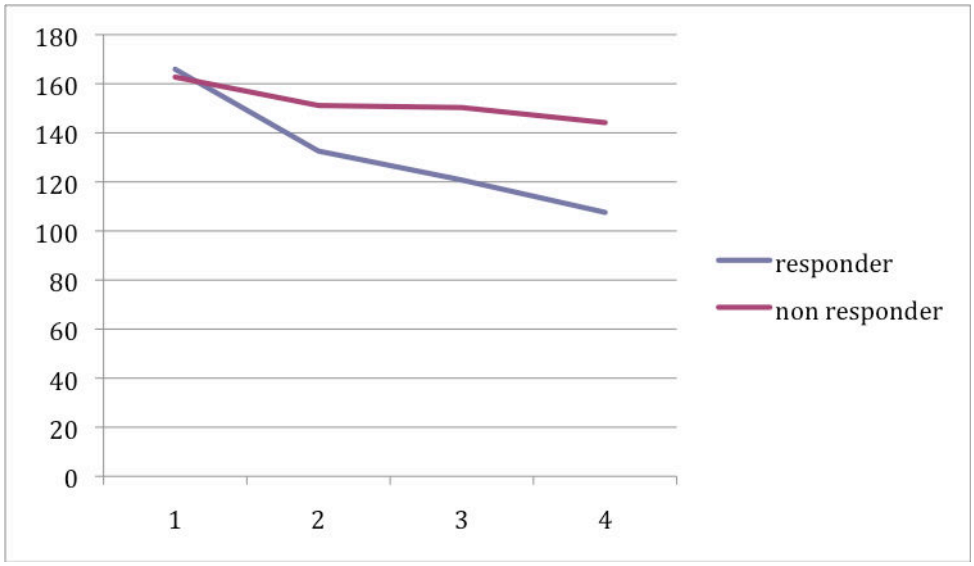
The baseline scores for responders and non-responders for the ICIQ-OAB and ICIQ-LUTSqol are not significantly different, implying that non-responders did not start the study with significantly worse symptoms. The symptom and QOL scores over time can be seen in figure four and five below:

Figure twenty-four: Mean ICIQ-OAB total scores in responders versus non-responders



Visit 1, 2, 3, 4 relate to week 0, 4, 8 and 12

Figure twenty-five: Mean ICIQ-LUTSqol total scores in responders versus non-responders



Visit 1, 2, 3, 4 relate to week 0, 4, 8 and 12

The demographics of the responders and non-responders are displayed in the table twenty-six below:

Responders				**Non-Responders**			
Mean age (yrs)		42		Mean age (yrs)		48.3	
Mean EDSS (MS patients)		4		Mean EDSS (MS patients)		3.7	
Group	Weekly	Daily		Group	Weekly	Daily	
MS	7	6	13 (72%)	MS	3	4	7 (44%)
IDO	2	3	5 (28%)	IDO	3	6	9 (56%)
	9	9			6	10	

There was no significant difference in age or EDSS of either group of responders or non-responders. Interestingly the majority, 13 (72%), of the responders had MS. There was a comparable number of responders and non-responders in the daily and weekly arms.

The table twenty-seven below shows the baseline characteristics which are comparable between the two groups.

Table twenty-seven: Study characteristics at baseline according to diagnosis

	Those who completed 12 weeks follow up N=34		Range
Characteristic:	Idiopathic N=15	MS N=19	
Sex (M/F)			
Daily treatment; N (%)	10 (66.7%)	10 (53.6%)	
Wet; N (%)	13 (86.7%)	17 (89.5%)	
Age; mean (SD)	41.9 (18.2)	48.3 (9.2)	
Perceived day frequency ICIQ-OAB; median (IQR)	2 (2, 2)	2 (1, 2.5)	0: 1 to 6 times 1: 7 to 8 times 2: 9 to 10 times 3: 11 to 12 times 4: 13 or more times
Perceived nocturia ICIQ-OAB; median (IQR)	3 (2, 3)	2 (1.5, 3)	0: none 1: one 2: two 3: three 4: four or more
Perceived urgency ICIQ-OAB; median (IQR)	3 (2, 3)	2 (2, 3)	0: never 1: occasionally 2: sometimes

			3: most of the time 4: all of the time
Perceived leakage ICIQ-OAB; median (IQR)	2 (2, 3)	2 (2, 3)	0: never 1: occasionally 2: sometimes 3: most of the time 4: all of the time
ICIQ OAB total; median (IQR)	41 (35, 46)	38 (28, 44)	0-56
ICIQ-LUTSqoL total; median (IQR)	145 (135, 235)	155 (118, 204)	0-276
Mean voided volume; median (IQR)	202 (151, 238)	150 (112, 184)	
24 hour freq (BD); median (IQR)	10.7 (8, 11)	12 (9, 14)	
Mean bladder urge (BD); median (IQR)	2.5 (2.3, 3.3)	2.9 (2.2, 3.1)	
Mean leakages (BD); median (IQR)	2.7 (1, 4)	1.7 (1, 4)	
Leakage severity (BD); median (IQR)	1.2 (1, 2)	1 (1, 1.3)	1: mild 2: moderate 3: severe

IQR: Interquartile range

BD: Bladder diary

From the bladder diary, patients with MS did have significantly smaller mean and maximum voided volumes compared to the patients with idiopathic OAB, at the end of study, from the three-day bladder diary (169 and 271 mls versus 312 and 466 mls respectively). This suggests that the patients with MS may have had smaller functional bladder capacities to the patients with idiopathic OAB.

Table twenty-eight: Mean (SD) absolute changes in the ICIQ questionnaire and bladder diary outcomes from baseline to week 12 according to diagnosis

Characteristic:	Idiopathic N=15	MS N=19	Overall N=34
Perceived day frequency change; mean (SD)	-0.62 (0.77)	-0.50 (0.89)	-0.55 (0.83)
Perceived nocturia change; mean (SD)	-0.69 (1.11)	-0.56 (0.73)	-0.62 (0.90)
Perceived urgency change; mean (SD)	-0.54 (0.88)	-0.88 (0.72)	-0.72 (0.80)
Perceived leakage change; mean (SD)	-0.69 (0.95)	-0.88 (0.89)	-0.79 (0.90)
ICIQ OAB total change; mean (SD)	-9.2 (10.4)	-10.6 (12.7)	-9.9 (11.5)
ICIQ QoL total change; mean (SD)	-20.3 (65.5)	-44.9 (55.4)	-34.0 (60.1)
Mean voided volume change; mean (SD)	43.4 (94.1)	-3.4 (55.7)	16.3 (75.7)
24 hour freq change (BD); mean (SD)	-1.03 (3.55)	-1.56 (2.76)	-1.32 (3.06)
Mean bladder urge change (BD); mean (SD)	-0.70 (0.93)	-0.56 (0.79)	-0.62 (0.83)
Mean leakages change (BD); mean (SD)	-0.70 (1.53)	-0.97 (1.48)	-0.85 (1.47)
Leakage severity change (BD); mean (SD)	-0.46 (0.59)	0.05 (0.67)	-0.23 (0.64)

BD: Bladder diary

(SD): standard deviation

The above table shows the absolute changes in the individual parameters, from end of study to baseline. For the ICIQ-OAB, ICIQ-LUTqol, and BD parameters, a negative value, suggests fewer symptoms and an improved outcome. For mean voided volume, only improvement was seen in the idiopathic OAB group, with no improvement observed for the patients with MS.

All of the below tables (twenty-nine to thirty-nine) have been produced using mixed effects models to take into account the multiple observations on each patient. These tables show the effect of time, diagnosis or treatment frequency on each of the outcome parameters.

Table twenty-nine: Change in perceived day frequency according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.04 (-0.06, -0.02)	0.001	-0.04 (-0.06, -0.02)	0.001
Diagnosis; MS vs IDO	-0.03 (-0.67, 0.61)	0.93	-0.02 (-0.68, 0.63)	0.95
Treatment; weekly vs daily	-0.04 (-0.66, 0.57)	0.88	-0.04 (-0.68, 0.60)	0.90

Table thirty: Change in perceived nocturia according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.05 (-0.08, -0.02)	<0.0001	-0.05 (-0.08, -0.02)	<0.0001
Diagnosis; MS vs IDO	-0.26 (-0.84, 0.32)	0.38	-0.29 (-0.88, 0.30)	0.34
Treatment; weekly vs daily	0.10 (-0.47, 0.68)	0.72	0.21 (-0.38, 0.79)	0.49

Table thirty-one: Change in perceived urgency according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.07 (-0.09, -0.04)	<0.0001	-0.07 (-0.09, -0.04)	<0.0001
Diagnosis; MS vs IDO	-0.32 (-0.75, 0.10)	0.14	-0.29 (-0.71, 0.13)	0.18
Treatment; weekly vs daily	-0.34 (-0.77, 0.09)	0.12	-0.27 (-0.69, 0.16)	0.22

Table thirty-two: Change in perceived urge leakage according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.06 (-0.09, -0.03)	<0.0001	-0.06 (-0.09, -0.03)	<0.0001
Diagnosis; MS vs IDO	-0.27 (-0.84, 0.30)	0.36	-0.23 (-0.79, 0.32)	0.41
Treatment; weekly vs daily	-0.32 (-0.86, 0.21)	0.24	-0.27 (-0.80, 0.26)	0.32

Table thirty-three: Change in ICIQ OAB total according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.82 (-1.13, -0.51)	<0.0001	-0.82 (-1.13, -0.51)	<0.0001
Diagnosis; MS vs IDO	-4.63 (-9.76, 0.51)	0.08	-4.65 (-9.84, 0.54)	0.08
Treatment; weekly vs daily	-2.98 (-8.19, 2.23)	0.26	-2.14 (-7.47, 3.19)	0.43

Table thirty-four: Change in ICIQ QoL total according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-3.22 (-5.04, -1.41)	0.001	-3.17 (-5.00, -1.35)	<0.0001
Diagnosis; MS vs IDO	-16.1 (-48.7, 16.5)	0.33	-15.3 (-47.1, 16.5)	0.35
Treatment; weekly vs daily	-27.3 (-57.9, 3.3)	0.08	-25.2 (-57.9, 7.5)	0.13

Table thirty-five: Change in mean voided volume according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	0.83 (-1.58, 3.23)	0.50	0.86 (-1.52, 3.23)	0.48
Diagnosis; MS vs IDO	-64.3 (-108.1, -20.6)	0.004	-65.0 (-109.2, -20.7)	0.004
Treatment; weekly vs daily	-17.2 (-63.3, 28.9)	0.47	-4.1 (-48.0, 39.8)	0.86

Table thirty-six: Change in 24 hour bladder frequency according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	0.09 (-0.33, -0.51)	0.68	0.07 (-0.33, -0.46)	0.74
Diagnosis; MS vs IDO	-2.34 (-9.20, 4.51)	0.50	-2.89 (-10.53, 4.75)	0.46
Treatment; weekly vs daily	3.32 (-2.82, 9.46)	0.29	3.77 (-3.28, 10.82)	0.30

Table thirty-seven: Change in mean urge according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.05 (-0.07, -0.02)	<0.0001	-0.05 (-0.07, -0.02)	<0.0001
Diagnosis; MS vs IDO	0.17 (-0.27, 0.61)	0.45	0.17 (-0.27, 0.62)	0.45
Treatment; weekly vs daily	-0.00 (-0.42, 0.41)	0.99	-0.00 (-0.42, 0.42)	0.99

Table thirty-eight: Change in mean leakages according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.06 (-0.12, -0.01)	0.03	-0.06 (-0.12, -0.01)	0.03
Diagnosis; MS vs IDO	-0.01 (-1.57, 1.55)	0.99	-0.00 (-1.55, 1.54)	0.99
Treatment; weekly vs daily	-0.03 (-1.60, 1.55)	0.97	-0.04 (-1.59, 1.51)	0.96

Table thirty-nine: Change in leakage severity according to baseline characteristics

	Unadjusted analysis		Adjusted analysis	
Characteristic:	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Time; per each extra week of therapy	-0.01 (-0.03, 0.01)	0.26	-0.01 (-0.03, 0.01)	0.36
Diagnosis; MS vs IDO	-0.00 (-0.26, 0.25)	0.98	0.05 (-0.23, 0.33)	0.72
Treatment; weekly vs daily	-0.17 (-0.41, 0.07)	0.16	-0.20 (-0.46, 0.07)	0.15

Clinical efficacy

It can seen that for all individual ICIQ-OAB, ICIQ-LUTSqol patient self reported parameters, there are significant improvements in symptoms of urinary frequency, nocturia, urgency and urge leakage and these are improvements are sustained for every week that the patient receives therapy. The clinical benefits appear to be cumulative for up to end of therapy at 12 weeks. These improvements are also related to the impact symptoms are having on patient QOL, suggesting reduced psychosocial burden which appears to improve significantly with each week of therapy.

MS versus Idiopathic

As can be seen in the tables above, for all ICIQ-OAB, ICIQ-LUTSqol and BD parameters, there is no significant difference between the performance of either group of patients in response to therapy. The TPTNS seems to have equal efficacy for idiopathic OAB and MS.

Weekly versus daily treatment

Likewise there are no significant differences in the efficacy using these parameters for patients in the weekly or daily arm. This suggests non-inferiority of weekly treatments compared to daily stimulations. This has important implications for clinical application, as can be seen from table three, one patient withdrew from treatment due to the onerous intensity of daily stimulation. Therefore weekly treatments would be more suitable for patient satisfaction, compliance and cost-effectiveness.

Using a multilevel regression model for all patients whether MS and Idiopathic OAB, and including those from both daily and weekly arms, all patients had significant improvements in ICIQ-LUTSqol at the week 4, 8 and 12 visits by 25.9, 28.2 and 40.8 points respectively compared to baseline. Likewise there were significant improvements for the ICIQ-OAB score at the week 4, 8, and 12 visits by -6.5, -7.4 and -10.2 points respectively. It can be seen that the most significant improvement for both questionnaires is by week 12. This suggests that treatment beyond 8 weeks, as carried out by some previous studies, and up to 12 weeks is justified, as the benefit from the stimulation is most evident at the end of study. This is illustrated in table fourty and fourty-one.

Table fourty and fourty-one: the absolute change in ICIQ-LUTSqol and ICIQ-OAB score over the visits.

	Change in ICIQ-LUTSqol total score from baseline	Std Error	P value	95% Confidence interval	
Week 4	-25.9	8.1	0.001	-41.7	-10.1
Week 8	-28.2	8.7	0.001	-45.2	-11.2
Week 12	-40.8	8.5	<0.0001	-57.4	-24.3

	Change in ICIQ-OAB total score from baseline	Std Error	P value	95% Confidence interval	
Week 4	-6.5	1.6	<0.0001	-9.8	-3.2
Week 8	-7.4	1.9	<0.0001	-11.0	-3.8
Week 12	-10.2	1.7	<0.0001	-13.5	-6.9

Patient satisfaction

Results of the 7-point Likert scale evaluating patient satisfaction suggests patients were satisfied with the ease of operating the device, comfort and mobility.

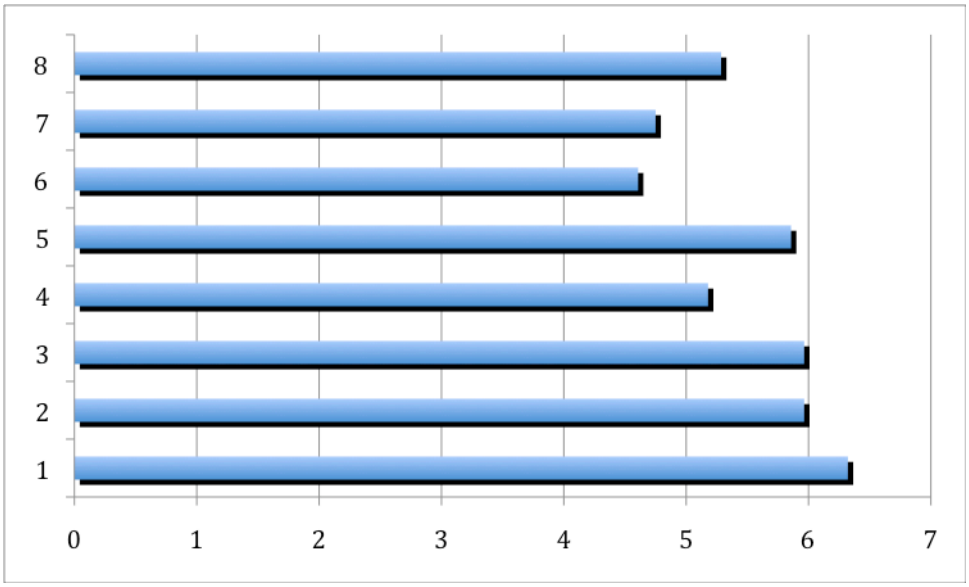


Figure twenty-six: Patient satisfaction survey. Y-axis 1-The device instructions are easy to understand; 2- The device is easy to attach and remove; 3- The device is easy to operate; 4. The device is comfortable to use; 5. I have full mobility when I am wearing the device; 6. The device improved my symptoms; 7. Overall I am satisfied with the device; 8. I would recommend the device to a friend for this use. X-axis seven point Likert scale (Strongly disagree- 1; Strongly agree- 7).

Urinary Neurotrophin Analysis

Nerve Growth Factor

In patients with MS who responded to therapy, mean NGF/creat levels changed from 30.1 at baseline to 32.4 at week 12. For the non-responders, mean levels were 33.8 at baseline to 30.5 at week 12.

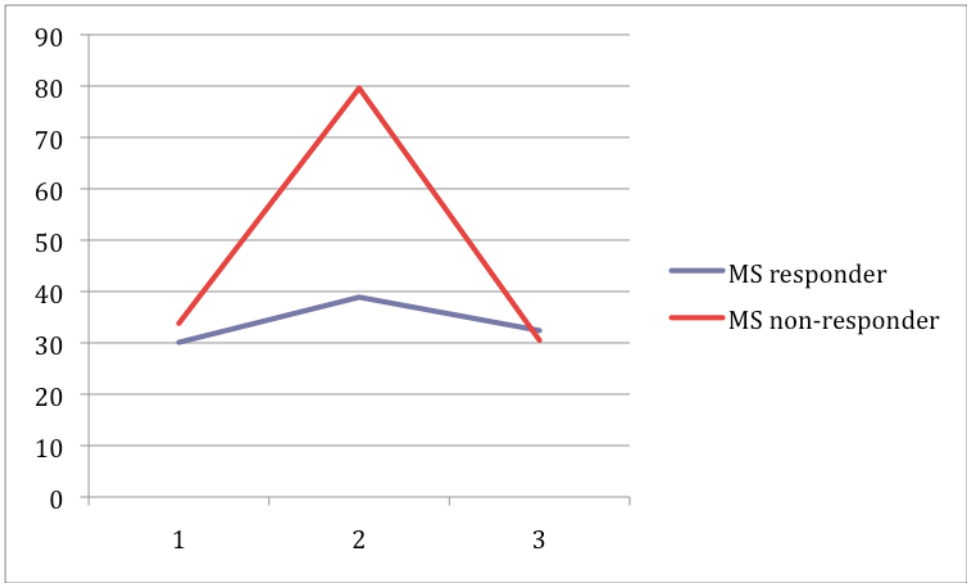


Figure twenty-seven: The change in NGF/Cr levels over the course of treatment in patients with MS

X-axis: Visit 1, 2 and 3 relate to visits at baseline, week 4 and 12

Y axis: urinary NGF/Creat level pg/millimol/L

In patients with Idiopathic OAB who responded to therapy, mean NGF/Creat levels were 11.3 (2.7-18.9) and 13.6 (1.8-22.4) pg/mg at baseline and week 12 respectively (p=0.99). Non-responders had a change of mean levels from 96.6 (40.6-97.8) to 48.4 (4.5-133.6) pg/mg at baseline and week 12 respectively (p=0.23). The NGF/Creat levels at baseline were significantly different between the responders and non-responders (p=0.05).

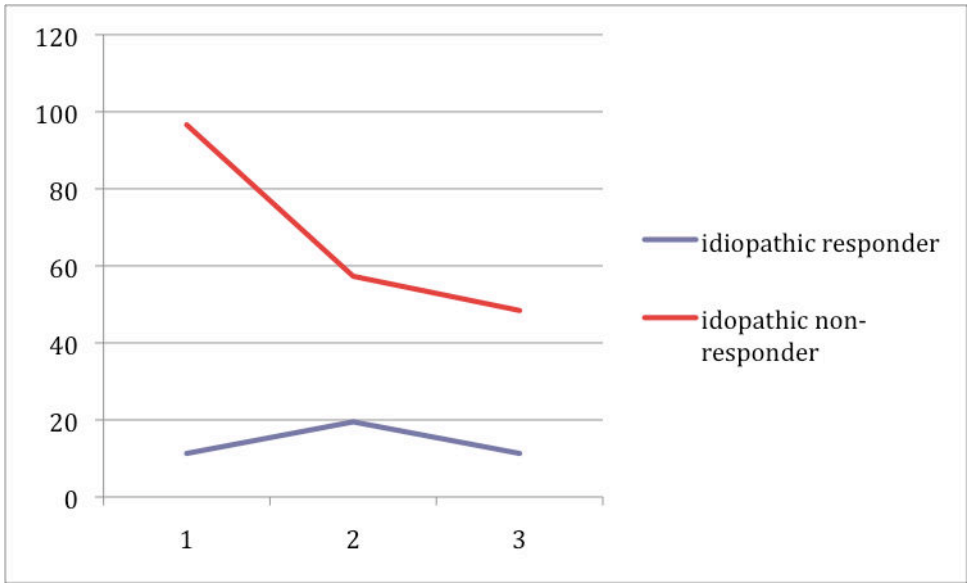


Figure twenty-eight: The change in NGF/Cr levels over the course of treatment in patients with idiopathic OAB

X-axis: Visit 1, 2 and 3 relate to visits at baseline, week 4 and 12

Y axis: urinary NGF/Creat level pg/millimol/L

Brain derived neurotrophic factor

In patients with MS who responded to therapy, mean BDNF/Cr levels changed from 67.2 at baseline to 46.8 at week 12 (p=0.49). For the non-responders, mean levels were 77.0 at baseline to 28.2 at week 12 (p=0.33).

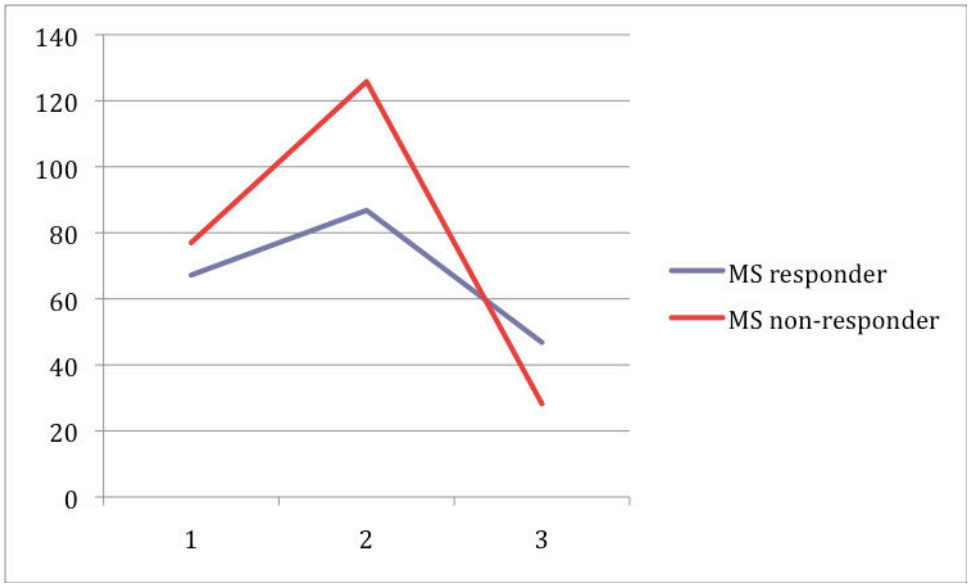


Figure twenty-nine: The change in BDNF/Cr levels over the course of treatment in patients with MS

X-axis: Visit 1, 2 and 3 relate to visits at baseline, week 4 and 12

Y axis: urinary BDNF/Cr level pg/millimol/L

In patients with Idiopathic OAB who responded to therapy, mean BDNF/Cr levels changed from 66.6 to 19.1pg/mg (p=0.03). Non-responders had mean levels of 117.7 (6.7-337.8) to 76.8 (0-182.5) pg/mg at week 12 (p=0.89).

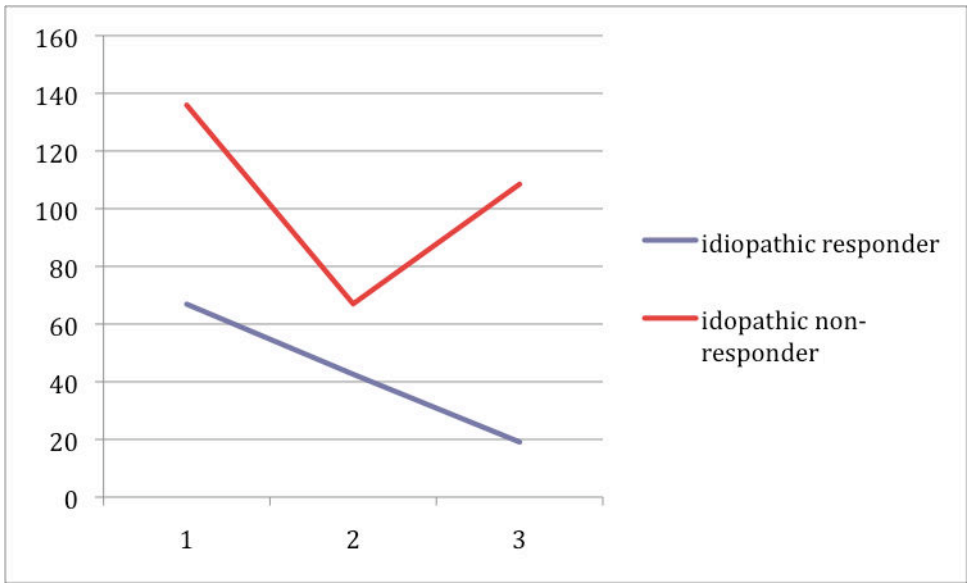


Figure thirty: The change in BDNF/Cr levels over the course of treatment in patients with idiopathic OAB

X-axis: Visit 1, 2 and 3 relate to baseline, week 4 and 12

Y axis: urinary BDNF/Cr level pg/millimol/L

DISCUSSION

This is the first study to assess the clinical efficacy of the geko^{TM} device as a mode of Transcutaneous Tibial Nerve Stimulation (TTNS) to manage OAB in idiopathic or patients with MS. Fifty three percent of patients completing 12 weeks of treatment had a positive response by reporting a moderate to significant improvement on the GRA scale. This is comparable to the improvement seen in the SUmiT study where 54.5% of the patients in the treatment arm reported moderate or marked improvements in bladder symptoms [40]. Patient using this form of TPTNS demonstrated that this treatment significantly improved their symptoms and its associated psychosocial burden.

Significant improvements were observed in this responder group with reductions in the symptoms of OAB and disturbance to QOL. A 40% reduction in perceived urgency, and 42%, 44% and 34% reduction in urge leakages, nocturia and daytime frequency respectively from the ICIQ-OAB questionnaire. These reductions in OAB symptom score were matched by 43% improvements in the bothersome QOL score. These benefits appear to be evident after the first week of therapy and are sustained and cumulative over the twelve-week treatment period.

The device also was demonstrated to be safe with minimal adverse effects, where only four out of thirty five patients experienced some discomfort with the device stimulations. The prevalence of OAB increases significantly with age [200] and there is a need for treatment alternatives that do not have any adverse effects for this group of patients who may already rely on numerous medications. With the efficacy of TPTNS, if proven with larger placebo controlled phase III studies, with its minimal adverse effect profile could be considered as a first line treatment in such populations.

No significant differences were seen in the performance of TPNS for OAB symptoms between patients with MS or idiopathic OAB. There was a trend for MS patients to perform better since 72% of the responders were MS patients. This efficacy of TTNS in patients with MS supports the findings from the study by de Seze et al, and the handful of small PTNS studies demonstrating success of symptom control in this neurogenic population of patients [51, 55-60, 75]. These findings also match the findings observed from the responses to PTNS described in chapter one.

The study design included weekly and daily arms to investigate whether one was superior to the other. The SUmiT study design treated patients on a weekly basis, whilst de Seze et al who studied another method of transcutaneous delivery used a daily treatment regime both over a three-month period [40, 75]. In this study patients receiving daily stimulations were equivalent and did no better than those on the weekly regime, and thus demonstrates non-inferiority of either treatment frequency. This may have significant cost implications as the cost of twelve devices over three months would be considerably less than that for 84 devices which would be required for daily use. This would also be favourable for patient compliance as weekly use would be much less intrusive to their daily routine, than a daily use of thirty minutes. As a result patient compliance to treatment may be more successful. It can be seen that one patient withdrew from the study due to the burden of daily stimulations. Also for patients who find the stimulations uncomfortable, which lead to three patients to withdraw, weekly use would be more bearable.

Urinary Neurotrophins

Interestingly no significant changes in neurotrophin levels were seen in the responders and non-responders with MS throughout the treatment programme. There appeared to be some

trend for neurotrophin level increase after four weeks of stimulation, especially with the non-responders to therapy, and the significance of this is unclear. This could possibly reflect some remodeling of the nerve activity at the level of the urothelium or the local neural circuitry. However as can be seen from our experience in chapter three, the reliability of urinary neurotrophins as a marker for OAB in patients with MS is unclear.

Whereas for the idiopathic OAB patients, baseline NGF levels were significantly higher in the non-responding patients compared to the responders. It is interesting to observe that all idiopathic patients who had comparable OAB symptom severity prior to starting therapy, yet there remains a large difference in the uNGF levels at baseline. This may reflect the severity of the underlying disease processes that are producing the symptoms. This may suggest that a very high baseline NGF/Cr score may be a poor predictor for response to treatment. No significant change in the NGF/Cr levels were seen however in the responders to therapy. The reason for this is unclear and this could be due to the small sample of patients, which could only identify trends and is not powered to offer accurate biomarker analyses. The other explanation for the lack of response seen in uNGF/Cr reason could suggest some evidence of placebo effect in this cohort, however this is confounded by the improvement in BDNF/Cr levels. Placebo effect is present to some degree in most clinical trials, however without a sham arm the actual extent of this is unknown.

With regards to BDNF/Cr levels in the idiopathic patients, the baseline levels were different with the non-responders having higher levels, similar to the NGF/Cr levels, however this was not to significance. However the BDNF/Cr levels significantly reduced by the end of study compared to baseline, in the clinical responders, suggesting that the BDNF levels may be of some use to monitor response to treatment. There was no reduction seen in the non-

responders to therapy. Further study of these biomarkers is required in larger numbers to uncover the role of urinary neurotrophins as a biomarker for assessing response to treatment. The evidence for its use for idiopathic OAB is growing, however this is largely lacking for the MS patients.

Study limitations

Only 34 out of an intended 48 patients completed this study, significantly reducing the sample size by almost 30%. The reasons for withdrawal were documented and eight of the fourteen patients were device related. Two of these were due to lack of efficacy and five due to adverse effects. However this study was not powered to draw significant statistical conclusions, but to be considered as a phase II pilot study to test the principle of this device as a potential treatment option, and to document its safety. A larger volume of patients will be recruited for any phase III study, which will be appropriately powered to adjust for the patients who withdraw.

Study outcomes were reliant on patient reporting and therefore not wholly objective. This is always a consideration for OAB trials as this is essentially a condition that is patient self-reported symptom complex. On the whole therefore the ICS recommends the use of validated tools that can assess symptoms and the impact on QOL [67]. Some studies evaluating PTNS have included urodynamic parameters to correlate with questionnaire scores. The three-day bladder diary is more objective than the symptom questionnaires, however this is also reliant on patients accurately measuring urine outputs and fluid intake over three 24hour periods, which some patients admittedly found difficult to do.

Urodynamic assessment of patients at baseline and end of therapy was not performed in this phase II study. Therefore no comment can be made on the detrusor pressures during filling, and the compliance and bladder capacity and its response to TTNS. Whether a new treatment modality can reduce detrusor pressures is important and this requires evaluation in a future study, as severe neurogenic DO may risk upper urinary tract function, and these candidates may therefore not be suitable for this therapy. However in an attempt to provide some objective evaluation of treatment response, urinary neurotrophins were analysed.

There was no placebo arm in this study and hence the extent of the placebo response is unknown. This form of peripheral nerve stimulation urgently requires large volume trials comparing the treatment to placebo. It has been noted during OAB drug trials that the placebo effect can effect up to 64% of patients for urinary incontinence symptoms and the actual placebo effect for this device needs to be appropriately evaluated [27]. This is particularly relevant as it has been demonstrated in a study that the effect of a sham device has a greater placebo effect than that of an inert pill. This study involved the use of an acupuncture needle for arm pain versus a placebo pill, over an eight-week period [201]. However the observed reduction in urinary BDNF/Cr levels in response to treatment in the responders, and the lack of any level reduction in the non-responders, would support that any placebo effect would be minimised.

Since the study duration was limited to 12 weeks, the continuation of efficacy past this period is unknown. Therefore the long-term reliability of this as a treatment needs further investigation with an extended study period to identify the optimal treatment period, and whether treatment efficacy is sustained with top up therapy as described in the STEP study with PTNS [71, 202].

Patient's who respond to antimuscarinic therapy for OAB may indicate that they have mild to moderate OAB. It is likely that the severe OAB patients would not respond to antimuscarinic therapy for sufficient symptomatic control, and would therefore require treatment with intravesical BTX-A or SNM. The patient cohort recruited for this study were either antimuscarinic naïve, responsive and those who had failed therapy. Since this is a heterogenous group of patients, although with comparable baseline symptom scores, some patients would have more significant disease that is resistant to therapy. If patients were all

responders to antimuscarinic therapy, then the overall performance and response to TTNS may be better than demonstrated in this study. To control the effect of antimuscarinic therapy, patients were asked to stop AM treatment with a two week washout period, so the risk of patients who may have been having a combination of AM and TTNS is avoided and the outcomes are not skewed as a result.

There was also heterogeneity in the group of patients with MS who had a range of EDSS scores. It has been demonstrated that the likelihood of a urodynamic abnormality in MS was higher in patients who had an EDSS score of more than 6.5, and hence more severe bladder dysfunction, and more resistant to treatment [163]. Therefore to limit the impact this may have on the range of baseline symptom severity, recruitment was restricted to patients with EDSS scores of less than 6.5 and free of relapses for the previous 3 months.

Twelve patients out of the sixty-two screened patients (19%) were unable to feel any sensory motor response once the patch was applied, and were defined as screen failures. All of these patients had intact sensory nerve examination of their lower limbs, both to touch and to pinprick. Fifty percent of this group had evidence of ankle oedema, which may have impeded signal transduction from the device. Eleven of these patients then went on to have a positive peripheral nerve evaluation with the PTNS needle system (UrgentPC), where a sensory motor response was successfully demonstrated. This would indicate that the needle is more effective at locating and delivering the stimulation to the posterior tibial nerve. This could possibly be due to the preferable anatomical proximity the needle has to access the deeper nerve, compared to a skin patch which is more superficial.

The nature of the stimulus may also be important to the clinical outcome. PTNS uses a current level of 0.5 to 9mA at 20Hz with a fixed pulse width of 200microseconds, and the current amplitude is adjusted to a setting that is found to be tolerable for the patient, which is

usually 1.5 times that sufficient to evoke fanning or planting flexion of toes. In comparison, the gekoTM device delivers single pulse of 27mA, with an adjustable width, at a frequency of 1Hz. The pulse width is adjustable to one of seven settings between 70 and 560μs. The level of electrical stimulation with the geko^{TM} is adjusted by changing the pulse width. The effect of the different modes of stimulation delivery is unknown. The optimal power and frequency of delivery of electrical stimulation that would be closest to the biology of a true action potential and provide the most significant clinical impact is also unknown. This is an area for further research investigation.

To address the issue with numerous patients unable to demonstrate a sensory motor response to the device, a newer modified device, called gekoT-2, has been developed with more effective stimulation delivery, and requires some clinical evaluation and validation. The lack of sensory motor response in 19% of patients screened immediately excludes patients from treatment, and the transcutaneous delivery of stimulation needs to optimized to minimize this patient loss.

Tolerance and Withdrawal

There were a few patients reporting local side effects to therapy with four out of 34 patients (12%) having discomfort with the device. One patient complained of skin redness as a hypersensitivity reaction to the adhesive of the device, which became apparent after only a few minutes of wearing the device. No patient reported any symptoms or signs of urinary retention, and the one patient who was self-catheterising did not recover voiding function at the end of study.

Patients found using the device to be easy to understand and operate, as well as relatively comfortable whilst allowing mobility during stimulation. This would be of significant benefit to patients to be able to continue their treatment at home, with minimal interruption to their

daily activities. Some patients reported that they were able to wear the device whilst at work or having dinner at a restaurant. In contrast, patients undergoing PTNS would need to attend an outpatient clinic weekly for their treatment. This may have implications for adherence and compliance to treatment. Some patients from this cohort of patients reported that they were able to wear the device whilst sitting at a desk at work or whilst sitting, dining at a restaurant. Satisfaction levels of the treatment and device itself were on the whole high and most would recommend this form of treatment to a friend.

Future directions

This study demonstrates that a 12-week course of transcutaneous stimulation of the posterior tibial nerve using the gekoTM device is safe and effective for the management of overactive bladder symptoms. However a larger, multicentric study is required to confirm the results of this pilot study, with a sham arm, to specifically assess the optimal frequency of device use. This study should include randomized arms into placebo, TTNS, PTNS and antimuscarinic therapy.

If TTNS shows non-inferiority to these other modalities, with a minimal adverse effect profile and high patient satisfaction scores, this could represent an ideal first line treatment.

The potential benefits over these other treatments are summarized in table fourty-two below:

	TPTNS	PTNS	Antimuscarinic therapy
Adverse effect	Similar minimal adverse effect profile to PTNS. Avoid the use of a needle puncture by using an adhesive pad.	Discomfort and bruising at needle point insertion.	Significant drop out rate due to intolerability. A recent systematic review reports that ongoing adherence and persistence of therapy is as low as 12% at 12 months after starting therapy, due to poor tolerability and lack of efficacy [203]. Signifincant adverse effect profile including dry eyes, mouth, constipation, cognitive decline, and contra-indicated with closed angle glaucoma.

Cost effectiveness	To be evaluated by further study. Weekly therapy equivalence to daily therapy will have significant cost benefits.	To be evaluated compared to the other modalities.	To be evaluated compared to the other modalities.
Convenience of treatment	Superior to PTNS as patients can avoid weekly outpatient visits, and self-apply treatment in the community, with minimal restriction to mobility.	Patients need to attend weekly to the outpatients department and are immobile for thirty minutes during the stimulation delivery.	May be less convenient especially for patients already taking numerous medications due to drug interactions.

CONCLUSION

Neuromodulation techniques have been evolving over the last two decades and the less invasive options will be welcome to patients, and change the approach to the OAB. The geko™ device can be safely used for stimulating the posterior tibial nerve at the ankle. A 12 week course of transcutaneous stimulation of the posterior tibial nerve using the geko™ device is effective in reducing the severity of OAB symptoms, and improving lower urinary tract related QOL, as assessed by standardized questionnaires of lower urinary tract functions and bladder diaries. Patients with both neurogenic OAB, due to MS, and idiopathic OAB show clinical improvement. Patients undergoing weekly stimulation showed an equivalent response compared to daily stimulation. Patients report a high level of satisfaction and tolerability, and would recommend the treatment to a friend. There were no significant safety concerns. There was a cohort of individuals, however, who were unable to feel the stimulation and were defined as screen failures. High baseline levels of urinary NGF/Cr may predict poor response to treatment in patients with idiopathic OAB. BDNF/Cr levels reduced with response to treatment in the clinical responders, but not with the non-responders to therapy. Further phase III, placebo controlled study is required to evaluate whether this device has a place in the management of the OAB.

REFERENCES

[1] Yoshimura N, Kaiho Y, Miyazato M, et al. Therapeutic receptor targets for lower urinary tract dysfunction. Naunyn Schmiedebergs Arch Pharmacol. 2008 Jun: **377**:437-48

[2] Apostolidis A, Dasgupta P, Fowler CJ. Proposed mechanism for the efficacy of injected botulinum toxin in the treatment of human detrusor overactivity. Eur Urol. 2006 Apr: **49**:644-50

[3] Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. Nat Rev Neurosci. 2008 Jun: **9**:453-66

[4] Kuo HC, Liu HT, Chancellor MB. Can urinary nerve growth factor be a biomarker for overactive bladder? Rev Urol. 2010 Spring: **12**:e69-77

[5] Abrams P. Describing bladder storage function: overactive bladder syndrome and detrusor overactivity. Urology. 2003 Nov: **62**:28-37; discussion 40-2

[6] Abrams P, Cardozo L, Fall M, 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**:167-78

[7] Coyne KS, Sexton CC, Irwin DE, Kopp ZS, Kelleher CJ, Milsom I. The impact of overactive bladder, incontinence and other lower urinary tract symptoms on quality of life, work productivity, sexuality and emotional well-being in men and women: results from the EPIC study. BJU Int. 2008 Jun: **101**:1388-95

[8] Milsom I, Abrams P, Cardozo L, Roberts RG, Thuroff J, Wein AJ. How widespread are the symptoms of an overactive bladder and how are they managed? A population-based prevalence study. BJU international. 2001 Jun: **87**:760-6

[9] Coyne KS, Wein A, Nicholson S, Kvasz M, Chen CI, Milsom I. Economic burden of urgency urinary incontinence in the United States: a systematic review. J Manag Care Pharm. 2014 Feb: **20**:130-40

[10] Marinkovic SP, Rovner ES, Moldwin RM, Stanton SL, Gillen LM, Marinkovic CM. The management of overactive bladder syndrome. BMJ. 2012: **344**:e2365

[11] Onukwugha E, Zuckerman IH, McNally D, Coyne KS, Vats V, Mullins CD. The total economic burden of overactive bladder in the United States: a disease-specific approach. Am J Manag Care. 2009 Mar: **15**:S90-7

[12] Stewart WF, Van Rooyen JB, Cundiff GW, et al. Prevalence and burden of overactive bladder in the United States. World J Urol. 2003 May: **20**:327-36

[13] Irwin DE, Kopp ZS, Agatep B, Milsom I, Abrams P. Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. BJU Int. 2011 Oct: **108**:1132-8

[14] Abrams P, Andersson KE, Birder L, et al. Fourth International Consultation on Incontinence Recommendations of the International Scientific Committee: Evaluation and treatment of urinary incontinence, pelvic organ prolapse, and fecal incontinence. Neurourol Urodyn. 2010: **29**:213-40

[15] Basra RK, Wagg A, Chapple C, et al. A review of adherence to drug therapy in patients with overactive bladder. BJU Int. 2008 Sep: **102**:774-9

[16] Kelleher CJ, Cardozo LD, Khullar V, Salvatore S. A medium-term analysis of the subjective efficacy of treatment for women with detrusor instability and low bladder compliance. Br J Obstet Gynaecol. 1997 Sep: **104**:988-93

[17] Brazzelli M, Murray A, Fraser C. Efficacy and safety of sacral nerve stimulation for urinary urge incontinence: a systematic review. J Urol. 2006 Mar: **175**:835-41

[18] Cruz F, Herschorn S, Aliotta P, et al. Efficacy and safety of onabotulinumtoxinA in patients with urinary incontinence due to neurogenic detrusor overactivity: a randomised, double-blind, placebo-controlled trial. Eur Urol. 2011 Oct: **60**:742-50
[19] Ginsberg D, Gousse A, Keppenne V, et al. Phase 3 efficacy and tolerability study of onabotulinumtoxinA for urinary incontinence from neurogenic detrusor overactivity. J Urol. 2012 Jun: **187**:2131-9
[20] Famm K, Litt B, Tracey KJ, Boyden ES, Slaoui M. Drug discovery: a jump-start for electroceuticals. Nature. 2013 Apr 11: **496**:159-61
[21] Woock JP, Yoo PB, Grill WM. Activation and inhibition of the micturition reflex by penile afferents in the cat. Am J Physiol Regul Integr Comp Physiol. 2008 Jun: **294**:R1880-9
[22] Farag FF, Martens FM, Rijkhoff NJ, Heesakkers JP. Dorsal genital nerve stimulation in patients with detrusor overactivity: a systematic review. Curr Urol Rep. 2012 Oct: **13**:385-8
[23] Purinton PT, Fletcher TF, Bradley WE. Innervation of pelvic viscera in the rat. Evoked potentials in nerves to bladder and penis (clitoris). Invest Urol. 1976 Jul: **14**:28-32
[24] Dalmose AL, Rijkhoff NJ, Kirkeby HJ, Nohr M, Sinkjaer T, Djurhuus JC. Conditional stimulation of the dorsal penile/clitoral nerve may increase cystometric capacity in patients with spinal cord injury. Neurourol Urodyn. 2003: **22**:130-7
[25] Ohlsson BL, Fall M, Frankenberg-Sommar S. Effects of external and direct pudendal nerve maximal electrical stimulation in the treatment of the uninhibited overactive bladder. Br J Urol. 1989 Oct: **64**:374-80
[26] Peters KM, Killinger KA, Boguslawski BM, Boura JA. Chronic pudendal neuromodulation: expanding available treatment options for refractory urologic symptoms. Neurourol Urodyn. 2010 Sep: **29**:1267-71
[27] van Leeuwen JH, Castro R, Busse M, Bemelmans BL. The placebo effect in the pharmacologic treatment of patients with lower urinary tract symptoms. Eur Urol. 2006 Sep: **50**:440-52; discussion 53
[28] Sajadi KP, Goldman HB. Percutaneous tibial nerve stimulation and overactive bladder. Curr Urol Rep. 2010 Sep: **11**:293-5
[29] Martinson M, MacDiarmid S, Black E. Cost of neuromodulation therapies for overactive bladder: percutaneous tibial nerve stimulation versus sacral nerve stimulation. J Urol. 2013 Jan: **189**:210-6
[30] Groen J, Blok BF, Bosch JL. Sacral neuromodulation as treatment for refractory idiopathic urge urinary incontinence: 5-year results of a longitudinal study in 60 women. J Urol. 2011 Sep: **186**:954-9
[31] M.G. Lucas DB, J.L.H.R. Bosch, , F. Burkhard FC, A.K. Nambiar, C.G. Nilsson, , D.J.M.K. de Ridder AT, R.S. Pickard European Urology Guidelines on Urinary Incontinence 2014
[32] Kacker R, Lay A, Das A. Electrical and mechanical office-based neuromodulation. Urol Clin North Am. 2013 Nov: **40**:581-9
[33] Fandel TT, E. Neuromodulation in Voiding Dysfunction: A historical overview of neurostimulation and its application. In Daneshgari F ed, Urologic Clinics of North America.Chapt 1, 2005:1-9
[34] Chang CJ, Huang ST, Hsu K, Lin A, Stoller ML, Lue TF. Electroacupuncture decreases c-fos expression in the spinal cord induced by noxious stimulation of the rat bladder. J Urol. 1998 Dec: **160**:2274-9
[35] Danisman A, Kutlu O, Akkaya E, Karpuzoglu G, Erdogru T. Tibial nerve stimulation diminishes mast cell infiltration in the bladder wall induced by interstitial cystitis urine. Scand J Urol Nephrol. 2007: **41**:98-102

[36] Tai C, Chen M, Shen B, Wang J, Roppolo JR, de Groat WC. Irritation induced bladder overactivity is suppressed by tibial nerve stimulation in cats. J Urol. 2011 Jul: **186**:326-30
[37] McPherson A. The effects of somatic stimuli on the bladder in the cat. J Physiol. 1966 Jul: **185**:185-96
[38] McGuire EJ, Zhang SC, Horwinski ER, Lytton B. Treatment of motor and sensory detrusor instability by electrical stimulation. J Urol. 1983 Jan: **129**:78-9
[39] Stoller MLC, S., Millard, R.J. et al. The efficacy of acupuncture in reversing the unstable bladder in pig-tailed monkeys., Vol. 137, 1987:104A
[40] Peters KM, Carrico DJ, Perez-Marrero RA, et al. Randomized trial of percutaneous tibial nerve stimulation versus Sham efficacy in the treatment of overactive bladder syndrome: results from the SUmiT trial. J Urol. 2010 Apr: **183**:1438-43
[41] Moossdorff-Steinhauser HF, Berghmans B. Effects of percutaneous tibial nerve stimulation on adult patients with overactive bladder syndrome: a systematic review. Neurourol Urodyn. 2013 Mar: **32**:206-14
[42] Peters KM, Macdiarmid SA, Wooldridge LS, et al. Randomized trial of percutaneous tibial nerve stimulation versus extended-release tolterodine: results from the overactive bladder innovative therapy trial. J Urol. 2009 Sep: **182**:1055-61
[43] Vecchioli-Scaldazza C, Morosetti C, Berouz A, Giannubilo W, Ferrara V. Solifenacin Succinate versus Percutaneous Tibial Nerve Stimulation in Women with Overactive Bladder Syndrome: Results of a Randomized Controlled Crossover Study. Gynecol Obstet Invest. 2013: **75**:230-4
[44] Finazzi-Agro E, Petta F, Sciobica F, Pasqualetti P, Musco S, Bove P. Percutaneous tibial nerve stimulation effects on detrusor overactivity incontinence are not due to a placebo effect: a randomized, double-blind, placebo controlled trial. J Urol. 2010 Nov: **184**:2001-6
[45] Karademir K, Baykal K, Sen B, Senkul T, Iseri C, Erden D. A peripheric neuromodulation technique for curing detrusor overactivity: Stoller afferent neurostimulation. Scand J Urol Nephrol. 2005: **39**:230-3
[46] Klingler HC, Pycha A, Schmidbauer J, Marberger M. Use of peripheral neuromodulation of the S3 region for treatment of detrusor overactivity: a urodynamic-based study. Urology. 2000 Nov 1: **56**:766-71
[47] Vandoninck V, van Balken MR, Finazzi Agro E, et al. Percutaneous tibial nerve stimulation in the treatment of overactive bladder: urodynamic data. Neurourol Urodyn. 2003: **22**:227-32
[48] Vandoninck V, Van Balken MR, Finazzi Agro E, et al. Posterior tibial nerve stimulation in the treatment of urge incontinence. Neurourol Urodyn. 2003: **22**:17-23
[49] van Balken MR, Vandoninck V, Gisolf KW, et al. Posterior tibial nerve stimulation as neuromodulative treatment of lower urinary tract dysfunction. J Urol. 2001 Sep: **166**:914-8
[50] Govier FE, Litwiller S, Nitti V, Kreder KJ, Jr., Rosenblatt P. Percutaneous afferent neuromodulation for the refractory overactive bladder: results of a multicenter study. J Urol. 2001 Apr: **165**:1193-8
[51] Amarenco G, Ismael SS, Even-Schneider A, et al. Urodynamic effect of acute transcutaneous posterior tibial nerve stimulation in overactive bladder. J Urol. 2003 Jun: **169**:2210-5
[52] van der Pal F, van Balken MR, Heesakkers JP, Debruyne FM, Bemelmans BL. Percutaneous tibial nerve stimulation in the treatment of refractory overactive bladder syndrome: is maintenance treatment necessary? BJU Int. 2006 Mar: **97**:547-50
[53] van Balken MR, Vergunst H, Bemelmans BL. Prognostic factors for successful percutaneous tibial nerve stimulation. Eur Urol. 2006 Feb: **49**:360-5

[54] Nuhoglu B, Fidan V, Ayyildiz A, Ersoy E, Germiyanoglu C. Stoller afferent nerve stimulation in woman with therapy resistant over active bladder; a 1-year follow up. Int Urogynecol J Pelvic Floor Dysfunct. 2006 May: **17**:204-7
[55] Kabay S, Kabay SC, Yucel M, et al. The clinical and urodynamic results of a 3-month percutaneous posterior tibial nerve stimulation treatment in patients with multiple sclerosis-related neurogenic bladder dysfunction. Neurourol Urodyn. 2009: **28**:964-8
[56] Gobbi C, Digesu GA, Khullar V, El Neil S, Caccia G, Zecca C. Percutaneous posterior tibial nerve stimulation as an effective treatment of refractory lower urinary tract symptoms in patients with multiple sclerosis: preliminary data from a multicentre, prospective, open label trial. Mult Scler. 2011 Dec: **17**:1514-9
[57] Kabay SC, Yucel M, Kabay S. Acute effect of posterior tibial nerve stimulation on neurogenic detrusor overactivity in patients with multiple sclerosis: urodynamic study. Urology. 2008 Apr: **71**:641-5
[58] Fjorback MV, van Rey FS, van der Pal F, Rijkhoff NJ, Petersen T, Heesakkers JP. Acute urodynamic effects of posterior tibial nerve stimulation on neurogenic detrusor overactivity in patients with MS. Eur Urol. 2007 Feb: **51**:464-70; discussion 71-2
[59] Zecca C, Digesu GA, Robshaw P, et al. Motor and sensory responses after percutaneous tibial nerve stimulation in multiple sclerosis patients with lower urinary tract symptoms treated in daily practice. Eur J Neurol. 2014 Mar: **21**:506-11
[60] Zecca C, Digesu GA, Robshaw P, Singh A, Elneil S, Gobbi C. Maintenance percutaneous posterior nerve stimulation for refractory lower urinary tract symptoms in patients with multiple sclerosis: an open label, multicenter, prospective study. J Urol. 2014 Mar: **191**:697-702
[61] Kabay SC, Kabay S, Yucel M, Ozden H. Acute urodynamic effects of percutaneous posterior tibial nerve stimulation on neurogenic detrusor overactivity in patients with Parkinson's disease. Neurourol Urodyn. 2009: **28**:62-7
[62] Fingerman JS, Finkelstein LH. The overactive bladder in multiple sclerosis. J Am Osteopath Assoc. 2000 Mar: **100**:S9-12
[63] Fowler CJ, Panicker JN, Drake M, et al. A UK consensus on the management of the bladder in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2009 May: **80**:470-7
[64] Kessler TM, La Framboise D, Trelle S, et al. Sacral neuromodulation for neurogenic lower urinary tract dysfunction: systematic review and meta-analysis. Eur Urol. 2010 Dec: **58**:865-74
[65] J. Donovan RB, M. Gotoh, S. Jackson, M. Naughton, S.Radley, L Valiquette, J.E. Bastista, K.Avery. Symptom and Quality of Life Assessment. In Paul Abrams LC, Saad Khoury, Alan Wein ed, Incontinence.Chapt 10, 2005:519-84
[66] Abrams P, Avery K, Gardener N, Donovan J. The International Consultation on Incontinence Modular Questionnaire: www.iciq.net. J Urol. 2006 Mar: **175**:1063-6; discussion 6
[67] Brubaker L, Chapple C, Coyne KS, Kopp Z. Patient-reported outcomes in overactive bladder: importance for determining clinical effectiveness of treatment. Urology. 2006 Aug: **68**:3-8
[68] MacDiarmid SA, Peters KM, Shobeiri SA, et al. Long-term durability of percutaneous tibial nerve stimulation for the treatment of overactive bladder. J Urol. 2010 Jan: **183**:234-40
[69] Patti F. Optimizing the benefit of multiple sclerosis therapy: the importance of treatment adherence. Patient Prefer Adherence. 2010: **4**:1-9
[70] Quinn P, Goka J, Richardson H. Assessment of an electronic daily diary in patients with overactive bladder. BJU Int. 2003 May: **91**:647-52

[71] Peters KM, Carrico DJ, Wooldridge LS, Miller CJ, MacDiarmid SA. Percutaneous tibial nerve stimulation for the long-term treatment of overactive bladder: 3-year results of the STEP study. J Urol. 2013 Jun: **189**:2194-201
[72] Goldman HB, Wyndaele JJ, Kaplan SA, Wang JT, Ntanios F. Defining response and non-response to treatment in patients with overactive bladder: a systematic review. Curr Med Res Opin. 2014 Mar: **30**:509-26
[73] Gaziev G, Topazio L, Iacovelli V, et al. Percutaneous Tibial Nerve Stimulation (PTNS) efficacy in the treatment of lower urinary tract dysfunctions: a systematic review. BMC Urol. 2013: **13**:61
[74] Wagg A, Compion G, Fahey A, Siddiqui E. Persistence with prescribed antimuscarinic therapy for overactive bladder: a UK experience. BJU Int. 2012 Dec: **110**:1767-74
[75] de Seze M, Raibaut P, Gallien P, et al. Transcutaneous posterior tibial nerve stimulation for treatment of the overactive bladder syndrome in multiple sclerosis: results of a multicenter prospective study. Neurourology and urodynamics. 2011 Mar: **30**:306-11
[76] Turner Warwick R. Clinical Urdoynamics. Urol Clin North Am. 1979: **6**:8-9
[77] Blaivas JG. The bladder is an unreliable witness. Neurourol Urodyn. 1996: **15**:443-5
[78] Chapple CR. Is a Voiding Diary Really Necessary in 2014? Eur Urol. 2014 May 15:
[79] Marschall-Kehrel D, Roberts RG, Brubaker L. Patient-reported outcomes in overactive bladder: the influence of perception of condition and expectation for treatment benefit. Urology. 2006 Aug: **68**:29-37
[80] Brubaker LC, C. Introduction to Patient-Reported Outcomes in Overactive Bladder Urology. 2006: **68**:1-2
[81] Coyne K, Kelleher C. Patient reported outcomes: the ICIQ and the state of the art. Neurourol Urodyn. 2010 Apr: **29**:645-51
[82] Coyne KS, Tubaro A, Brubaker L, Bavendam T. Development and validation of patient-reported outcomes measures for overactive bladder: a review of concepts. Urology. 2006 Aug: **68**:9-16
[83] Avery K, Donovan J, Peters TJ, Shaw C, Gotoh M, Abrams P. ICIQ: a brief and robust measure for evaluating the symptoms and impact of urinary incontinence. Neurourol Urodyn. 2004: **23**:322-30
[84] Cotterill N, Goldman H, Kelleher C, Kopp Z, Tubaro A, Brubaker L. What are the best outcome measures when assessing treatments for LUTD?--achieving the most out of outcome evaluation: ICI-RS 2011. Neurourol Urodyn. 2012 Mar: **31**:400-3
[85] Flood AB, Lorence DP, Ding J, McPherson K, Black NA. The role of expectations in patients' reports of post-operative outcomes and improvement following therapy. Med Care. 1993 Nov: **31**:1043-56
[86] Bright E, Cotterill N, Drake M, Abrams P. Developing and Validating the International Consultation on Incontinence Questionnaire Bladder Diary. Eur Urol. 2014 Mar 11:
[87] Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. Urology. 2003 Jan: **61**:37-49
[88] Bates CP, Corney CE. Synchronous cine-pressure-flow cystography: a method of routine urodynamic investigation. Br J Radiol. 1971 Jan: **44**:44-50
[89] Mosso AP, P. . Sur les fonctions de la vessie. Archives Italiennes de Biologie. 1882: **1**:97-128
[90] Hosker GR, P. Gajewski, J. Sand, P. Szabo, L. Capewell, A. Dynamic Testing. In Abrams PC, L. Khoury, S. Wein, A. ed, Incontinence, 4th Edition edn, 2009:417-42

[91] Wyndaele JJ. Normality in urodynamics studied in healthy adults. J Urol. 1999 Mar: **161**:899-902
[92] J. Christian Winters RRD, Howard B. Goldman, C.D. Anthony , Herndon KCK, Stephen R. Kraus, Gary E. Lemack, Victor W. Nitti, , Eric S. Rovner AJW. Adult Urodynamics AUA/SUFU guideline. American Urological Association Education and Research, 2012:1-30
[93] Levi-Montalcini R, Calissano P. The nerve-growth factor. Sci Am. 1979 Jun: **240**:68-77
[94] Ochodnicky P, Cruz CD, Yoshimura N, Michel MC. Nerve growth factor in bladder dysfunction: Contributing factor, biomarker, and therapeutic target. Neurourol Urodyn. 2011 Apr 21:
[95] Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol. 2002 Jul: **187**:116-26
[96] Liu HT, Chancellor MB, Kuo HC. Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol. 2009 Oct: **56**:700-6
[97] Michel MC, Chapple CR. Basic mechanisms of urgency: roles and benefits of pharmacotherapy. World J Urol. 2009 Dec: **27**:705-9
[98] Kuo HC, Liu HT, Chancellor MB. Can urinary nerve growth factor be a biomarker for overactive bladder? Rev Urol. Spring: **12**:e69-77
[99] Liu HT, Chen CY, Kuo HC. Urinary nerve growth factor levels in overactive bladder syndrome and lower urinary tract disorders. J Formos Med Assoc. 2010 Dec: **109**:862-78
[100] Birder LA, Wolf-Johnston AS, Chib MK, Buffington CA, Roppolo JR, Hanna-Mitchell AT. Beyond neurons: Involvement of urothelial and glial cells in bladder function. Neurourol Urodyn. 2010: **29**:88-96
[101] Vizzard MA. Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. Exp Neurol. 2000 Jan: **161**:273-84
[102] Hu VY, Zvara P, Dattilio A, et al. Decrease in bladder overactivity with REN1820 in rats with cyclophosphamide induced cystitis. J Urol. 2005 Mar: **173**:1016-21
[103] Ho DR, Chen CS, Lin WY, Chang PJ, Huang YC. Effect of hyaluronic acid on urine nerve growth factor in cyclophosphamide-induced cystitis. Int J Urol. 2011 Jul: **18**:525-31
[104] Yoshimura N, Bennett NE, Hayashi Y, et al. Bladder overactivity and hyperexcitability of bladder afferent neurons after intrathecal delivery of nerve growth factor in rats. J Neurosci. 2006 Oct 18: **26**:10847-55
[105] Schnegelsberg B, Sun TT, Cain G, et al. Overexpression of NGF in mouse urothelium leads to neuronal hyperinnervation, pelvic sensitivity, and changes in urinary bladder function. Am J Physiol Regul Integr Comp Physiol. 2010 Mar: **298**:R534-47
[106] Lee SR, Hong CH, Choi YD, Kim JH. Increased urinary nerve growth factor as a predictor of persistent detrusor overactivity after bladder outlet obstruction relief in a rat model. J Urol. 2010 Jun: **183**:2440-4
[107] Zvara P, Kliment J, Jr., DeRoss AL, et al. Differential expression of bladder neurotrophic factor mRNA in male and female rats after bladder outflow obstruction. J Urol. 2002 Dec: **168**:2682-8
[108] Ha US, Park EY, Kim JC. Effect of botulinum toxin on expression of nerve growth factor and transient receptor potential vanilloid 1 in urothelium and detrusor muscle of rats with bladder outlet obstruction-induced detrusor overactivity. Urology. 2011 Sep: **78**:721 e1-e6

[109] Seki S, Sasaki K, Fraser MO, et al. Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. J Urol. 2002 Nov: **168**:2269-74
[110] Seki S, Sasaki K, Igawa Y, et al. Suppression of detrusor-sphincter dyssynergia by immunoneutralization of nerve growth factor in lumbosacral spinal cord in spinal cord injured rats. J Urol. 2004 Jan: **171**:478-82
[111] Giannantoni A, Di Stasi SM, Nardicchi V, et al. Botulinum-A toxin injections into the detrusor muscle decrease nerve growth factor bladder tissue levels in patients with neurogenic detrusor overactivity. J Urol. 2006 Jun: **175**:2341-4
[112] Liu HT, Kuo HC. Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology. 2007 Sep: **70**:463-8
[113] Lowe EM, Anand P, Terenghi G, Williams-Chestnut RE, Sinicropi DV, Osborne JL. Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol. 1997 Apr: **79**:572-7
[114] Henry JL. Substance P and inflammatory pain: potential of substance P antagonists as analgesics. Agents Actions Suppl. 1993: **41**:75-87
[115] Mayeux R. Biomarkers: potential uses and limitations. NeuroRx. 2004 Apr: **1**:182-8
[116] Chan R, Munoz A, Wenker EP, Stewart J, Boone T, Khavari R. The Association of Urinary Nerve Growth Factor Levels With Bladder Outlet Obstruction in Women. Female Pelvic Med Reconstr Surg. 2014 Sep 2:
[117] Liss MA, Gordon A, Morales B, et al. Urinary nerve growth factor as an oncologic biomarker for prostate cancer aggressiveness. Urol Oncol. 2014 Jul: **32**:714-9
[118] Aydogmus Y, Sunay M, Arslan H, Aydin A, Adiloglu AK, Sahin H. Acupuncture versus Solifenacin for Treatment of Overactive Bladder and Its Correlation with Urine Nerve Growth Factor Levels: A Randomized, Placebo-Controlled Clinical Trial. Urol Int. 2014 Jul 16:
[119] Shalom DF, Pillalamarri N, Xue X, et al. Sacral nerve stimulation reduces elevated urinary nerve growth factor levels in women with symptomatic detrusor overactivity. Am J Obstet Gynecol. 2014 Jul 11:
[120] Antunes-Lopes T, Pinto R, Barros SC, et al. Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. J Urol. 2013 Jan: **189**:359-65
[121] Liu HT, Kuo HC. Increased urine and serum nerve growth factor levels in interstitial cystitis suggest chronic inflammation is involved in the pathogenesis of disease. PLoS One. 2012: **7**:e44687
[122] Liu HT, Lin H, Kuo HC. Increased serum nerve growth factor levels in patients with overactive bladder syndrome refractory to antimuscarinic therapy. Neurourol Urodyn. 2011 Aug 8:
[123] Liu HT, Chen CY, Kuo HC. Urinary nerve growth factor in women with overactive bladder syndrome. BJU Int. 2011 Mar: **107**:799-803
[124] Pinto R, Lopes T, Frias B, et al. Trigonal injection of botulinum toxin A in patients with refractory bladder pain syndrome/interstitial cystitis. Eur Urol. 2010 Sep: **58**:360-5
[125] Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor level is increased in patients with interstitial cystitis/bladder pain syndrome and decreased in responders to treatment. BJU Int. 2009 Nov: **104**:1476-81
[126] Kuo HC, Liu HT, Chancellor MB. Urinary nerve growth factor is a better biomarker than detrusor wall thickness for the assessment of overactive bladder with incontinence. Neurourol Urodyn. 2010 Mar: **29**:482-7

[127] Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor but not prostaglandin E2 increases in patients with interstitial cystitis/bladder pain syndrome and detrusor overactivity. BJU Int. 2010 Dec: **106**:1681-5
[128] Liu HT, Chancellor MB, Kuo HC. Decrease of urinary nerve growth factor levels after antimuscarinic therapy in patients with overactive bladder. BJU Int. 2009 Jun: **103**:1668-72
[129] Liu HT, Liu AB, Chancellor MB, Kuo HC. Urinary nerve growth factor level is correlated with the severity of neurological impairment in patients with cerebrovascular accident. BJU Int. 2009 Oct: **104**:1158-62
[130] Liu HT, Kuo HC. Urinary nerve growth factor levels are elevated in patients with overactive bladder and do not significantly increase with bladder distention. Neurourol Urodyn. 2009: **28**:78-81
[131] Liu HT, Chancellor MB, Kuo HC. Urinary nerve growth factor level could be a biomarker in the differential diagnosis of mixed urinary incontinence in women. BJU Int. 2008 Nov: **102**:1440-4
[132] Yokoyama T, Kumon H, Nagai A. Correlation of urinary nerve growth factor level with pathogenesis of overactive bladder. Neurourol Urodyn. 2008: **27**:417-20
[133] Jacobs BL, Smaldone MC, Tyagi V, et al. Increased nerve growth factor in neurogenic overactive bladder and interstitial cystitis patients. Can J Urol. 2010 Feb: **17**:4989-94
[134] Liu HT, Kuo HC. Urinary nerve growth factor levels are increased in patients with bladder outlet obstruction with overactive bladder symptoms and reduced after successful medical treatment. Urology. 2008 Jul: **72**:104-8; discussion 8
[135] Liu HT, Kuo HC. Urinary nerve growth factor level could be a potential biomarker for diagnosis of overactive bladder. J Urol. 2008 Jun: **179**:2270-4
[136] Kim JC, Park EY, Seo SI, Park YH, Hwang TK. Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder. J Urol. 2006 May: **175**:1773-6; discussion 6
[137] Kim JC, Park EY, Hong SH, Seo SI, Park YH, Hwang TK. Changes of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol. 2005 Oct: **12**:875-80
[138] Mukerji G, Yiangou Y, Grogono J, et al. Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical correlations. J Urol. 2006 Jul: **176**:367-73
[139] Clemow DB, Steers WD, Tuttle JB. Stretch-activated signaling of nerve growth factor secretion in bladder and vascular smooth muscle cells from hypertensive and hyperactive rats. J Cell Physiol. 2000 Jun: **183**:289-300
[140] Steers WD, Creedon DJ, Tuttle JB. Immunity to nerve growth factor prevents afferent plasticity following urinary bladder hypertrophy. J Urol. 1996 Jan: **155**:379-85
[141] Michael GJ, Averill S, Nitkunan A, et al. Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. J Neurosci. 1997 Nov 1: **17**:8476-90
[142] Pinto R, Frias B, Allen S, et al. Sequestration of brain derived nerve factor by intravenous delivery of TrkB-Ig2 reduces bladder overactivity and noxious input in animals with chronic cystitis. Neuroscience. 2010 Mar 31: **166**:907-16
[143] Antunes--Lopes T. PR, Carvalho-Barros S., Diniz P., Martins-Silva C., , Duarte-Cruz C, Cruz F. Urinary levels of brain derived neurotrophic factor (BDNF) in women with overactive bladder (OAB) syndrome correlate with the severity of symptoms. European Urology Supplements. 2011: **10**:277-8

[144] Wang LW, Han XM, Chen CH, Ma Y, Hai B. Urinary brain-derived neurotrophic factor: a potential biomarker for objective diagnosis of overactive bladder. Int Urol Nephrol. 2014 Feb: **46**:341-7
[145] Koven NS, Collins LR. Urinary brain-derived neurotrophic factor as a biomarker of executive functioning. Neuropsychobiology. 2014: **69**:227-34
[146] Collins LR, Koven NS. Urinary BDNF-to-creatinine ratio is associated with aerobic fitness. Neurosci Lett. 2014 Jan 24: **559**:169-73
[147] Antunes-Lopes T, Carvalho-Barros S, Cruz CD, Cruz F, Martins-Silva C. Biomarkers in overactive bladder: a new objective and noninvasive tool? Adv Urol. 2011: **2011**:382431
[148] Okragly AJ, Niles AL, Saban R, et al. Elevated tryptase, nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor levels in the urine of interstitial cystitis and bladder cancer patients. J Urol. 1999 Feb: **161**:438-41; discussion 41-2
[149] Apostolidis A, Jacques TS, Freeman A, et al. Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. Eur Urol. 2008 Jun: **53**:1245-53
[150] Bonini S, Lambiase A, Angelucci F, Magrini L, Manni L, Aloe L. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. Proc Natl Acad Sci U S A. 1996 Oct 1: **93**:10955-60
[151] Serrano-Sanchez T, Robinson-Agramonte MA, Lorigados-Pedre L, Diaz-Armesto I, Gonzalez-Fraguela ME, Dorta-Contreras AJ. [Endogenous nerve growth factor in patients with Alzheimer s disease]. Rev Neurol. 2001 May 1-15: **32**:825-8
[152] Bruns DE. The STARD initiative and the reporting of studies of diagnostic accuracy. Clin Chem. 2003 Jan: **49**:19-20
[153] Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. Clin Chem. 2003 Jan: **49**:1-6
[154] Melcon MO, Correale J, Melcon CM. Is it time for a new global classification of multiple sclerosis? J Neurol Sci. 2014 Jun 29:
[155] Orton SM, Herrera BM, Yee IM, et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol. 2006 Nov: **5**:932-6
[156] Confavreux C, Vukusic S, Moreau T, Adeleine P. Relapses and progression of disability in multiple sclerosis. N Engl J Med. 2000 Nov 16: **343**:1430-8
[157] Betts CD, D'Mellow MT, Fowler CJ. Urinary symptoms and the neurological features of bladder dysfunction in multiple sclerosis. J Neurol Neurosurg Psychiatry. 1993 Mar: **56**:245-50
[158] Hemmett L, Holmes J, Barnes M, Russell N. What drives quality of life in multiple sclerosis? QJM. 2004 Oct: **97**:671-6
[159] Kalsi V, Gonzales G, Popat R, et al. Botulinum injections for the treatment of bladder symptoms of multiple sclerosis. Ann Neurol. 2007 Nov: **62**:452-7
[160] Buljevac D, Flach HZ, Hop WC, et al. Prospective study on the relationship between infections and multiple sclerosis exacerbations. Brain. 2002 May: **125**:952-60
[161] Stohrer M, Blok B, Castro-Diaz D, et al. EAU guidelines on neurogenic lower urinary tract dysfunction. Eur Urol. 2009 Jul: **56**:81-8
[162] Hashim H, Abrams P. How should patients with an overactive bladder manipulate their fluid intake? BJU Int. 2008 Jul: **102**:62-6
[163] Wiedemann A, Kaeder M, Greulich W, et al. Which clinical risk factors determine a pathological urodynamic evaluation in patients with multiple sclerosis? an analysis of 100 prospective cases. World J Urol. 2013 Feb: **31**:229-33

[164] Litwiller SE, Frohman EM, Zimmern PE. Multiple sclerosis and the urologist. J Urol. 1999 Mar: **161**:743-57
[165] de Seze M, Ruffion A, Denys P, Joseph PA, Perrouin-Verbe B. The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines. Mult Scler. 2007 Aug: **13**:915-28
[166] Y. Homma, J. Batista, S. Bauer, et al. Urodynamics. In Abrams P CL, Koury S, Wein A. ed, Incontinence, Second edn.Chapt Seven, 2001
[167] Fry CH, Sahai A, Vahabi B, Kanai AJ, Birder LA. What is the role for biomarkers for lower urinary tract disorders? ICI-RS 2013. Neurourol Urodyn. 2014 Jun: **33**:602-5
[168] Qu HC, Yan S, Zhang XL, Zhu XW, Liu YL, Wang P. Urinary nerve growth factor levels could be a biomarker for overactive bladder symptom: a meta-analysis. Genet Mol Res. 2014 Apr 14: **13**
[169] Comelli MC, Guidolin D, Seren MS, et al. Time course, localization and pharmacological modulation of immediate early inducible genes, brain-derived neurotrophic factor and trkB messenger RNAs in the rat brain following photochemical stroke. Neuroscience. 1993 Jul: **55**:473-90
[170] Obata K, Noguchi K. BDNF in sensory neurons and chronic pain. Neurosci Res. 2006 May: **55**:1-10
[171] Frias B, Allen S, Dawbarn D, Charrua A, Cruz F, Cruz CD. Brain-derived neurotrophic factor, acting at the spinal cord level, participates in bladder hyperactivity and referred pain during chronic bladder inflammation. Neuroscience. 2013 Mar 27: **234**:88-102
[172] Gold SM, Schulz KH, Hartmann S, et al. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. J Neuroimmunol. 2003 May: **138**:99-105
[173] Haab F, Richard F, Amarenco G, et al. Comprehensive evaluation of bladder and urethral dysfunction symptoms: development and psychometric validation of the Urinary Symptom Profile (USP) questionnaire. Urology. 2008 Apr: **71**:646-56
[174] Jacobs BL, Smaldone MC, Tyagi V, et al. Increased nerve growth factor in neurogenic overactive bladder and interstitial cystitis patients. Can J Urol. Feb: **17**:4989-94
[175] Lipman NS, Jackson LR, Trudel LJ, Weis-Garcia F. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. ILAR J. 2005: **46**:258-68
[176] Crowther JR. The ELISA guidebook: Humana press, 2001
[177] Mao J, Chen S, Na Z, Xhang Y, Huang Y, Li Y. Frozen storage of urine samples before ELISA measurement of retinol-binding protein. Clin Chem. 1996 Mar: **42**:466-7
[178] Kaufman J, Tyagi V, Anthony M, Chancellor MB, Tyagi P. State of the art in intravesical therapy for lower urinary tract symptoms. Rev Urol. 2010 Fall: **12**:e181-9
[179] Lewczuk P, Beck G, Esselmann H, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. Clin Chem. 2006 Feb: **52**:332-4
[180] Nanda SK, Scholz WK. A distinctive surface for phospholipid assays. Clin Chem. 2000 Sep: **46**:1508-10
[181] Valdo P, Stegagno C, Mazzucco S, et al. Enhanced expression of NGF receptors in multiple sclerosis lesions. J Neuropathol Exp Neurol. 2002 Jan: **61**:91-8
[182] Laudiero LB, Aloe L, Levi-Montalcini R, et al. Multiple sclerosis patients express increased levels of beta-nerve growth factor in cerebrospinal fluid. Neurosci Lett. 1992 Nov 23: **147**:9-12
[183] Acosta CM, Cortes C, MacPhee H, Namaka MP. Exploring the role of nerve growth factor in multiple sclerosis: implications in myelin repair. CNS Neurol Disord Drug Targets. 2013 Dec: **12**:1242-56

[184] Kalinowska-Lyszczarz A, Losy J. The role of neurotrophins in multiple sclerosis-pathological and clinical implications. Int J Mol Sci. 2012: **13**:13713-25
[185] Linker RA, Lee DH, Demir S, et al. Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. Brain. 2010 Aug: **133**:2248-63
[186] Bonini S, Lambiase A, Levi-Schaffer F, Aloe L. Nerve growth factor: an important molecule in allergic inflammation and tissue remodelling. Int Arch Allergy Immunol. 1999 Feb-Apr: **118**:159-62
[187] Gigliotti P, Lofaro D, Leone F, et al. High nerve growth factor blood concentration in renal transplantation: a new prognostic marker? Transplant Proc. 2013 Sep: **45**:2654-6
[188] Hellweg R, von Richthofen S, Anders D, et al. The time course of nerve growth factor content in different neuropsychiatric diseases--a unifying hypothesis. J Neural Transm. 1998: **105**:871-903
[189] Aloe L, Bracci-Laudiero L, Alleva E, Lambiase A, Micera A, Tirassa P. Emotional stress induced by parachute jumping enhances blood nerve growth factor levels and the distribution of nerve growth factor receptors in lymphocytes. Proc Natl Acad Sci U S A. 1994 Oct 25: **91**:10440-4
[190] Vijaya G, Cartwright R, Derpapas A, Gallo P, Fernando R, Khullar V. Changes in nerve growth factor level and symptom severity following antibiotic treatment for refractory overactive bladder. Int Urogynecol J. 2013 Sep: **24**:1523-8
[191] Cruz CD. Neurotrophins in bladder function: what do we know and where do we go from here? Neurourol Urodyn. 2014 Jan: **33**:39-45
[192] Silva-Ramos M, Silva I, Oliveira O, et al. Urinary ATP may be a dynamic biomarker of detrusor overactivity in women with overactive bladder syndrome. PLoS One. 2013: **8**:e64696
[193] Burton C, Sajja A, Latthe PM. Effectiveness of percutaneous posterior tibial nerve stimulation for overactive bladder: a systematic review and meta-analysis. Neurourol Urodyn. 2012 Nov: **31**:1206-16
[194] Wein AJ. Re: the efficacy of posterior tibial nerve stimulation for the treatment of overactive bladder in women: a systematic review. J Urol. 2013 Jun: **189**:2202-3
[195] Furgala A, Thor PJ, Kolasinska-Kloch W, Krygowska-Wajs A, Kopp B, Laskiewicz J. The effect of transcutaneous nerve stimulation (TENS) on gastric electrical activity. J Physiol Pharmacol. 2001 Dec: **52**:603-10
[196] Ohannessian A, Kabore FA, Agostini A, et al. [Transcutaneous tibial nerve stimulation in the overactive bladder syndrome in patients with Parkinson's syndromes]. Prog Urol. 2013 Sep: **23**:936-9
[197] Schreiner L, dos Santos TG, Knorst MR, da Silva Filho IG. Randomized trial of transcutaneous tibial nerve stimulation to treat urge urinary incontinence in older women. Int Urogynecol J. 2010 Sep: **21**:1065-70
[198] Warwick DJ, Shaikh A, Gadola S, et al. Neuromuscular electrostimulation viathe common peroneal nerve promotes lower limb blood flow in a below-kneecast: A potential for thromboprophylaxis. Bone Joint Res. 2013: **2**:179-85
[199] Hope M. The geko device for reducing the risk of venous thromboembolism. 2014 [cited; Available from: http://guidance.nice.org.uk/MT/196
[200] Irwin DE, Milsom I, Hunskaar S, et al. Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol. 2006 Dec: **50**:1306-14; discussion 14-5
[201] Kaptchuk TJ, Stason WB, Davis RB, et al. Sham device v inert pill: randomised controlled trial of two placebo treatments. BMJ. 2006 Feb 18: **332**:391-7

[202] Peters KM, Carrico DJ, MacDiarmid SA, et al. Sustained therapeutic effects of percutaneous tibial nerve stimulation: 24-month results of the STEP study. Neurourol Urodyn. 2013 Jan: **32**:24-9
[203] Veenboer PW, Bosch JL. Long-term adherence to antimuscarinic therapy in everyday practice: a systematic review. J Urol. 2014 Apr: **191**:1003-8

Reference to Chapter 1: Tibial nerve stimulation for the management of the neurogenic and idiopathic overactive bladder: A safe and effective treatment

1. Detail of the patient population needs to be expanded on – reflecting on the heterogeneity of their past treatments and how this may influence outcomes of the studies

This prospective study evaluates an non-invasive treatment for overactive bladder (OAB). This treatment has been evaluated in patients with idiopathic OAB, but less so in patients with neurogenic OAB. Our patient cohort represents a heterogenous group of 74 patients. Twenty-five patients (33.8%) had idiopathic OAB, 19 (25.7%) had multiple sclerosis (MS), and 30 (40.5%) other neurological diagnoses at entry.

The 30 patients with 'other neurological diagnoses' do encompass a wide range of diagnoses. This may indeed represent a variety of severity of bladder dysfunction, however urodynamic evaluation was not carried out in our study as it is not standard of practice to perform invasive pressure-flow studies prior to offering treatment with PTNS.

The variety of clinical diagnoses may certainly affect response to treatments in an unpredictable way, with the patients with more severe disease having less of a response to treatment.

However the common aspect that all of these patients share is that lack of response to first line measures of treatment of OAB which are antimuscarinic medication and hence all patients are referred to having 'refractory OAB'.

It was also confirmed that idiopathic and neurogenic groups had comparable symptom severity scores prior to starting treatment.

2. The difference in OAB between neuropathic and idiopathic forms needs to be drawn out

Authors agree that distinction between diagnosis of idiopathic and neurogenic is an important one to make as both groups may represent a different pathophysiology behind the condition. Whereas OAB symptoms following a suprapontine lesion, such as in conditions such as PD, is due to uninhibited detrusor contractions, that following spinal cord pathology is thought to be due to the emergence of previously dormant C-fibres resulting in spinal cord mediated micturition reflex detrusor contractions. The cause for DO in patients with idiopathic DO is uncertain, with potential myogenic or urothelial mechanisms hypothesized. Investigations are unable to identify the pathophysiology for DO, however this study seems to suggest that irrespective of the underlying cause for OAB, PTNS was effective in managing symptoms.

3. a. Write up needs to emphasise the rigorous ensurance of data collection

Quality assurance (QA):

Data collection was performed by the research team. Qualitative outcomes were measure using patient reported outcomes (PROMS) with the ICIQ-OAB, ICIQ-LUTSqol and patient satisfaction survey.

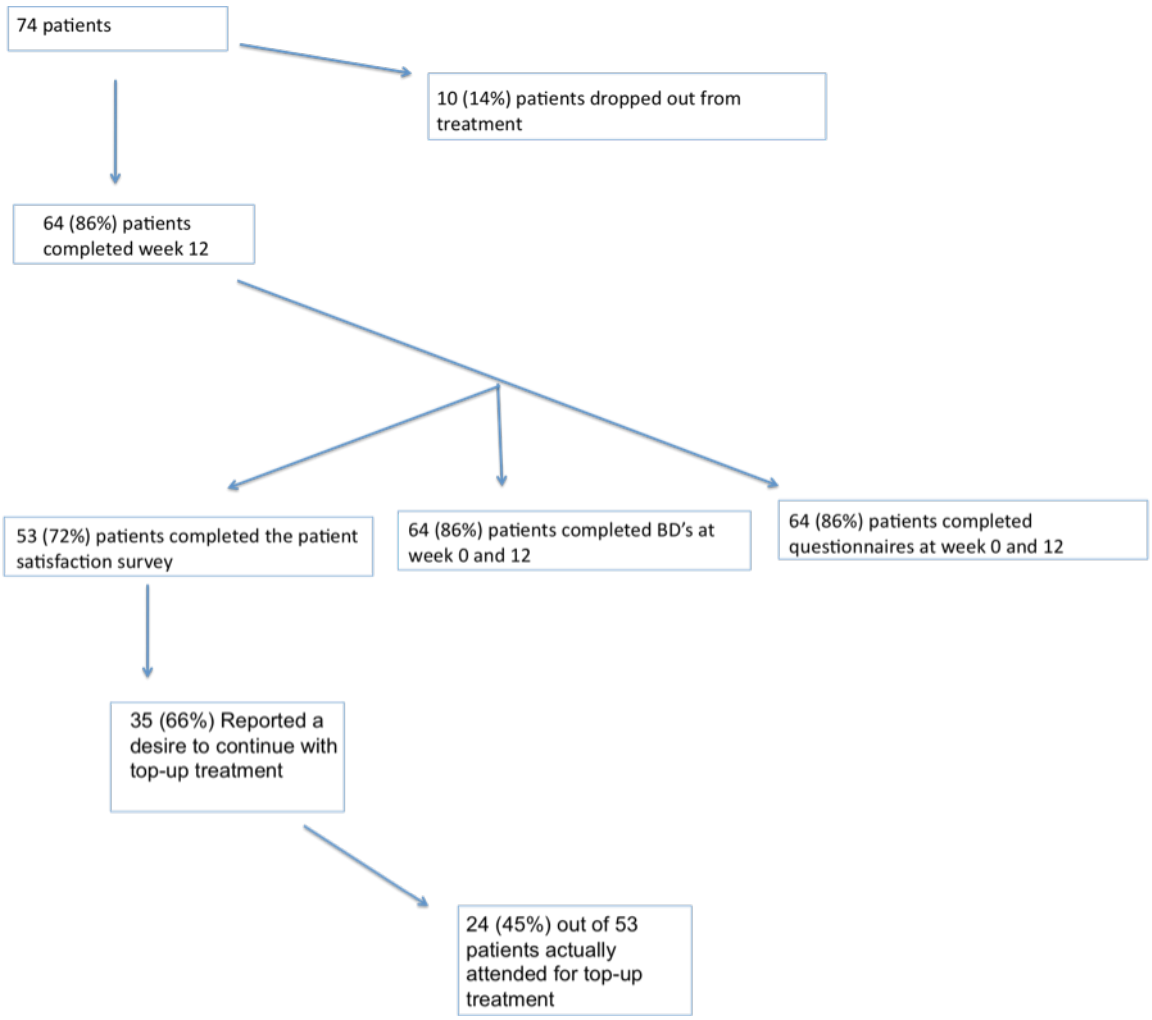
More quantitative assessment was performed patient completed 3-day bladder diaries. Patients were all given the same advice and instruction on how to complete these.

The QA measures was achieved by using a standardised protocol in that all patients were completing the PROMS at baseline and at 12 weeks after end of treatment as shown in figure 4 of pg 58 of the thesis.

Quality control: All collected data was carefully collated and stored in patient case report files and then uploaded into a study database which was secured in a password protected trust computer.

3b. Display outcomes in a flow chart.

Pls find below



3.c Discuss the long-term time course of response year by year for group treatment

The time of writing the thesis and end of data collection was at 12 months follow up, and so we are unable to give long term year by year response data. 24 (45%) patients attended for top-up therapy in that first one year after end of treatment at 12 weeks.

Long term data is important to establish longevity of treatment. We can refer the examiners to The STEP study which identified that at least 75% of the patients who had a positive response to the first 12-weeks of therapy, went on to have sustained symptom improvements for up to three years with an average top up treatment of one per month. However this data amongst neuropathic patients has not yet been established.

Peters KM, Carrico DJ, Wooldridge LS, Miller CJ, MacDiarmid SA. Percutaneous tibial nerve stimulation for the long-term treatment of overactive bladder: 3-year results of the STEP study. J Urol. 2013 Jun: 189:2194-201

3.d. Impact of the literature review – what would you do in relation to findings from the review.

There is emerging evidence to show that PTNS can be a beneficial, non-invasive treatment for patients suffering from OAB symptoms. Its benefits which outweigh some of the alternative treatments such as onabotulinumtoxinA injections are the safety of the treatment with no adverse effects such as urinary tract infections, urinary retention and the need to

perform clean intermittent self-catheterisation. Therefore it may represent a more desirable treatment for patients with less associated risk.

Also it is far less invasive that sacral neuromodulation and clam-ileocystoplasty.

PTNS clearly needs further evaluation for its cost-effectiveness, longevity of effect and comparison data against the other treatment alternatives, which form fertile ground for future study, especially in the neuropathic population.

Based on the literature review, I would recommend continue PTNS as a treatment alternative, to our patients who do not wish to consider the alternative more invasive treatment options, as also recommened by the National Institute of Clinical Excellence (NICE), PTNS commissioning guidance.

4a. The statistical test used to test for normality need to be specified.

QOL Data was tested for normality by assessing spread of scores and standardizing these with the mean and standard deviations and creating a histogram to confirm it follows a normal distribution.

4b. Regression analysis – were other factors missing in neuro patients that need consideration?

There are a number of other factors that could have been considered in the other neurological patients include urodynamic demonstration of detrusor overactivity or bladder outflow

obstruction. Furthermore how their neurological condition affects other aspects of managing with incontinence such as mobility which if impaired may affect their bother/number of pads worn/ and overall QOL impact. However these factors were not accounted for in the data collection or included in the regression analysis and this limitation has now been included in the discussed .

4d. How did dropouts bias the outcomes?

10 patients (14%) dropped out from the study. Their baseline QOL data was incorporated into the analysis, however the 12-week data will be absent from these patients. All patients were comparable at baseline for symptom severity and an attempt was made to follow up those who dropped out, to understand the reasons for attrition and these are listed in the results section of chapter 4. Only a per protoocl analysis had been performed in this pilot study. This does limit the interpretation of the efficacy outcomes of the study, and has been included in the discussion.

4e. How was missing data handled and how did it influence outcomes?

There was no missing data for the patient reported ICIQ-QOL and ICIQ-OAB scores. However only 53 patients completed the patient satisfaction survey out of the 64 patients who completed the treatment. As a result we don't have a comprehensive overview of the entire cohorts view regarding satisfaction and desire for top-up treatments, reducing the sample size for this observation. We can however draw conclusions from those number of patients who completed the satisfaction survey of which 66% reported a desire to return for top-up

treatments which is a meaningful observation that is comparative of the result observed in the SumIT study.

4f. Discuss the limitations of having no control group?

The lack of a control group certainly opens questions of the influence of placebo effect on subjective outcomes. Indeed it is challenging to introduce a placebo arm in a study evaluating TTNS and this is a recommendation for future studies that has been proposed in the thesis. It is notable however that data from the SuMIT study has proven the efficacy of PTNS over a validated sham, on which the basis of PTNS is recommended in clinical guidelines such as NICE.

4g. Discuss the mechanistic possibilities and potential sexual/gastrointestinal effects of therapy?

For OAB symptoms, it is unclear how PTNS has its affect on resolution of symptoms. There is scientific evidence in animals that PTNS can reduce c-fos expression (a marker of neuronal metabolic activity) in the sacral spinal cord 1. Urodynamics studies as discussed in the literature review have demonstrated increased bladder capacity in both cats and humans.

The three main pathways have been debated for mechanism of action [33]:

1. Direct stimulation of the hypogastric nerve through activation of sympathetic fibres at low bladder filling volumes

2. Direct stimulation of the nuclei of the pudendal nerve in the spinal cord at maximal bladder filling

3. Supraspinal inhibition of the detrusor

Sexual function:

There has been evidence reported in the literature to suggest that PTNS can have a positive impact in female sexual function from a prospective series of 41 patients, self reporting outcomes using the female sexual function index (FSFI) 2.

Gastro-intestinal:

There is also some evidence that PTNS can reduce the number of faecal episodes of faecal incontinence from 7 to 3 per week 3. And improvements appear to be mainly to bowel symptoms over global pelvic floor complaints 4. However there is paucity of long-term data beyond 12 months of follow-up. In the recent CONFIDeNT trial, a double blind randomized controlled trial of PTNS versus sham for faecal incontinence, involving 227 patients, showed no benefit of PTNS over sham 5.

The effects of PTNS on patients with chronic constipation were also recently reported, in groups with both slow transit and rectal evacuation difficulty, however there was no benefit to patients who had 12-weekly sessions of treatment 6.

4h. How to interpret the Fowler data re 100msec timelag for conduction? What would be the influence of a full bladder during treatment in terms of afferent stimulation?

The results of the study by Fowler et al. carried out a few years demonstrating prolonged latency following sacral root stimulation suggest that the anal contractions are an afferent mediated response. Studies evaluating the putative mechanism of action of tibial nerve

stimulation are few however afferent stimulation is likely to result in modulation of reflexes at the level of the spinal cord and cortex [7].

5.a Study 2

How about looking at markers of immune activation – a neuro-immune effect of treatment?

This has been done to some extent. Multiple inflammatory mediators have been examined in various animal models. Such as a upregulation of Prostaglandin receptors (PGE2) in the urothelium of rats with cyclophosphamide induced OAB [8]. The role of inflammation and OAB has also been suggested with the reduction of spinal cord and bladder TRPV1 (Transient receptor potential vanilloid receptor 1) and CGRP (a marker of neuronal inflammation) after intrathecal administration of resiniferatoxin in spinal cord injured rats, ameliorating detrusor overactivity [9]. Furthermore the histological evidence of chronic urothelial inflammation with evidence of mast cells and apoptosis in patients with OAB compared to controls has been reported [10]. Indeed the inflammatory and neurotrophin pathways are felt to be linked in an unclear way. None of these inflammatory targets have yet been successfully targeted in any form of treatment, butfurtuer developments are to be awaited.

5.b.

Discuss the effect of antimuscarinic drugs on measured levels.

There is evidence, from scientific/histological studies that antimuscarinic use does reduce the level of neurotrophin in urine [11]. As a result this effects must be considered when measuring levels in patients who may have been taking these, as is discussed in the thesis.

5c. Consider OAB vs interstitial Cystitis (IC) data

There certainly does appear to be overlap in the clinic spectrum of IC and OAB. IC traditionally is a chronic condition that can be debilitating and histologically also described the diffuse infiltration of mast cells and lymphocytes. It has been questioned whether patients with OAB actually have early IC. It has been shown in scientific study that NGF levels from bladder biopsy tissue is actually equivalent between IC and OAB patients, although significantly raised compared to controls [12].

5d. Discuss the time course of nerve growth factor response to treatment

The concept of urinary NGF levels over time is interesting as there are many factors that need to be considered. It seems that the levels vary slightly on a day to day basis and is dynamic according to severity of urothelial inflammation/overactivity, symptom severity, and whether patient is taking any treatment. It has been shown that of patients with OAB taking treatment with tolterodine, with urinary NGF levels measured at baseline, 1, 2 and 3 months, it took three months for levels to significantly reduce, despite symptoms improving far earlier [11, 13]. However after discontinuing medication for 1month, and urinary NGF levels are re-assayed it can seen that levels become significantly elevated as with return of symptoms. It has also been shown in a cohort of patients being treated with onabotulinumtoxinA, that levels significantly reduce with symptom amelioration, however on symptomatic return,

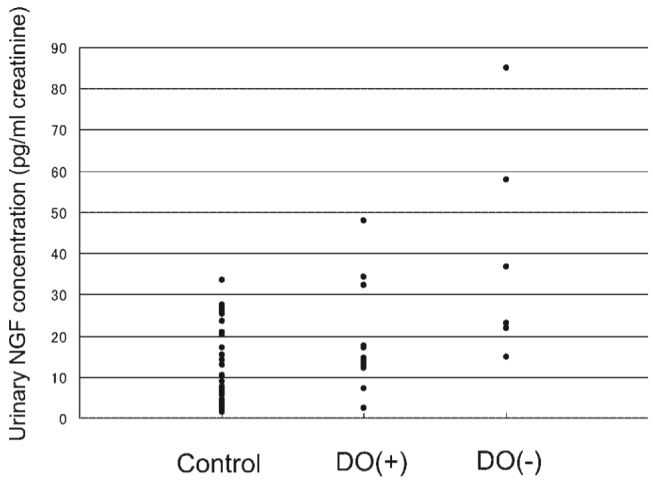
biologically detectable levels/indicator of disease also return to the baseline high levels [14]. The response of uNGF therefore to treatment is a temporary one, which returns to baseline once therapy is either withdrawn or wears off.

5e. Use a figure to display the degree of overlap between OAB and healthy controls

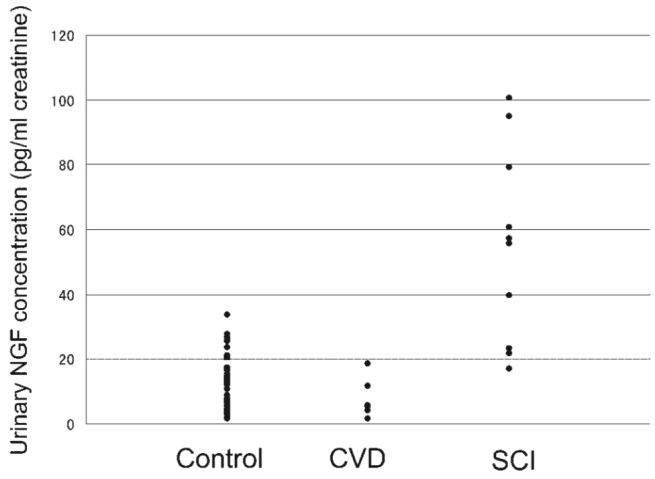
This graph 1 below taken from a study by Yokoyama et al 2008, shows that there is a clear overlap in uNGF levels between healthy controls and pts with idiopathic OAB.

Whereas graph 2, also from the same publication, demonstrates a more significant rise in levels in patients with neuropathic bladder due to spinal cord injury, when compared with healthy controls.

The explanation for these differences may be due to the nature of the pathophysiology of the detrusor overctivity in patients with idiopathic OAB and neuropathic OAB. Those with the former may be explained with the myogenic theory where there are alterations in smooth muscle excitability, with potential genetic or hormonal components to the condition. Whereas the neurogenic cause, is felt to be due to enhanced urothelial c-fibre activity, triggered by a sodium ion channelopathy, increased electrical excitability and lower threshold, raised neurotrophin levels and the emergence of a c-fibre spinal reflex circuit [15].



DO: Detrusor overactivity



CVD: cerebrovascular disease

SCI: spinal cord injury

5f. Discuss the philosophical role of a biomarker – is it better than symptoms to predict response?

Biomarkers are a measurable characteristic that reflects a particular physiological state. Whilst with the case of uNGF and the OAB, certainly symptoms are the most reliable marker of disease activity and patient related Quality of Life impact. However the exploration of the

behaviour of potential biomarkers is essential to a) understand aetiology, b) monitor or predict response to potential treatments and c) to pave opportunity to develop new drug/therapy targets to slow or reverse the disease process.

5g. How will biomarkers fluctuate with symptom variation – another question regarding utility

In the studies reported uptodate, biomarker (uNGF) levels reflect symptom severity. Since NGF is thought to drive c-fibre growth in the urothelium, which is part of the pathophysiology of OAB symptoms, once symptoms are effectively treated with resultant reduced c-fibre growth, there is a reflection of reduced NGF activity and quantity detected in the urine. However how uNGF levels would vary day to day in patients with varying OAB symptoms, has not as yet been evaluated or described. It is also feasible that during symptom improvement due to lifestyle measures may cause less neurotrophin release from areas in the central nervous system, accompanied with the increased sensation of well-being and improved LUTS.

5h. Discuss the effect of diurnal variation

One study examined uNGF variation throughout the daytime, morning afternoon and evening, in healthy volunteers which identified stable values throughout the day and no circadian variation [16].

6. Study 3

a. We agree with this and has been noted

6b. ROC statistics need clarifying

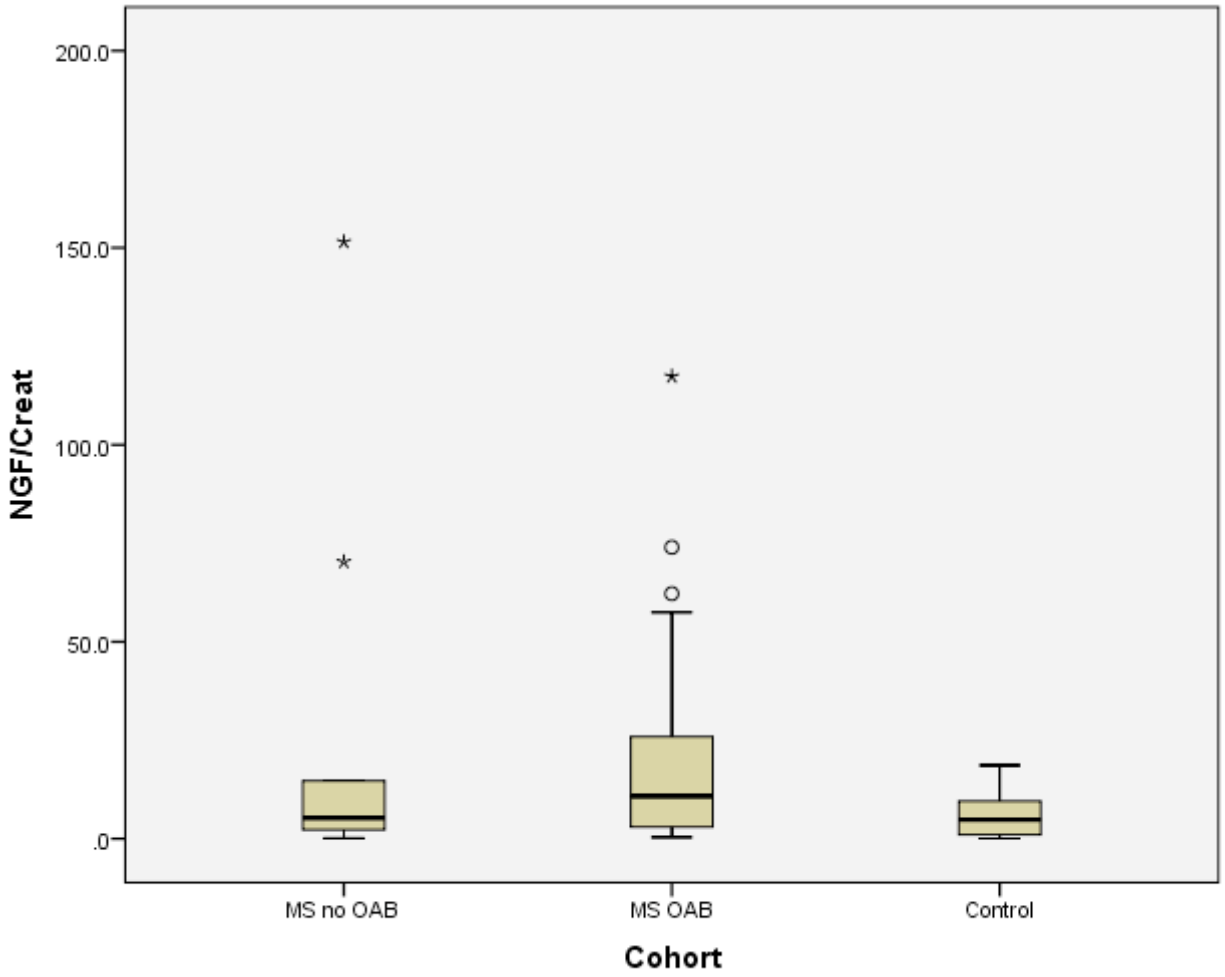
Using the Youden Index, the optimized cut-off point in NGF/Cr levels is 18.97. This gives a 37% sensitivity and a 93% specificity for predicting that the individual will have OAB symptoms.

The optimized cut-off point for BDNF/Cr levels is 17.37. This point gives a 70% sensitivity and a 67% specificity for predicting that the individual will have OAB symptoms.

Both of these values compare poorly to that demonstated in a group of women with idiopathic OAB where the optimized cut off points for NGF/Cr was 0.14 for a 54% sensitivity and 96% specificity for predicting OAB, and a BDNF/Cr value of 4.6 for a 89% sensitivity and 100% specificity

6c. Show table 19 as box and whisker plot

Please see box plot performed



6d. Summarise lesson learnt from study succinctly

Urinary NGF/creatinine and BDNF/creatinine levels were significantly raised in patients with MS and OAB as would be expected, when compared with healthy controls. Levels were also marginally raised in patients with MS and no OAB, when compared with controls. The raised levels therefore cannot be specific to OAB as having MS as a diagnosis also may lead to elevated detectable levels.

There was no strong correlation with urinary neurotrophin level and symptom severity, with some patients complaining of mild symptoms having higher levels than those severely afflicted. This is contrary to the findings within an idiopathic population were higher levels

were found in those with worse symptom profiles [17]. These molecules are clearly relevant in the pathophysiology of the OAB, however at this stage further study is required to understand their exact role and other molecules considered before an biomarker is found.

6e. Consider the composite of NGF and EDSS as a possible measure

There was indeed a more positive correlation in OAB score and EDSS than there was for uNGF. This mirrors a finding in another study which demonstrated higher levels of uNGF correlating with higher severity of neurological deficit after CVA [18]. This was not evaluated in this study, however since there is a correlation with EDSS and biological neurotrophin activity in the urine, it may well have a role as a predictor of bladder involvement. This warrants further study.

6f. Could symptoms be better defined by urodynamic studies to stratify patients better.

Urodynamics at present would represent a gold standard at providing an accurate evaluation of lower urinary tract symptoms. This allows a further differentiation between the OAB and detrusor overactivity. As we know from previous study that between 50-90% of patients with clinical OAB have urodynamic DO [19], depending on symtpoms severity. However this is an invasive test, with associated morbidity and therefore its use minimized. We did not use urodynamics in this study to stratify our patient population as it would not have changed our management. Furthermore in a previous study, the presence of DO did not strongly associate with higher uNGF levels compared to absence of DO, where it was the symptom of urgency that was more relevant affiliation with raised levels [20].

7. b. Is there enough data to support a non-controlled study?

There is a paucity of evidence to support evidence for efficacy of transcutaneous tibial nerve stimulation. The main aim of this study was to acquire safety and acceptibity data as a phase two pilot, to base a larger, placebo controlled study on.

c. Discuss the recent Knowles et al lancet paper and its implications in the findings of this chapter

This recent high level muticentre placebo controlled study, examines PTNS for patients with faecal incontinence [21]. This shows no significant difference between sham and treatment arms for symptom improvement. This demonstrates again the power of the placebo effect which is not measurable in our study, however our study was only powered as a pilot study to acquire initial safety data to base a larger study on. This highlights the importance of designing in a sham arm in any future efficacy study.

d. Discuss the relationship between responders and those adherent to this therapy

Examining the usage diaries, those in both the weekly and daily arms, the usage/adherence for those who completed the study was >90%. 34/48 patients completed the study, of which 18 were responders. It is unlikely from this study there is evidence to suggest that those more adherent to therapy had higher response rates as our adherence rates were very high.

e. Where could the treatment fit in the pathway – before anticholinergics? Specify the studies that would be needed

Certainly if this treatment option was proven to be efficacious ,the next phase would be to proves cost-effectiveness against other firstline treatment options. By its nature of having a minimal adverse effect profile and being non-invasive, would make it a highly desirable and acceptable treatment from a patient perspective as a first line option. Head to head or crossover studies between transcutaneous TNS and firstline medical therapy would need to be designed, with intermediate term follow-up to prove its place in the treatment ladder.

References

1. Chang, C. J., Huang, S. T., Hsu, K. et al.: Electroacupuncture decreases c-fos expression in the spinal cord induced by noxious stimulation of the rat bladder. J Urol, **160:** 2274, 1998
2. Musco, S., Serati, M., Lombardi, G. et al.: Percutaneous Tibial Nerve Stimulation Improves Female Sexual Function in Women With Overactive Bladder Syndrome. J Sex Med, **13:** 238, 2016
3. Govaert, B., Pares, D., Delgado-Aros, S. et al.: A prospective multicentre study to investigate percutaneous tibial nerve stimulation for the treatment of faecal incontinence. Colorectal Dis, **12:** 1236, 2010
4. Kelly, S. L., Radley, S. C., Brown, S. R.: Does percutaneous tibial nerve stimulation improve global pelvic function in women with faecal incontinence? Colorectal Dis, **18:** O158, 2016
5. Horrocks, E. J., Bremner, S. A., Stevens, N. et al.: Double-blind randomised controlled trial of percutaneous tibial nerve stimulation versus sham electrical stimulation in the treatment of faecal incontinence: CONtrol of Faecal Incontinence using Distal NeuromodulaTion (the CONFIDeNT trial). Health Technol Assess, **19:** 1, 2015
6. Kumar, L., Liwanag, J., Athanasakos, E. et al.: Effectiveness of percutaneous tibial nerve stimulation in managing refractory constipation. Colorectal Dis, **19:** 45, 2017
7. Finazzi-Agro, E., Peppe, A., D'Amico, A. et al.: Effects of subthalamic nucleus stimulation on urodynamic findings in patients with Parkinson's disease. J Urol, **169:** 1388, 2003
8. Chuang, Y. C., Yoshimura, N., Huang, C. C. et al.: Expression of E-series prostaglandin (EP) receptors and urodynamic effects of an EP4 receptor antagonist on cyclophosphamide-induced overactive bladder in rats. BJU Int, **106:** 1782, 2010
9. Cruz, C. D., Charrua, A., Vieira, E. et al.: Intrathecal delivery of resiniferatoxin (RTX) reduces detrusor overactivity and spinal expression of TRPV1 in spinal cord injured animals. Exp Neurol, **214:** 301, 2008
10. Wang, C. C., Kuo, H. C.: Urothelial Dysfunction and Chronic Inflammation in Diabetic Patients with Overactive Bladder. Low Urin Tract Symptoms, 2016
11. Liu, H. T., Chancellor, M. B., Kuo, H. C.: Decrease of urinary nerve growth factor levels after antimuscarinic therapy in patients with overactive bladder. BJU Int, **103:** 1668, 2009
12. Lowe, E. M., Anand, P., Terenghi, G. et al.: Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol, **79:** 572, 1997
13. Kuo, H. C., Liu, H. T., Guan, Z. et al.: Promise of Urinary Nerve Growth Factor for Assessment of Overactive Bladder Syndrome. Low Urin Tract Symptoms, **3:** 2, 2011
14. Liu, H. T., Kuo, H. C.: Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology, **70:** 463, 2007
15. Steers, W. D.: Pathophysiology of overactive bladder and urge urinary incontinence. Rev Urol, **4 Suppl 4:** S7, 2002
16. Antunes-Lopes, T., Pinto, R., Barros, S. C. et al.: Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. J Urol, **189:** 359, 2013
17. Liu, H. T., Chen, C. Y., Kuo, H. C.: Urinary nerve growth factor in women with overactive bladder syndrome. BJU Int, **107:** 799, 2011

18. Liu, H. T., Liu, A. B., Chancellor, M. B. et al.: Urinary nerve growth factor level is correlated with the severity of neurological impairment in patients with cerebrovascular accident. BJU Int, **104:** 1158, 2009
19. Hyman, M. J., Groutz, A., Blaivas, J. G.: Detrusor instability in men: correlation of lower urinary tract symptoms with urodynamic findings. J Urol, **166:** 550, 2001
20. Yokoyama, T., Kumon, H., Nagai, A.: Correlation of urinary nerve growth factor level with pathogenesis of overactive bladder. Neurourol Urodyn, **27:** 417, 2008
21. Knowles, C. H., Horrocks, E. J., Bremner, S. A. et al.: Percutaneous tibial nerve stimulation versus sham electrical stimulation for the treatment of faecal incontinence in adults (CONFIDeNT): a double-blind, multicentre, pragmatic, parallel-group, randomised controlled trial. Lancet, **386:** 1640, 2015

http://www.real-statistics.com/tests-normality-and-symmetry/graphical-tests-normality-symmetry/

NHS
National Research Ethics Service
East Central London REC 1
South House, Block A
Royal Free Hospital
Pond Street
London
NW3 2QG

Telephone: 020 7794 0552
Facsimile: 020 7794 0714

09 July 2010

Mr Jai Seth

12 Oakley Avenue
Barking
Essex
IG11 9JD

Dear Mr Seth

Full title of study: **Evaluation of a method to measure urinary Nerve Growth Factor (NGF) as a biomarker for detrusor overactivity (DO) in patients with neurological disease**
REC reference number: **10/H0721/33**
Protocol number: **1**

Thank you for your email of 17th June 2010. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 28 April 2010. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1	
Participant Information Sheet	2	17 June 2010
Response to Request for Further Information		17 June 2010
Participant Consent Form	2	17 June 2010

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

10/H0721/33 **Please quote this number on all correspondence**

Yours sincerely

Mr John Doherty
Committee Co-ordinator

NRES Committee London - Surrey Borders
HRA
Research Ethics Committee (REC) London Centre
Ground Floor
Skipton House
80 London Road
London SE1 6LH

Telephone: 020 7972 2554
Facsimile: 020 7972 2592

08 January 2013

Dr Jalesh Panicker
Consultant Neurologist and Honorary Senior Lecturer
The National Hospital for Neurology and Neurosurgery
and UCL Institute of Neurology
Department of Uro-Neurology,
Internal Mailbox 71
The National Hospital for Neurology and Neurosurgery
Queen Square,
London WC1N 3BG

Dear Dr Panicker

Study title:	**Single centre randomised pilot study of two regimens (30mins daily or weekly for 12 weeks) of transcutaneous tibial nerve stimulation of patients with overactive bladder (OAB) syndrome**
REC reference:	**12/LO/1613**
Protocol number:	**OAB-JP-001**
IRAS project ID:	**105169**

Thank you for your letter of 25th October 2012. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 24 October 2012

Documents received

The documents received were as follows:

Document	*Version*	*Date*
GP/Consultant Information Sheets	1.0	05 September 2012
Other: Letter responding to favourable with conditions opinion by Dr Jai Seth		25 October 2012
Participant Consent Form	1.1; tracked changes	13 November 2012
Participant Information Sheet	1.1; tracked changes	06 November 2012
Protocol	1.1; tracked changes	06 November 2012

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Evidence of insurance or indemnity		30 July 2012
GP/Consultant Information Sheets	1.0	05 September 2012
Other: Questionnaire EDSS		
Other: 3 day bladder diary	1	05 September 2012
Other: Usage diary	1	05 September 2012
Other: letter from Joint Research Office		05 September 2012
Other: Instructions for use		
Other: Letter responding to favourable with conditions opinion by Dr Jai Seth		25 October 2012
Participant Consent Form	1.1; tracked changes	13 November 2012
Participant Information Sheet	1.1; tracked changes	06 November 2012
Protocol	1.1; tracked changes	06 November 2012
Questionnaire: ICIQ-OAB		
Questionnaire: NBD		
Questionnaire: ICIQ-QoL		
REC application	1	07 September 2012

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

12/LO/1613 **Please quote this number on all correspondence**

Yours sincerely

Mrs Alka Bhayani
Committee Co-ordinator

E-mail: NRESCommittee.London-SurreyBorders@nhs.net

Copy to:

Mr David Wilson
Database and Information Officer
University College London
Joint Research Office
R & D (1st Floor Maple House)
Rosenheim Wing
Ground Floor
25 Grafton Way
London WC1E 5DB

P.T.O

Copy to:

Ms Anjani Parmar
UCL Joint Research Office
1st Floor, Maple House – Suite B
149 Tottenham Court Road
London W1T 7DN

University College London Hospitals NHS
NHS Foundation Trust

Joint UCLH/UCL/RoyalFree Biomedical Research (R&D) Unit

Office Location:
1st Floor Maple House
149 Tottenham Court Road
London W1T 7DN

Postal Address:
Rosenheim Wing, Ground Floor
25 Grafton Way
London WC1E 6DB

Mr Jai Seth
University College London Hospital Trust
Department of Uro-Neurology
The National Hospital for Neurology and Neurosurgery
Queen Square
London

9th November 2010

Dear Mr Seth

Project ID: **10/0099** (Please quote in all correspondence)
REC Ref: 10/H0721/33
Title: Evaluation of a method to measure urinary nerve growth factor (NGF) as a biomarker for detrusor overactivity (DO) in patients with neurological disease

Thank you for registering the above study (non-IMP) with the UCL/UCLH/RF Joint Biomedical Research Unit (UCLH Site). I am pleased to inform you that your study now has local R&D conditioned approval to proceed and recruit participants at University College London Hospitals NHS Foundation Trust.

Please note that this approval is granted on the condition **that you may not work on this study until you have a valid honorary contract with the UCLH Trust,** looking forward to receiving a copy as soon as this is available.

The approval is also granted on the basis of the key document provided:

- All documentation as listed in the REC notice of favourable opinion dated **6th May 2010**

As Chief Investigator you are required to ensure that your study is conducted in accordance with the Department of Health's Research Governance Framework for Health and Social Care (2nd edition 2005) and that all members of the research team are aware of their responsibilities under the Framework.

Please note that you are also required:

- To comply with the Data Protection Act, Caldicott Principles and Trust Information Governance Policy.
- To ensure all researchers taking part in this study have up-to-date and appropriate honorary contracts.
- To ensure that a signed and dated copy of the consent form is kept in the study file and a copy also given to the participant.
- To maintain an investigator file to store all study documentation to be made available for audit.
- Where applicable to acknowledge NIHR/CBRC funding and support and collaboration with the BioStatistics Group.

If applicable to the methodology and conduct of the proposed study:

- There is a legal obligation to abide by the Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments as well as any other applicable regulations.

- Medical Devices research regulated by the MHRA should be conducted in accordance to the Device Regulations.

- Tissue research must be conducted in accordance to the Human Tissue Act 2004 and the Codes of Practice issued by the Human Tissue Authority, with special relevance to Code 9 Research. Where tissue is used for human application please ensure that you abide by the Human Tissue (Quality and Safety for Human Application) Regulations 2007.

This R&D approval is conditional upon you complying with all requirements of the Research Ethics Committee notice of favourable opinion (**6th May 2010**) and notifying the UCL/UCLH/RF Biomedical Research Unit of the following as they arise:

- Serious Adverse Events & untoward research incidents
- Amendments (including a request to extend the study)
- Annual Progress Reports
- Any change in staff, their duties and their time on the study
- End of or suspension of study notification
- Planned audits by the Sponsor
- Publications

Please do not hesitate to contact a member of the team with regards to assistance and guidance for your research.

Yours sincerely

pp Professor Monty Mythen
Director of Research and Development
UCL/UCLH/Royal Free Biomedical Research Unit

cc: Professor Clare Fowler

Investigator File
List of important documents

For the purposes of audit it is strongly recommended that you maintain a file of study documentation relating to your research. The list below is designed to help you put together your investigator file.

1. Approvals (R&D, REC, GTAC, MHRA, ARSAC, NIGB, HTA licence)
2. Fully signed copy of REC, R&D and SSI forms
3. Protocol (including amendments)
4. Patient Information Sheet, Consent form, GP letter, Invitation letter (including amendments)
5. Details of peer review, authorisations from Divisional Clinical Director of support departments (e.g. pathology, radiology, pharmacy)
6. Agreements (mCTA, mNCA, mCIA, MTA, funding, indemnity, insurance, Sponsor letter)
7. List of study personnel, delegation log*, CVs, honorary contracts, GCP training certificates
8. Screening log*, recruitment log*
9. Original signed consent forms
10. Serious Adverse Events, SUSARS, untoward incidents, complaints
11. Correspondence, minutes of study meetings
12. REC Annual Progress Reports
13. End of study/trial notification
14. Publication

* Please do not hesitate to contact the Joint Biomedical Research Unit for a template log if required.

University College London Hospitals NHS
NHS Foundation Trust

National Hospital for Neurology and Neurosurgery
Department of Uro-Neurology
Queen Square London
WC1N 3BG
Direct Line: 020 7 829 8713
Fax: 020 7813 4587

INFORMED CONSENT FORM

REC Study Number:	12/LO/1613	Patient Information Sheet Version and Date: Version 1.1; 13 November 2012
Patient Number:		

Title of Study: Single centre randomised pilot study of two regimens (30mins daily or weekly for 12 weeks) of Transcutaneous Tibial Nerve Stimulation for the treatment of patients with Overactive Bladder (OAB) Syndrome

Name of Chief Investigator (CI): Dr Jalesh Panicker

Please initial box

1. I confirm that I have read and understand the information sheet dated 06 November 2012 (version number 1.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

4. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by the sponsor (UCL) as well as responsible individuals from regulatory authorities from the NHS Trust for purposes of monitoring and auditing, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I Consent to the Research Team approaching me after the study if they wish to check on my wellbeing or in the future about this device if it has been of benefit. ☐

6. I agree for my GP to be informed about the study. ☐

7. I agree to take part in the above study ☐

Name of patient	Date	Signature
_____	_____	_____

Name of person taking consent	Date	Signature
_____	_____	_____

Comments or concerns during the study: If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated while partaking in the study, you should write or get in touch with the Complaints Manager, UCL hospitals.

When completed: 1 form for the patient: 1 to be kept as part of the study documentation for the trial master/investigator site file: 1 original to be kept with the hospital medical notes.

Treatment Flow Chart – What will we assess at each visit

	Screening	Randomisation/ start of treatment	W1-W3	W4	W5-7	W8	W9-W11	W12/end of study
	Visit 0	Visit 1	Telephone	Visit 2	Telephone	Telephone	Telephone	Visit 3
Signed Consent Form	X							
Vital Signs, Height, Weight, BMI	X							
Pregnancy Test (if required)	X							X
Bladder diary	X			X		X		X
Questionnaires	X			X		X		X
Usage diary		X	X	X	X	X	X	X
Patient Satisfaction Questionnaire and GRA								X

University College London Hospitals NHS
NHS Foundation Trust

Dear Dr.

National Hospital for Neurology and Neurosurgery
Department of Uro-Neurology
Queen Square London
WC1N 3BG
Direct Line: 020 34484713
Fax: 020 7813 4587

I am writing to inform you that your patient has expressed an interest in taking part in a study entitled "Randomised pilot study of Transcutaneous Tibial Nerve Stimulation for patients with Overactive Bladder Syndrome". This will be a prospective study using a new device called geko™ for patients with overactive bladder syndrome. It is a UK single centre study sponsored by UCL as part of the UCL/UCLH/Royal Free Joint Research Office.

REC Study Number:	12/LO/1613	GP letter Sheet Version and Date: Version 1.0; 05 September 2012
Patient Number:		

After consent, there will be a baseline assessment period (days -3 to 0 with bladder diary and questionnaires), 12 weeks treatment protocol and finally post treatment assessment (repeat 3 day bladder diary and questionnaires). Your patient will apply the geko™ device daily for the 12 weeks period and the duration of application will depend on randomisation (30 mins daily or 30 minutes weekly). They will be contacted on a weekly basis.

Your patient will be allowed to continue taking medications which are considered necessary for their welfare. They should not be started on any new treatment that can affect bladder functions.

I am enclosing a copy of the patient information leaflet. For the period of this study I will be grateful if you could inform me, should you see the patient at your surgery for any reason. This will enable me to record and report any adverse event, change in medical condition or medication and comply with International Good Clinical Practice Guidelines.

If you have any concerns or comments regarding this participation please feel free to contact me or members of my department at the above number, or at 08451555000 and dial one of the following extensions:

Dr Jalesh Panicker	Consultant	ext 83148
Dr. Jai Seth	Honorary Research Assistant	ext 83139
Mrs Juliana Ochulor	Clinical Nurse Specialist	ext 84713
Mrs Collette Haslam	Clinical Nurse Specialist	ext 84713
Miss Gwen Gonzales	Clinical Nurse Specialist	

Yours Sincerely,

Dr Jalesh N. Panicker

Urinary Symptom Profile - USP©

➢ **Before starting the questionnaire, please fill in today's date:**

/__/__/ /__/__/ /__/__/

Day Month Year

The following questions concern the intensity and frequency of urinary symptoms that you have had over the past 4 weeks.

To answer the following questions, please tick the box which best applies to you. There are no "right" or "wrong" answers. If you are not quite sure how to answer, choose the answer which best applies to you.

Please answer this questionnaire somewhere quiet and preferably on your own. Take as long as you need to fill it in.

Once you have finished, put the questionnaire into the envelope provided and hand it to your doctor.

Thank you for your cooperation.

You may sometimes experience urine leaks during physical effort. This effort could be strenuous (such as doing sport or having a violent coughing fit), moderate (climbing or coming down the stairs) or even light (walking or changing position).

1. <u>Over the past 4 weeks</u>, please specify the number of times a week you have had leaks during physical effort:

Please tick one box for each of the lines 1a, 1b and 1c.

	No urine leaks	**Less than one urine leak a week**	**Several urine leaks a week**	**Several urine leaks a day**
1a. During **strenuous** physical effort	☐0	☐1	☐2	☐3
1b. During **moderate** physical effort	☐0	☐1	☐2	☐3
1c. During **light** physical effort	☐0	☐1	☐2	☐3

For the doctor only: note the sum of items 1a + 1b + 1c on the scale below

"STRESS URINARY INCONTINENCE" SCORE

Over the past 4 weeks and under everyday conditions of social, professional or family life:

2. How many times a week have you had to rush to the toilet to urinate because you urgently needed to go?

☐0	☐1	☐2	☐3
Never	**Less than once a week**	**Several times a week**	**Several times a day**

3. When you have had an urgent need to urinate, for how many minutes on average have you been able to hold on?

☐0	☐1	☐2	☐3
More than 15 minutes	**From 6 to 15 minutes**	**From 1 to 5 minutes**	**Less than 1 minute**

4. How many times a week have you experienced a urine leak preceded by an urgent need to urinate that you were unable to control?

☐0	☐1	☐2	☐3
Never	**Less than once a week**	**Several times a week**	**Several times a day**

4 a. In the above case, what kind of leaks did you have?

☐0	☐1	☐2	☐3
No leaks in this case	**A few drops**	**Light leaks**	**Heavy leaks**

Over the past 4 weeks and under everyday conditions of social, professional or family life:

5. During the day, in general, how long elapsed between urinating?

☐0	☐1	☐2	☐3
2 hours or more	**Between 1 and 2 hours**	**Between 30 minutes and 1 hour**	**Less than 30 minutes**

6. How many times on average have you been <u>woken up</u> during the night by <u>a need</u> to urinate?

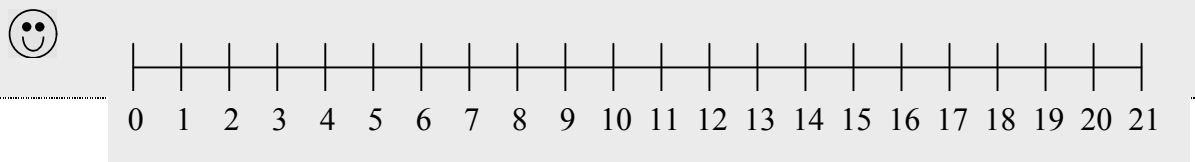
☐0	☐1	☐2	☐3
Never or Once	**Twice**	**3 or 4 times**	**More than 4 times**

7. How many times a week have you had a urine leak while asleep or have you woken up wet?

☐0	☐1	☐2	☐3
Never	**Less than once a week**	**Several times a week**	**Several times a day**

For the doctor only: note the sum of items 2 + 3 + 4 + 4a + 5 + 6 + 7 on the scale below

"OVERACTIVE BLADDER" SCORE



Over the past 4 weeks and under everyday conditions of social, professional or family life:

8. How would you describe your usual urination over these past 4 weeks?

☐0	☐1	☐2	☐3
Normal	**Needed to push with abdominal (stomach) muscles or lean forward (or required a change of position) to urinate**	**Needed to press on the lower stomach with my hands**	**Used a catheter**

9. In general, how would you describe your urine flow?

☐0	☐1	☐2	☐3
Normal	**Weak**	**Drop by drop**	**Used a catheter**

10. In general, how has your urination been?

☐0	☐1	☐1	☐2	☐3
Normal and quick	**Difficult to start, then normal**	**Easy at first but slow to finish**	**Very slow from start to finish**	**Used a catheter**

For the doctor only: note the sum of items 8 + 9 + 10 on the scale below

"LOW STREAM" SCORE

Please check that you have answered all the questions.

Thank you for your cooperation

Initial number

ICIQ-OAB (UK English) 11/05

CONFIDENTIAL

DAY MONTH YEAR

Today's date

Overactive bladder

Many people experience urinary symptoms some of the time. We are trying to find out how many people experience urinary symptoms, and how much they bother them. We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1. Please write in your date of birth:

DAY MONTH YEAR

2. Are you *(tick one)***:** Female ☐ Male ☐

3a. How often do you pass urine during the day?

1 to 6 times ☐	0
7 to 8 times ☐	1
9 to 10 times ☐	2
11 to 12 times ☐	3
13 or more times ☐	4

3b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all a great deal

4a. During the night, how many times do you have to get up to urinate, on average?

none ☐	0
one ☐	1
two ☐	2
three ☐	3
four or more ☐	4

4b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all a great deal

5a. Do you have to rush to the toilet to urinate?

never	☐	0
occasionally	☐	1
sometimes	☐	2
most of the time	☐	3
all of the time	☐	4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all a great deal

6a. Does urine leak before you can get to the toilet?

never	☐	0
occasionally	☐	1
sometimes	☐	2
most of the time	☐	3
all of the time	☐	4

6b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all a great deal

Thank you very much for answering these questions.

Initial number

CONFIDENTIAL

DAY MONTH YEAR
Today's date

Quality of life

Below are some daily activities that can be affected by urinary problems. How much does your urinary problem affect you? We would like you to answer every question. Simply tick the box that applies to you.

We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1. Please write in your date of birth:

DAY MONTH YEAR

2. Are you *(tick one)***:** Female ☐ Male ☐

3a. To what extent does your urinary problem affect your household tasks (e.g. cleaning, shopping, etc.)

not at all ☐ 1
slightly ☐ 2
moderately ☐ 3
a lot ☐ 4

3b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

4a. Does your urinary problem affect your job, or your normal daily activities outside the home?

not at all ☐ 1
slightly ☐ 2
moderately ☐ 3
a lot ☐ 4

4b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

5a. Does your urinary problem affect your physical activities (e.g. going for a walk, run, sport, gym, etc.)?

not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

6a. Does your urinary problem affect your ability to travel?

not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

6b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

7a. Does your urinary problem limit your social life?

not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

7b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

8a. Does your urinary problem limit your ability to see/visit friends?

not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

8b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

9a. Does your urinary problem affect your relationship with your partner?

not applicable	☐	8
not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

9b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

10a. Does your urinary problem affect your sex life?

not applicable	☐	8
not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

10b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

11a. Does your urinary problem affect your family life?

not applicable	☐	8
not at all	☐	1
slightly	☐	2
moderately	☐	3
a lot	☐	4

11b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

12a. Does your urinary problem make you feel depressed?

not at all	☐	1
slightly	☐	2
moderately	☐	3
very much	☐	4

12b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

13a. Does your urinary problem make you feel anxious or nervous?

not at all	☐	1
slightly	☐	2
moderately	☐	3
very much	☐	4

13b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

14a. Does your urinary problem make you feel bad about yourself?

not at all	☐	1
slightly	☐	2
moderately	☐	3
very much	☐	4

14b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

15a. Does your urinary problem affect your sleep?

never	☐	1
sometimes	☐	2
often	☐	3
all the time	☐	4

15b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

16a. Do you feel worn out/tired?

never	☐	1
sometimes	☐	2
often	☐	3
all the time	☐	4

16b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

Do you do any of the following? If so, how much?

17a. Wear pads to keep dry?

never ☐ 1
sometimes ☐ 2
often ☐ 3
all the time ☐ 4

17b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

18a. Be careful how much fluid you drink?

never ☐ 1
sometimes ☐ 2
often ☐ 3
all the time ☐ 4

18b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

19a. Change your underclothes when they get wet?

never ☐ 1
sometimes ☐ 2
often ☐ 3
all the time ☐ 4

19b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

20a. Worry in case you smell?

never ☐ 1
sometimes ☐ 2
often ☐ 3
all the time ☐ 4

20b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

21a. Get embarrassed because of your urinary problem?

never ☐ 1
sometimes ☐ 2
often ☐ 3
all the time ☐ 4

21b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

22. Overall, how much do urinary symptoms interfere with your everyday life?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

Thank you very much for answering these questions.

Bladder Diary

Patient's Name –

Hospital Number –

Date of Birth –

Instructions

Please complete this diary as accurately as possible. This will enable us to assess the severity of your bladder symptoms.

Fill the diary columns each time you pass water.

Date - Start the diary with the first time you pass water after you usually get out of the bed in the morning. Continue to record the volume and sensation each time you get up at night to pass water.

Time - You can mention the time using am/pm or the hours (0000 - 2400)

Voided Volume – You can use any measuring container to record the volume in milliliters (mL).

Residual Volume - is measured if you self-catheterize, and is the volume that is drained using a catheter after you have voided when you had a desire to pass water.

Sensation - What is the reason you went to urinate (pass water)? This can be graded as:

Grade	Definition
0	Convenience (no urge)
1	Mild urge (can hold more than 1 hour)
2	Moderate urge (can hold for 10 to 60 minutes)
3	Severe urge (can hold less than 10 minutes)
4	Desperate urge (must go immediately)

After completing, please return to:

Department of Uro-neurology
Internal Mail Box 71
National Hospital for Neurology & Neurosurgery
Queen Square London WC1N 3BG

Tel - 02078373611 ext 3418
Fax - 02078134587

Day 1; date:

Time out of bed - **Time to bed –**

Time	Voided Volume mL	Residual volume mL	Urinary leakage (none, slight, moderate, heavy)	Fluid intake	Bladder sensation (0 to 4)

Day 2; date:

Time out of bed - **Time to bed -**

Time	Voided Volume mL	Residual volume mL	Urinary leakage (none, slight, moderate, heavy)	Fluid intake	Bladder sensation (0 to 4)

Day 3; date:

Time out of bed - **Time to bed –**

Time	Voided Volume mL	Residual volume mL	Urinary leakage (none, slight, moderate, heavy)	Fluid intake	Bladder sensation (0 to 4)

Patient Satisfaction Questionnaire

Please complete the questions to describe your experience of using the device

The device instructions are easy to understand

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

The device is easy to attach and remove

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

The device is easy to operate

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

The device is comfortable to use

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

I have full mobility when I am wearing the device

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

The device improved my symptoms

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

Overall I am satisfied with the device

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

I would recommend the device to a friend for this use

Strongly Disagree---1------2------3------4-----5-----6------7---Strongly Agree

Global Response Assessment

As compared to when you started the study [treatment], how would you rate your bladder symptoms now?"

3 Markedly improved

2 Moderately improved

1 Slightly improved

0 No change

-1 Slightly worse

-2 Moderately worse

-3 Markedly worse