Multi-methodological approaches to investigate lower urinary tract function in health and disease

Multi-methodological approaches to investigate lower urinary tract function in health and disease

Matthias Walter

2018

ISBN: 978-94-6295-874-6
Universitaire Pers Maastricht

Layout and print: Datawyse bv, Maastricht

Multi-methodological approaches to investigate lower urinary tract function in health and disease

DISSERTATION

to obtain the degree of doctor at Maastricht University,
on the authority of the Rector Magnificus,
Prof. dr. Rianne M. Letschert
in accordance with the decision of the Board of Deans,
to be defended in public on
Monday March 5th 2018, at 14:00 hours

by

Matthias Walter

born in Eilenburg, Germany

Promotores

Prof. dr. P. E. van Kerrebroeck
Prof. dr. G. A. van Koeveringe

Copromotor

Prof. dr. A. Curt (University of Zurich)

Degree committee members

Prof. dr. H.W.M. Steinbusch, chairman
Prof. dr. M. van Kleef
Prof. dr. G.M. Rommers
Prof. dr. J.J. de la Rosette (University of Amsterdam)
Prof. dr. J.D. Steeves (University of British Columbia, Canada)

Table of Contents

CHAPTER 1

General Introduction

BACKGROUND

Lower urinary tract – overview

The human lower urinary tract (LUT) comprises two main compartments: the urinary bladder as well as the functional unit of the bladder neck, urethra, and urethral sphincter. The urinary bladder acts as a reservoir for urine, while the functional unit represents the reservoir's outlet [1, 2]. Both compartments are involved in the two distinct functions of the LUT – low pressure continent storage of urine and periodical, self-determined, complete release of the stored urine [1, 2]. To proper execute both, the LUT relies on an intact innervation, which is ensured by a complex network [1, 3]. The interaction between the storage and voiding phase is facilitated through aligned smooth and striated muscle activation [1-3]. In health, changing from storage to voiding is based on the individuals' arbitrary decision once a certain filling state of the bladder is perceived as well as emotional and social parameters are met [1, 4]. This dependency of the LUT on the complex central neuronal circuits makes it unique in comparison to other visceral functions, such as the gastrointestinal tract or cardiovascular, but also more vulnerable to neurological disorders [1, 5]. Impairment or loss of LUT control inevitably results in LUT dysfunction and consequently reduces the quality of life [1].

Innervation of the lower urinary tract

The peripheral innervation of the LUT is facilitated by three different peripheral nerves: pelvic (parasympathetic), hypogastric (sympathetic), and pudendal (somatic) nerves [2]. The sympathetic innervation (hypogastric nerves) originates in the thoracolumbar outflow of the spinal cord (T11 to L2) and is responsible (through norepinephrine) [4] for 1) detrusor *smooth* muscle relaxation of the urinary bladder (inhibitory), i.e. beta-adrenoceptors and 2) the contraction of the internal urethral sphincter *smooth* muscle (excitatory), i.e.

alpha-adrenoceptors, enabling urine storage [2]. The parasympathetic (pelvic) and somatic (pudendal nerves) innervation, both emerge from sacral segments of the spinal cord (S2–S4) [2]. The parasympathetic innervation is responsible for the 1) detrusor *smooth* muscle contraction of the urinary bladder (excitatory) mainly mediated through acetylcholine acting on muscarinic receptors [4] and 2) relaxation of the urethral *smooth* muscle (inhibitory), both allowing urinary flow [2]. Somatic fibres (pudendal nerves) from the motor neurons in Onuf's nucleus (anterior horn at S2 to S4) elicit contraction of the external urethral sphincter *striated* muscle (excitatory), i.e. by acetylcholine on nicotinic receptors to avoid loss of urine [2-4]. Afferent sensory input is transmitted differently to the spinal cord - from the urinary bladder (pelvic and hypogastric nerves) and from the bladder neck and the urethra (pudendal and hypogastric nerves) [3]. Afferent nerves can either have myelinated (A delta) or unmyelinated (C) axons. The cell bodies of A delta and C fibres are found in the dorsal root ganglia (DRG) at the level T11–L2 and S2–S4 [3]. A delta fibres react to passive distension of the urinary bladder and active contraction of the detrusor. [2, 3]. C fibres respond primarily to noxious stimuli such as chemical irritation or cooling, but remain unresponsive to bladder filling under physiological conditions [2, 3]. Furthermore, there is evidence for a network of nerve fibres within the urinary bladder's suburothelium, with a high concentration at the bladder neck but hardly found at the bladder dome, responsible for sensory afferent input [3]. Preganglionic neurons (receiving afferent input from the LUT through the dorsal horn) are located in laminae V to VII of the grey matter lumbar (sympathetic, T11 to L2) and sacral (parasympathetic, S2 to S4) segments of the spinal cord [3]. Somatic motor neurons (responsible for the innervation of the external urethral sphincter) are found in lamina IX of the ventral grey matter (Onuf 's nucleus, primarily S2) [3]. From animal studies, using different electrophysiological, pharmacological, label-

ling or tracing techniques, we have learned more about spinal and supraspinal pathways [2, 3]. Spinal afferent pathway comprises tracts of the anteriolateral and dorsal column [6].

Lower urinary tract function

The control of the LUT requires intact afferent and efferent pathways to a variety of brain areas and the spinal cord involving the autonomic (sympathetic and parasympathetic) and somatic nervous systems.

Storage phase

Storage is capturing the transition from an empty to a full bladder, that is more than 99% of the time, and primarily controlled by spinal reflexes [3]. In an empty bladder, almost no stimulation of the bladder wall, i.e. distension, is present. As the bladder volume starts to increase, either naturally or through retrograde filling during an urodynamic investigation (UDI), a gradual distension of the bladder wall is developing over time. Subsequently, afferent signals are submitted through the sacral dorsal horns into the spinal cord [4]. This transmission process involves two afferent axons, the A delta fibres (slightly myelinated, mechanoreceptive) and the C fibres (unmyelinated, nociceptive) [3, 7]. In healthy subjects, this information is conveyed by the A delta fibres, while C-fibres remain silent, i.e. rather respond to cooling, chemical, and noxious stimulation [8, 9]. After reaching the sacral dorsal horns, afferent signals are transmitted three-fold:

1) Within the sacral spinal cord (S2 – S4) via interneurons to the Onuf's nucleus lying within the anterior horn. Somatic efferent (pudendal nerve), which originate from here, release acetylcholine leading to a contraction of striated muscles in the external urethral sphincter [4]. When afferent activity is enhanced due to ongoing bladder filling, a) urethral sphincter tone is increased through the spinal reflex pathway [3], also known as the guarding reflex

("*bladder-to-urethral sphincter excitatory reflex*") [3, 4, 10], and b) inhibitory interneuronal synapses with preganglionic parasympathetic neurons are activated ("*urethral sphincter-to-bladder inhibitory reflex*") leading to a suppression of detrusor contraction) [4], which obviates involuntary loss of urine.
2) To the spinal cord segments T11 to L2, where a "*sympathetically mediated reflex*", i.e. releasing norepinephrine via sympathetic efferences (hypogastric nerve), contributes to a continent urine storage by acting on alpha adrenoceptors to cause contractions of the bladder neck and smooth muscles of the internal urethral sphincter, and on beta adrenoceptors to relax the detrusor [3, 4].
3) To the periaqueductal gray (PAG) [11-16], which acts as a relay centre to pass signals to higher supraspinal regions [3] that are thought to be involved in conscious perception of bladder sensations and subsequent deciding processes, i.e. maintain storage and/or initiate voiding, including the prefrontal cortex (PFC), the insula, the (anterior) cingulate cortex, the cerebellum, the thalamus, the hypothalamus, the pons, and the basal ganglia [17]. These brain areas have been consistently observed to show activity in a variety of neuro-imaging studies during urinary storage and are considered to play an important role of the current understanding of the central LUT control [3, 17].

Voiding phase

In contrast to storage, voiding requires only a short amount of time and usually takes place, when a desire to void is perceived and the individual's emotional state and social parameters are deemed appropriate [1, 4]. This synergic voiding in healthy controls, i.e. complete emptying of the bladder, is depending on a synchronization of contraction of the detrusor and relaxation of the internal and external urethral sphincter and the pelvic floor muscles [4]. In case of a strong desire to void (SDV), the "*bladder-to-urethral sphincter inhibitory reflex*" suspends the activation of the urethral sphincter [4]. This is

ensued by an increase of the detrusor pressure, as initiated by the "*bladder-to-bladder excitatory reflex*" [4]. Thereafter, a contraction of the detrusor (mediated through efferent parasympathetic nerve fibres originating from the intermediolateral column of the spinal cord S2 – S4) commences voiding [4]. However, this spinobulbospinal voiding reflex is under voluntary supraspinal control and as mentioned before, is only executed when surrounding situation meets the subject's requirement to feel comfortable [4], otherwise involuntary voiding, i.e. incontinence, would commence when a sensory threshold is reached [18], as seen in infants and young children [3]. In contrast, patients with a non-neurogenic overactive bladder (OAB) often exhibit a desire to void at a smaller bladder volume. In this condition, the process of withholding urine is no longer suspensible, hence the feeling of urgency with or without urinary incontinence. To enable a synergic voiding, several supraspinal areas are involved [17, 18].

Assessment of lower urinary tract

Thorough assessment of a subject with suspected LUT dysfunction starts with obtaining past and present medical history including bowel, sexual, and neurological history (level of evidence (LE) 4, grade of recommendation (GR) A) [19]. Bladder diaries and specific questionnaires on patient's quality of life (QoL) as well as urinary, bowel, sexual, and neurological symptoms such as Qualiveen [20] and I-QoL [21] have been shown to provide valued information (LE 1a, GR A) [19]. Physical examination (LE 4, GR A), which includes the palpation of genital and reproductive organs should be completed by a neuro-urologic status. The latter explores the sensations and reflexes mediated through the spinal cord from L1 to S5) [19]. To round up the non-invasive assessments, urinalysis (e.g. urinary tract infection and hematuria), blood chemistry (e.g. renal insufficiency), free uroflowmetry (e.g. bladder outlet ob-

struction), and urinary tract imaging such as ultrasound (e.g. post void residual urine) are recommended to detect LUT dysfunctions (LE 4, GR A) [19]. Prior to any invasive diagnostic assessment, the presence of any physical and intellectual disability should be taken into consideration to plan accordingly [19]. There are several invasive diagnostic tools available to assess the LUT. The two best known options, urethro-cystoscopy and urodynamic investigation (UDI), are used to identify LUT dysfunctions worldwide. The former, either performed with a rigid or flexible cystoscope, enables the investigator to detect anatomical changes of LUT structures such as urethral stenosis, bladder stones, benign and malign lesions (LE 3, GR B) [22]. The latter, considered to be the gold standard to assess the LUT (LE 1b, GR A), allows the investigator to obtain information during artificial bladder filling (i.e. mimicking storage phase) and voiding, which is crucial for the decision-making process to initiate appropriate therapy [23]. To ensure accurate results, UDI should always be performed according to the International Continence Society Good Urodynamic Practices [24].

Lower urinary tract dysfunction

Lower urinary tract dysfunction in humans with neurogenic (Table 1) and non-neurogenic conditions is highly prevalent, causes an enormous economic burden for every health care system, and also significantly impairs QoL of the affected [1, 25-27]. Although there are several concepts regarding LUT function and neuronal control, the exact pathophysiological mechanisms involved are yet to be discovered completely [1, 3]. Depending on the location and severity of the damage to the nervous system, the pattern and extent of LUT dysfunction vary [1, 19, 28].

Neurogenic lower urinary tract dysfunction

Suprapontine and pontine lesions

A lesion at or above the pons can incorporate these conditions: Parkinsonian syndrome (including idiopathic Parkinson's disease and multiple system atrophy), dementias (Alzheimer's disease, vascular or other), cerebrovascular accidents (stroke), cerebral palsy, brain tumors, and traumatic brain injuries [1, 19]. Injury to pathways involved in LUT control at this stage can influence the storage phase. Individuals usually suffer from urinary incontinence as a result of neurogenic detrusor overactivity (NDO, i.e. spontaneous involuntary detrusor contractions after the loss of the supraspinally induced tonic inhibition) as detected during a urodynamic investigation and a reduced bladder capacity [3, 29]. However, urgency urinary incontinence (UUI) can also be a consequence of cognitive impairment and behavioral changes or immobility in different degrees of severity [1].

Spinal (infrapontine) lesions

A spinal cord injury (SCI), traumatic or non-traumatic SCI, can interfere with both storage and voiding phase [1, 19]. After sustaining an SCI (acute stage), individuals experience the inability to pass urine (i.e. urinary retention) due to the spinal shock [1, 30, 31]. Four to six weeks following SCI, detrusor-sphincter-dssynergia (i.e. a simultaneous contraction of the detrusor and urethral sphincter) and NDO can be detected during urodynamic investigation [1, 29]. In contrast to NDO resulting from a supraspinal lesion, this one is a consequence of the spinal cord damage. This enables a spinal reflex to evolve at the sacral segment which is driven by afferent C fibres [1]. If no adequate treatment is applied, the persistence of high intravesical pressures can lead to deterioration of the upper urinary tract (UUT), subsequently result in renal failure [1, 19]. Spina bifida, a congenital condition that can also affect the spinal cord, is a common cause of LUT dysfunction in children (i.e. more than

90%) [1]. Depending on the extent of the lesion (i.e. spina bifida occulta, meningocele, and myelomeningocele), the onset and severity of symptoms can vary from infancy to adulthood [32]. Both storage and voiding symptoms can result from spina bifida [33]. Since these children are at a high risk of developing UUT deterioration, they must be monitored closely their entire life, hence to ensure the transition from a pediatric urologist to a urologist [34].
The conus medullaris is the lower end of the spinal cord located between the first and second lumbar vertebrae (L1-2) [35]. The filum terminale constitutes the connection between the conus medullaris and the cauda equina. The latter is hallmarked by nerve roots branching out to the spinal segments (L2-S5) [35]. Situated in the subarachnoid space, the cauda equina connects the central (CNS) and the peripheral nervous system (PNS). It terminates at the level of the second sacral vertebra (S2) [35]. Damage to the conus medullaris, which is more frequent in younger age (i.e. <40 years) [36] and has a sudden onset and bilateral presentation [35], is considered as a CNS lesion. The cauda equina syndrome, which appears more gradually and unilaterally [35], yet can cause long-term disturbances in a patient's life [37], falls into the category of PNS pathologies.

Multiple sclerosis

Certain conditions affecting the central nervous system cannot be categorized to just one of the aforementioned anatomical area as associated lesions can be found at multiple levels. In multiple sclerosis (MS), a well-known disseminating CNS disease, it is very common that individuals present with LUTS [38, 39]. Depending on the location of demyelination, individuals can show a variety of storage and voiding dysfunction [38, 39]. However, prevalence of LUTS in MS increases over time and with greater extent of spinal cord damage [1, 39]. There are four risk factors for UUT damage – duration of disease

of more than 15 years, indwelling urinary catheters, high-amplitude uninhibited detrusor contractions and high detrusor pressure [39].

Peripheral lesions

There are several conditions/diseases (Table 1) that can affect the peripheral innervation of the LUT [1, 19]. Most commonly, voiding phase is affected, hence NDO or acontractility can be detected during urodynamic investigation resulting in an increased post-void residual volume or at worst, urinary retention as well as impaired sensation or pain [31, 35, 38, 40, 41].

Non-neurogenic lower urinary tract dysfunction

There are a variety of causes for LUTS in women and men without an underlying neurological disorder [22, 42]. I would like to point out two.

Idiopathic overactive bladder syndrome

One is idiopathic overactive bladder (OAB) syndrome [42, 43]. This condition is defined as urinary urgency, with or without urgency urinary incontinence, and usually with frequency and nocturia [43, 44]. By affecting millions of men and women (i.e. prevalence >20%) and disease-related costs of more than $50 Billion in the United States alone, OAB causes an enormous individual, social and economic burden [44].

Fowler's syndrome

When a young female individual without any underlying neurological or urological disorder is presenting herself in a state of complete urinary retention, a condition known as Fowler's syndrome could be the reason [31]. As a result of absent urethral sphincter relaxation, this condition is marked by an inhibition of detrusor contractions [31, 45]. Using urethral sphincter electromyography, the gold standard test for Fowler's syndrome, a specific pattern comprising complex repetitive discharge and decelerating bursts, which would to

a naïve person would sound like a 'helicopters' and 'whales', can be revealed [31, 45].

Table 1 – Distribution of neurogenic lower urinary tract dysfunction depending on the injury's anatomical location

Anatomical region / neurologic disorder	Individuals* (%) affected by LUTS at any stage	LUTS* at any stage
Suprapontine and pontine lesions		
Parkinsonian syndrome	~ 80%	
Idiopathic - Parkinson's Disease		UUI, OAB, NDO, nocturia
Non-idiopathic incl. MSA		UUI, OAB, NDO, MSA-specific (impaired contractility and open bladder neck)
Dementia (all types)	~ 25%	UUI, OAB, NDO
Cerebrovascular (Stroke)	~ 80%	UUI, OAB, NDO, nocturia
Cerebral palsy	~ 70%	UI, NDO, UTI
Brain/spinal cord tumors		Various symptoms depending on location
Traumatic brain injury	~ 45%	Storage and voiding symptoms
Spinal (infrapontine) lesions		
Spinal cord injury	~ 95%	NDO, DSD, detrusor underactivity
Conus medullaris syndrome		Impaired sensation, various symptoms
Spina bifida	~ 96%	Impaired sensation, various symptoms
Multiple sclerosis	~ 86%	Impaired sensation, UUI, NDO, DSD, detrusor underactivity
Peripheral nervous system lesions		
Cauda equina syndrome	~88%	Impaired sensation, various symptoms
Diabetes mellitus		Impaired sensation, various symptoms
Guillain-Barré syndrome, alcohol		Impaired sensation, various symptoms
Disk prolapse/spinal canal stenosis	~ 83%	Impaired sensation, acontractile detrusor
Degenerative/inflammation	~ 26%	Detrusor underactivity
Iatrogenic lesions (pelvic nerves)		
Postoperative (rectal cancer)	~ 50%	Urinary retention
Postoperative (cervical cancer)	~ 30%	Voiding symptoms

* According to the literature referenced in this chapter.

CNS = central nervous system, DSD = detrusor-sphincter-dssynergia, LUTS = lower urinary tract symptoms, MSA = multiple system atrophy, NDO = neurogenic detrusor overactivity, OAB = overactive bladder, UI = urinary incontinence, UUI = urgency urinary incontinence.

Secondary complications from lower urinary tract dysfunction

When LUT dysfunction (foremost in individuals with an underlying neurological disorder) is mismanaged or not treated at all, the risk of long-term UUT damage leading to renal failure is high [46]. However, there are other potential secondary complications such as development of LUT malignancies, e.g. bladder cancer [47].

Individuals with a SCI as well as MS patients with severe spinal cord damage are prone to autonomic dysfunctions [48]. Cardiovascular disease as a consequence of autonomic dysfunction is the leading cause of death in individuals with SCI [48]. Those with an injury at or above the spinal segment T6 are at risk to experience two specific kinds of autonomic dysfunctions - orthostatic hypotension (OH) [49] and autonomic dysreflexia (AD) [50]. The latter is elicited through noxious or innocuous stimuli originating from below the lesion level [50]. Bladder (i.e. NDO) and bowel management are very frequent causes for AD [51]. This potentially life-threatening situation, characterized by a sudden increase in systolic blood pressure (SPB) >20 mmHg from baseline [50], can result in brain hemorrhage, stroke, or even death [52]. Clinical symptoms such as headache, flushing, piloerection, stuffy nose, sweating above the level of the lesion, vasoconstriction below the level of the lesion, and dysrhythmias can accompany AD [50].

Neuro-imaging of lower urinary tract control

Our understanding of central LUT control in humans has been continuously growing over the last two decades as a variety of neuroimaging studies investigating the human supraspinal network involved in LUT control emerged [17, 18]. There are several neuroimaging techniques available to investigate functional supraspinal properties including functional magnetic resonance imaging (fMRI), positron emission tomography (PET), single photon emission com-

puted tomography (SPECT), and near infrared spectroscopy (NIRS). Since increased neuronal activity within a particular brain region leads to a higher metabolic turnover and change in the hemodynamics of the brain, these neuroimaging techniques are capable of detecting specific cerebral areas that are activated in response to a particular stimulus or condition. For example, fMRI measures neuronal activity by detecting a blood oxygenation level dependent (BOLD) contrast signal [53], while PET [54] and SPECT [55] take advantage of changes of regional glucose consumption, and regional cerebral blood flow, respectively. In recent years, fMRI, has been preferred to 1) SPECT and PET due to its superior spatial resolution, its non-invasive nature, and lack of a need to administer radioactive isotopes and 2) NIRS, as this technique is limited to only record cortical activity, i.e. PFC activity. Not only did we learn more about the supraspinal LUT control in healthy subjects, but multiple neuro-imaging studies with different techniques investigated individuals with different underlying neurologic conditions such as Parkinson's disease (PD) [56-60], multiple system atrophy (MSA [61], and individuals with spinal cord injury (SCI) [62]. Taken together, a subtle network of different brain areas seems to be responsible for supraspinal LUT control in humans [17, 18]. Since previous studies represent an inhomogeneous body (i.e. study designs using very different subject populations, investigation methods, and scan protocols), it is not surprising to see different patterns and/or extents of supraspinal activation. Despite the increasing amount of literature in this field [17, 18], one still lacks important information regarding the relation between supraspinal structures and their involvement in LUT control. Therefore, standardized neuro-imaging studies with sophisticated analytic strategies are needed to improve our understanding of the supraspinal network that controls storage and voiding in humans.

OBJECTIVES AND OUTLINE OF THE THESIS

The overall objective of this dissertation was two-fold: ***1)*** To use standardized diagnostic assessments from daily clinical practice to investigate long-term outcome in individuals with neurogenic lower urinary tract dysfunction (NLUTD) and healthy subjects, as well as ***2)*** to design standardized neuro-imaging study protocols and utilize a novel infusion-drainage device (IDD) to investigate supraspinal responses to LUT stimulation.

Four original research studies (Chapters 2, 3, 4, and 5 to 7) were carried out between 2011 and 2016. The objective of the first study ***(Chapter 2)*** was to examine the significance of surveillance urethro-cystoscopy in patients with NLUTD. Although it is generally agreed that individuals with NLUTD are at increased risk for bladder cancer, reports from previous literature are conflicting. In the second study ***(Chapter 3)***, we investigated the incidence of AD and cardiovascular changes during same session repeat UDI in women with suprasacral SCI using continuous cardiovascular monitoring. The repeatability of cardiovascular changes during same session repeat urodynamic investigation (UDI) is unknown and there is a complete lack of women-specific data on autonomic dysreflexia (AD). The third study ***(Chapter 4)*** addressed the question whether UDI - the gold standard to assess refractory LUTS - is a valid means to confirm healthy volunteers as control subjects for comparative studies. The fourth study ***(Chapters 5 to 7)*** comprised two aims: First, to outline two study protocols to investigate supraspinal responses to LUT stimulation using neuro-imaging in a) healthy subjects and individuals with non-neurogenic LUTS ***(Chapter 5)*** and b) individuals with spinal cord injury (SCI) before and after treatment of NLUTD ***(Chapter 6)***. Second, to evaluate the applicability and precision of a novel IDD to investigate supraspinal responses to LUT stimulation using fMRI according to the two study protocols ***(Chapter 7)***.

COLLABORATIONS

The studies were conducted at the Department of Neuro-Urology and Neurology, Spinal Cord Injury Center and Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland in close collaboration with:

- Institute of Neuro-Radiology and Surgical Pathology, University of Zürich, University Hospital Zürich, Zürich, Switzerland
- Aroflex AG, Märstetten, Switzerland
- Department of Urology, Maastricht University Medical Center, Maastricht, the Netherlands
- Department of Brain Repair and Rehabilitation, Institute of Neurology, University College London, London, UK

REFERENCES

1. Panicker, J.N., C.J. Fowler, and T.M. Kessler, *Lower urinary tract dysfunction in the neurological patient: clinical assessment and management.* Lancet Neurol, 2015. 14(7): p. 720-32.
2. Yoshimura, N., et al., *Integrated physiology of the lower urinary tract*, in *Textbook of the Neurogenic Bladder*, J. Corcos, D. Ginsberg, and G. Karsenty, Editors. 2016, CRC Press - Taylor & Francis Group. p. 33 - 47.
3. Fowler, C.J., D. Griffiths, and W.C. de Groat, *The neural control of micturition.* Nat Rev Neurosci, 2008. 9(6): p. 453-66.
4. Griffiths, D. and A. Apostolidis, *Neurological control of the bladder in health and disease*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 1 - 24.
5. Blok, B.F., *Brain control of the lower urinary tract.* Scand J Urol Nephrol Suppl, 2002(210): p. 11-5.
6. Aldousari, S. and J. Corcos, *Simplifies anatomy of the vesicourethral functional unit*, in *Textbook of the Neurogenic Bladder*, J. Corcos, D. Ginsberg, and G. Karsenty, Editors. 2016, CRC Press - Taylor & Francis Group. p. 3 - 8.
7. Holstege, G., *Micturition and the soul.* J Comp Neurol, 2005. 493(1): p. 15-20.
8. Fall, M., S. Lindstrom, and L. Mazieres, *A bladder-to-bladder cooling reflex in the cat.* J Physiol, 1990. 427: p. 281-300.
9. Habler, H.J., W. Janig, and M. Koltzenburg, *Activation of unmyelinated afferent fibres by mechanical stimuli and inflammation of the urinary bladder in the cat.* J Physiol, 1990. 425: p. 545-62.
10. Park, J.M., D.A. Bloom, and E.J. McGuire, *The guarding reflex revisited.* Br J Urol, 1997. 80(6): p. 940-5.
11. Athwal, B.S., et al., *Brain responses to changes in bladder volume and urge to void in healthy men.* Brain, 2001. 124(2): p. 369-377.
12. Matsuura, S., et al., *Human brain region response to distention or cold stimulation of the bladder: A positron emission tomography study.* Journal of Urology, 2002. 168(5): p. 2035-2039.
13. Griffiths, D., et al., *Brain control of normal and overactive bladder.* Journal of Urology, 2005. 174(5): p. 1862-1867.
14. Kuhtz-Buschbeck, J.P., et al., *Cortical representation of the urge to void: A functional magnetic resonance imaging study.* Journal of Urology, 2005. 174(4 I): p. 1477-1481.
15. Griffiths, D., et al., *Cerebral control of the bladder in normal and urge-incontinent women.* NeuroImage, 2007. 37(1): p. 1-7.
16. Tadic, S.D., et al., *Brain responses to bladder filling in older women without urgency incontinence.* Neurourology and Urodynamics, 2013. 32(5): p. 435-440.
17. Fowler, C.J. and D.J. Griffiths, *A decade of functional brain imaging applied to bladder control.* Neurourol Urodyn, 2010. 29(1): p. 49-55.
18. Griffiths, D. and S.D. Tadic, *Bladder control, urgency, and urge incontinence: evidence from functional brain imaging.* Neurourol Urodyn, 2008. 27(6): p. 466-74.
19. Blok, B., et al. *EAU Guidelines on Neuro-Urology*. 2017 2017/04/14]; Available from: http://uroweb.org/guideline/neuro-urology/.
20. Costa, P., et al., *Quality of life in spinal cord injury patients with urinary difficulties. Development and validation of qualiveen.* Eur Urol, 2001. 39(1): p. 107-13.
21. Wagner, T.H., et al., *Quality of life of persons with urinary incontinence: development of a new measure.* Urology, 1996. 47(1): p. 67-71; discussion 71-2.
22. Gravas, S., et al. *EAU Guidelines on Non-neurogenic male LUTS including benign prostatic obstruction*. 2017 2017/04/14]; Available from: http://www.uroweb.org/guidelines.

23. Mangera, A. and C. Chapple, *Modern evaluation of lower urinary tract symptoms in 2014.* Curr Opin Urol, 2014. 24(1): p. 15-20.

24. Schafer, W., et al., *Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies.* Neurourol Urodyn, 2002. 21(3): p. 261-74.

25. Reynolds, W.S., J. Fowke, and R. Dmochowski, *The Burden of Overactive Bladder on US Public Health.* Curr Bladder Dysfunct Rep, 2016. 11(1): p. 8-13.

26. Flack, C. and C.R. Powell, *The Worldwide Economic Impact of Neurogenic Bladder.* Curr Bladder Dysfunct Rep, 2015. 10(4): p. 350-354.

27. Tang, D.H., et al., *Impact of urinary incontinence on healthcare resource utilization, health-related quality of life and productivity in patients with overactive bladder.* BJU Int, 2014. 113(3): p. 484-91.

28. Averbeck, M.A. and H. Madersbacher, *Follow-up of the neuro-urological patient: a systematic review.* BJU Int, 2015. 115 Suppl 6: p. 39-46.

29. Schops, T.F., et al., *Neurogenic lower urinary tract dysfunction (NLUTD) in patients with spinal cord injury: long-term urodynamic findings.* BJU Int, 2015. 115 Suppl 6: p. 33-8.

30. Game, X. and R. Hamid, *Spinal cord injury*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 241 - 254.

31. Panicker, J., et al., *Urinary retention*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 293 - 306.

32. Veenboer, P.W., et al., *Upper and lower urinary tract outcomes in adult myelomeningocele patients: a systematic review.* PLoS One, 2012. 7(10): p. e48399.

33. Kessler, T. and G. Kiss, *Spina bifida and tethered cord syndrome*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 255 - 265.

34. Ouyang, L., et al., *Characteristics and survival of patients with end stage renal disease and spina bifida in the United States renal data system.* J Urol, 2015. 193(2): p. 558-64.

35. Shenot, P.J. and M. Louis Moy, *Cauda equina injury*, in *Textbook of the Neurogenic Bladder*, J. Corcos, D. Ginsberg, and G. Karsenty, Editors. 2016, CRC Press - Taylor & Francis Group. p. 275 - 280.

36. Podnar, S., *Epidemiology of cauda equina and conus medullaris lesions.* Muscle Nerve, 2007. 35(4): p. 529-31.

37. Podnar, S., B. Trsinar, and D.B. Vodusek, *Bladder dysfunction in patients with cauda equina lesions.* Neurourol Urodyn, 2006. 25(1): p. 23-31.

38. Podnar, S. and C. Fowler, *Pelvic organ dysfunction following cauda equina damage*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 266 - 277.

39. de Seze, M., et al., *The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines.* Mult Scler, 2007. 13(7): p. 915-28.

40. Panicker, J. and H. Manji, *Neuromuscular disorders*, in *Pelvic Organ Dysfunction in Neurological Disease: Clinical Management and Rehabilitation*, C. Fowler, J. Panicker, and A. Emmanuel, Editors. 2010, Cambridge University Press: Cambridge, UK. p. 278 - 292.

41. Shenot, P.J. and M. Louis Moy, *Intervertebral disk prolapse*, in *Textbook of the Neurogenic Bladder*, J. Corcos, D. Ginsberg, and G. Karsenty, Editors. 2016, CRC Press - Taylor & Francis Group. p. 269 - 274.

42. Burkhard, F.C., et al. *EAU Guidelines on Urinary Incontinence in Adults*. 2017 2017/06/27]; Available from: http://www.uroweb.org/guidelines.

43. Wein, A.J. and E.S. Rovner, *Definition and epidemiology of overactive bladder.* Urology, 2002. 60(5 Suppl 1): p. 7-12; discussion 12.

44. Reynolds, W.S., J. Fowke, and R. Dmochowski, *The Burden of Overactive Bladder on US Public Health.* Current bladder dysfunction reports, 2016. 11(1): p. 8-13.

45. Fowler, C.J., R.S. Kirby, and M.J. Harrison, *Decelerating burst and complex repetitive discharges in the striated muscle of the urethral sphincter, associated with urinary retention in women.* Journal of Neurology, Neurosurgery, and Psychiatry, 1985. 48(10): p. 1004-1009.

46. Lawrenson, R., et al., *Renal failure in patients with neurogenic lower urinary tract dysfunction.* Neuroepidemiology, 2001. 20(2): p. 138-43.

47. Zhou, J., et al., *Lower Urinary Tract Symptoms and Risk of Bladder Cancer in Men: Results From the Health Professionals Follow-up Study.* Urology, 2015. 85(6): p. 1312-8.

48. Cragg, J.J., et al., *Cardiovascular disease and spinal cord injury: results from a national population health survey.* Neurology, 2013. 81(8): p. 723-8.

49. Kaufmann, H., *Consensus statement on the definition of orthostatic hypotension, pure autonomic failure and multiple system atrophy.* Clin Auton Res, 1996. 6(2): p. 125-6.

50. Krassioukov, A., et al., *International standards to document remaining autonomic function after spinal cord injury.* J Spinal Cord Med, 2012. 35(4): p. 201-10.

51. Faaborg, P.M., et al., *Autonomic dysreflexia during bowel evacuation procedures and bladder filling in subjects with spinal cord injury.* Spinal Cord, 2014. 52(6): p. 494-8.

52. Wan, D. and A.V. Krassioukov, *Life-threatening outcomes associated with autonomic dysreflexia: a clinical review.* J Spinal Cord Med, 2014. 37(1): p. 2-10.

53. Ogawa, S., et al., *Brain magnetic resonance imaging with contrast dependent on blood oxygenation.* Proc Natl Acad Sci U S A, 1990. 87(24): p. 9868-72.

54. Lenzi, G.L., T. Jones, and R.S. Frackowiak, *Positron emission tomography: state of the art in neurology.* Prog Nucl Med, 1981. 7: p. 118-37.

55. Fazio, F., et al., *Tomographic assessment of cerebral perfusion using a single-photon emitter (krypton-81m) and a rotating gamma camera.* J Nucl Med, 1980. 21(12): p. 1139-45.

56. Winge, K., et al., *Relationship between nigrostriatal dopaminergic degeneration, urinary symptoms, and bladder control in Parkinson's disease.* European Journal of Neurology, 2005. 12(11): p. 842-850.

57. Sakakibara, R., et al., *SPECT imaging of the dopamine transporter with [(123)I]-beta-CIT reveals marked decline of nigrostriatal dopaminergic function in Parkinson's disease with urinary dysfunction.* J Neurol Sci, 2001. 187(1-2): p. 55-9.

58. Kitta, T., et al., *Brain activation during detrusor overactivity in patients with Parkinson's disease: A positron emission tomography study.* Journal of Urology, 2006. 175(3): p. 994-998.

59. Herzog, J., et al., *Improved sensory gating of urinary bladder afferents in Parkinson's disease following subthalamic stimulation.* Brain, 2008. 131(1): p. 132-145.

60. Herzog, J., et al., *Subthalamic stimulation modulates cortical control of urinary bladder in Parkinson's disease.* Brain, 2006. 129(12): p. 3366-3375.

61. Sakakibara, R., et al., *Reduced cerebellar vermis activation during urinary storage and micturition in multiple system atrophy: 99mTc-labelled ECD SPECT study.* European Journal of Neurology, 2004. 11(10): p. 705-708.

62. Zempleni, M.Z., et al., *Cortical substrate of bladder control in SCI and the effect of peripheral pudendal stimulation.* NeuroImage, 2010. 49(4): p. 2983-2994.

CHAPTER 2

Do we need surveillance urethro-cystoscopy in patients with neurogenic lower urinary tract dysfunction?

Sammer U*, **Walter M***, Knüpfer SC, Mehnert U, Bode-Lesniewska B, Kessler TM
PLoS One. 2015 Oct 29;10(10):e0140970. (*Equal contribution, i.e. shared-first authorship)

ABSTRACT

Purpose

To examine the value of surveillance urethro-cystoscopy in patients with neurogenic lower urinary tract dysfunction (NLUTD) in regard to the conflicting literature as it is generally agreed that patients with NLUTD are at increased risk for bladder cancer.

Materials and Methods

In a cross-sectional study, a consecutive series of 129 patients (50 females, 79 males, mean age 51, range 18 - 88) suffering from NLUTD for at least 5 years was prospectively investigated using urethro-cystoscopy and bladder washing cytology at a single university spinal cord injury (SCI) center.

Results

Due to suspicious urethro-cystoscopy and/or bladder washing cytology findings, 13 (10%) of 129 patients underwent transurethral resection of the bladder lesion and/or random bladder biopsies. Overall, 9 relevant histological findings were found in 5% (7/129) of our patients: bladder melanosis (n=1), nephrogenic adenoma (n=3), keratinizing squamous metaplasia (n=1), intestinal metaplasia (n=3), and muscle-invasive adenocarcinoma of the bladder (n=1).

Conclusions

Using surveillance urethro-cystoscopy, we found relevant histological findings in 5% of our patients suffering from NLUTD for at least 5 years. Thus, surveillance urethro-cystoscopy might be warranted, although the ideal starting point and frequency remain to be determined in further prospective studies.

INTRODUCTION

Bladder cancer, as defined by the 10th revision of the International Classification of Diseases (ICD-10) [1], is a common neoplasia in the general population with a recently reported incidence of 151 and a mortality of 52 per 100'000 adults in Europe for the year 2012 [2]. Men are more frequently affected than women with a higher incidence ratio ranging between 1.3 and 6.3 [3]. In men, bladder cancer is the sixth leading cancer worldwide [4]. There are several risk factors associated with bladder cancer, that is, tobacco smoking, increased age, male gender, chronic bladder infection, chronic irritation from indwelling catheters, and exposure to aromatic amines, polycyclic aromatic hydrocarbons and chlorinated hydrocarbons, benzole, cyclophosphamide and radiation [5]. It is generally agreed that patients with neurogenic lower urinary tract dysfunction (NLUTD) due to spinal cord injury (SCI) are at increased risk for bladder cancer [6]. However, there is no consensus on the type and frequency of investigations to detect urological malignancies at an early stage in patients suffering from NLUTD and there is a lack of specific recommendations in the guidelines of the American Urological Association (AUA) and European Association of Urology (EAU) to address this issue. Considering the conflicting literature, we aimed to examine the value of surveillance urethro-cystoscopy in patients with NLUTD due to different neurological disorders including SCI.

MATERIALS AND METHODS

Patients

From January 2011 to November 2013, a consecutive series of 129 patients suffering from NLUTD for at least 5 years was prospectively investigated in a cross-sectional study using urethro-cystoscopy and bladder washing cytology at the SCI Center, University of Zürich, Balgrist University Hospital, Zürich,

Switzerland. Study exclusion criteria was current urinary tract infection (UTI), gross hematuria and age <18 years. This study was approved by the local ethics committee, i.e. Kantonale Ethikkommision (KEK) Zürich, Switzerland; study identification number: KEK-ZH-NR: 2010-0207/0 and all patients provided written informed consent according to the Helsinki II declaration.

Investigations

According to the EAU guidelines, urethro-cystoscopy and bladder washing cytology are both established routine follow-up investigations for patients with NLUTD [5]. At our department, we performed both in addition to a widespread neuro-urological evaluation that includes renal ultrasound and urodynamics. Furthermore, we obtained the medical history, in particular the method of bladder management and the exposure to risk factors for developing bladder cancer such as tobacco smoking, aromatic amines, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, ionizing radiation, cyclophosphamide, pioglitazone [5]. Patients with a UTI were excluded and adequately treated according to the antibiotic sensitivity pattern before urethro-cystoscopy. For local anesthesia, lidocaine gel (Instillagel® 2x 10 mL, Farco-Pharma GmbH, Germany) was instilled into the urethra and exposed for 10 minutes. Urethro-cystoscopy was performed in a lithotomy or supine position using a rigid or flexible cystoscope in women and men, respectively. After urethro-cystoscopy, we routinely performed a bladder washing cytology. In the case of suspicious urethro-cystoscopy and/or bladder washing cytology findings, the patient was scheduled for transurethral resection of the bladder (TUR-B) and/or random cold biopsies in spinal or general anesthesia. Histological and cytological findings were reported by board certified pathologists and cytologists, respectively, according to findings on routinely processed and stained specimen.

Outcome measure

The outcome measures were suspicious urethro-cystoscopy and/or bladder washing cytology findings and relevant histological findings defined as bladder cancer or potentially premalignant lesions.

Statistical analysis

Data were normally distributed and are presented as mean and standard deviation (SD). Descriptive statistical analyses were performed using IBM® SPSS® Statistics Version 19.

RESULTS

Mean age of the 129 patients was 51 ± 16 years (range 18 to 88). 50 (39%) and 79 (61%) were women and men, respectively. The cause of NLUTD was SCI in 89 patients (69%), multiple sclerosis in 20 (16%), myelomeningocele in 8 (6%), cerebral palsy in 4 (3%) and other neurological disorders in 8 (6%). Patients' characteristics are shown in Table 1.

Table 1 – Patients' characteristics.

Neurological disorder	Number of patients (females)	Age at cystoscopy in years*	Duration of neurological disorder in years*	Bladder management		
				I	II	III
Spinal cord injury (69%)	89 (25)	50±15 (18-88)	15±9 (5-45)	29	43	17 (10/7)
Multiple sclerosis (16%)	20 (16)	55±11 (36-73)	22±10 (6-47)	7	5	8 (8/0)
Myelomeningocele (6%)	8 (3)	30±19 (21-75)	30±19 (21-75)	3	5	0
Cerebral palsy (3%)	4 (3)	56±8 (45-65)	56±8 (45-65)	3	0	1 (1/0)
Other (6%)	8 (3)	70±12 (49-88)	19±25 (5-80)	5	0	3 (3/0)
All	129 (50)	51±16 (18-88)	19±14 (5-80)	47	53	29 (22/7)

Bladder management: I, spontaneous voiding, II, aseptic intermittent self-catheterization and III, indwelling catheter (suprapubic/transurethral); * Data presentation: mean ± standard deviation (range).

Overall, 58 (45%) of the 129 patients were exposed to at least one risk factor for bladder cancer (Table 2). Due to suspicious urethro-cystoscopy and/or bladder washing cytology findings, 13 (10%) of the 129 patients underwent transurethral resection of the bladder lesion and/or random bladder biopsies (Table 3). In total, 9 relevant histological findings were found in 5% (7/129) of our patients: Bladder melanosis (n=1, Figure 1), nephrogenic adenoma (n=3, Figure 2), keratinizing squamous metaplasia (n=1, Figure 3), intestinal metaplasia (n=3, Figure 4) and muscle-invasive adenocarcinoma of the bladder (n=1, Figure 5). The patient with bladder cancer underwent bilateral pelvic lymphadenectomy and radical cystoprostatovesiculectomy with a continent catheterizable reservoir and final histology revealed pT3b pN0 (0/15) cM0 G3 mucus-producing adenocarcinoma of the bladder.

Table 2 – Patients' exposure to risk factors for bladder cancer.

Neurological disorder	Exposure to risk factors*	Tobacco	Aromatic amines	Polycyclic aromatic hydrocarbons	Chlorinated hydrocarbons	Ionizing radiation
Spinal cord injury (n=89)	45 (51%)	40	12	5	3	3
Multiple sclerosis (n=20)	5 (25%)	5	0	0	0	0
Myelomeningocele (n=8)	3 (38%)	1	1	1	0	0
Cerebral palsy (n=4)	1 (25%)	1	0	0	0	0
Other (n=8)	4 (50%)	3	1	0	0	2
All (n=129)	58 (45%)	50	14	6	3	5

* Patients were exposed to at least one risk factor (multiple exposures possible).

Table 3 – Characteristics of the patients undergoing transurethral resection of the bladder lesion and/or random bladder biopsies

No.	Gender	Age	Neurological disorder	Injury level	AIS	Duration of neurological disorder	Bladder management	Cystoscopy	Cytology	Histopathology	History of tobacco consumption (pack years)	Exposure to other risk factors
1	Male	59	SCI	T5	A	5	II	Suspicious reddish bladder lesions	Squamous metaplasia	Follicular cystitis	No	No
2	Male	26	SCI	T5	A	7	II	Suspicious reddish bladder lesions	Squamous metaplasia	Follicular cystitis	Yes (7)	No
3	Female	45	SCI	T10	D	9	I	Exophytic bladder tumor	Squamous metaplasia	Papillary inflammatory lesions	Yes (39)	No
4	Male	69	SCI	C4	D	14	II	Suspicious reddish bladder lesions	Squamous metaplasia	Nonkeratinizing squamous metaplasia & follicular cystitis	Yes (30)	Yes[1]
5	Male	43	SCI	C5	C	16	III (transurethral)	Exophytic bladder tumor	Inflammatory cells	Eosinophilic cystitis	Yes (20)	Yes[1]
6	Male	52	SCI	C8	A	20	II	Exophytic bladder tumor	Inflammatory cells	Nephrogenic adenoma & cystitis cystica	No	No
7	Male	43	SCI	T6	A	24	II	Suspicious reddish bladder lesions	Squamous metaplasia	Keratinizing squamous metaplasia	Yes (3)	Yes[1,2]
8	Male	59	SCI	T5	A	33	II	Exophytic bladder tumor	Squamous metaplasia	Muscle-invasive adenocarcinoma of the bladder & intestinal metaplasia	No	No
9	Female	62	MS	-	-	30	III (suprapubic)	Exophytic bladder tumor	Inflammatory cells	Nephrogenic adenoma, nonkeratinizing squamous metaplasia & intestinal metaplasia	No	No
10	Female	21	MMC	-	-	21	II	Exophytic bladder tumor	Inflammatory cells	Nephrogenic adenoma	No	No
11	Female	45	CP	-	-	45	III (suprapubic)	Exophytic bladder tumor	Inflammatory cells	Intestinal metaplasia & cystitis cystica et glandularis	No	No
12	Female	56	CP	-	-	56	I	Multiple black bladder spots	Melanosis	Melanosis of the bladder	No	No
13	Male	88	Other (GBS)	-	-	10	III (suprapubic)	Exophytic bladder tumor	low grade urothelial cell atypia	Chronic cystitis	No	No

Bladder management: I, spontaneous voiding, II, aseptic intermittent self-catheterization and III, indwelling catheter (suprapubic/transurethral); AIS, American Spinal Injury Association Impairment Scale; CP, Cerebral palsy; GBS, Guillain-Barré syndrome; MMC, Myelomeningocele; MS, Multiple sclerosis; SCI, Spinal cord injury. [1] Exposure to aromatic amines; [2] Exposure to polycyclic aromatic hydrocarbons.

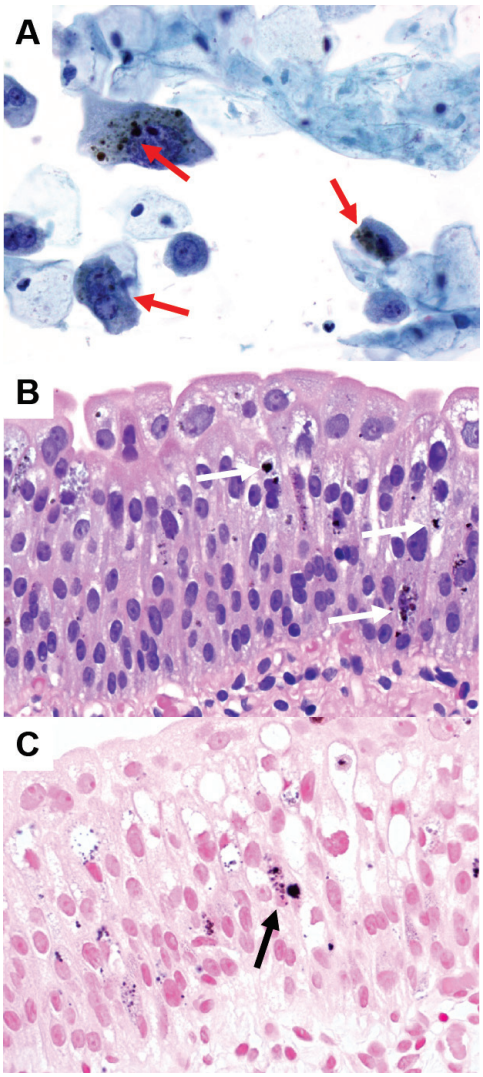

Figure 1 – Melanosis of the urothelium in a 56 years old woman (Table 3, Patient 12).
A - Bladder washing with dark brown and black pigment in urothelial cells (arrows) (Papanicolaou stain; original magnification 400x). B - Bladder mucosa biopsy containing dark brown and black pigment in urothelial cells of all levels (arrows) (Hematoxylin and eosin stain; original magnification 400x). C - Negativity of the intraepithelial pigment in an iron stain (arrow) excluding hemosiderin pigment deposits (Iron stain; original magnification 400x).

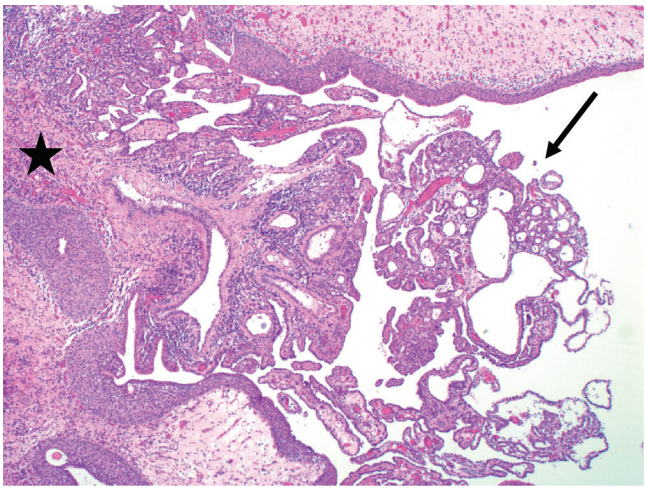

Figure 2 – Nephrogenic adenoma in a 21 years old woman (Table 3, Patient 10).
Intraluminal polypoid mass (arrow), covered by cylindrical non-dysplastic epithelium and containing bland glands, accompanied by some inflammatory cells. The bladder mucosa at the base of the lesion (asterisk) shows chronic inflammation (Hematoxylin and eosin stain; original magnification 25x).

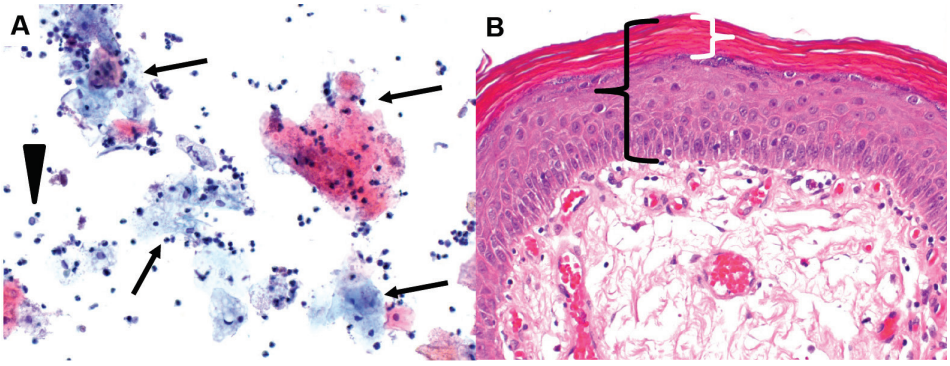

Figure 3 – Extensive keratinizing squamous metaplasia of the urothelium in a 43 years old man (Table 3, Patient 7).
A - Bladder washing with numerous, often anucleated squamous cells (arrows). Only rare, degenerated urothelial cells (arrowhead) and many neutrophils in the background (Papanicolaou stain; original magnification 200x). B - On biopsy, bladder mucosa is covered by non-dysplastic squamous epithelium (black bracket) with a thick anucleated keratinized superficial layer (white bracket) (Hematoxylin and eosin stain; original magnification 200x).

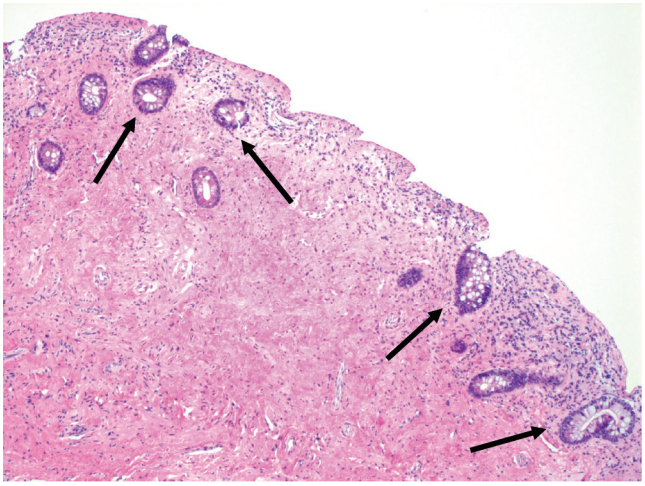

Figure 4 – Intestinal metaplasia in a 45 years old woman (Table 3, Patient 11).
Intestinal type glands in the bladder biopsy (arrows). Denuded surface of the mucosa containing some inflammatory cells (Hematoxylin and eosin stain; original magnification 50x).

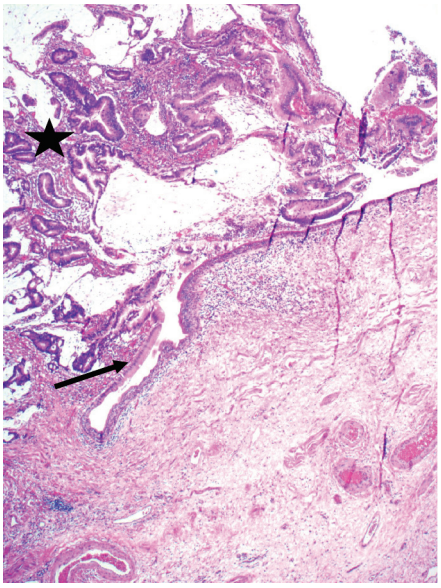

Figure 5 – Mucinous adenocarcinoma in a 59 years old man (Table 3, Patient 8).
Intraluminal polypoid mass (asterisk) consisting of malignant glands with intra- and extracellular mucus and infiltrating growth. The arrow points to the transition zone between urinary type epithelium and cylinder cells of the intestinal metaplasia (Hematoxylin and eosin stain; original magnification 25x).

DISCUSSION

Using surveillance urethro-cystoscopy, we detected relevant histological findings in 5% of our patients suffering from NLUTD for at least 5 years. Considering this important percentage of clinically relevant findings, surveillance urethro-cystoscopy might be warranted, although the ideal patient population, starting point and frequency remain to be determined in further prospective studies. In comparison to the general population, an up to 25 times higher risk of developing bladder cancer has been reported in patients with SCI relying on chronic indwelling catheters [7]. This method of bladder management leads to chronic irritation of the bladder mucosa [8] and chronic urinary tract infections (UTI) [6] while serving as an independent risk factor for bladder cancer in SCI patients [7]. Bladder cancer has been reported to be present in SCI patients at a lower age and a higher stage, when diagnosed [6], compared to the general population. Gross hematuria, a well-known phenomenon in SCI patients with indwelling catheters, has a significantly higher incidence the longer the duration of catheterization is [9]. The initiation of a routine screening protocol might be useful with the objective of minimizing morbidity and mortality of bladder cancer in patients suffering from NLUTD, since typical clinical symptoms of bladder cancer such as gross hematuria may be absent [9]. Although the literature is limited, microscopic hematuria has been reported to be present in 15% of SCI patients [10]. Yet, the specificity of urine dipstick is restricted as microscopic hematuria may result from various reasons, that is calculi, infections or use of certain drugs [11]. Therefore, the interpretation of microscopic hematuria is challenging in this population and needs a patient-tailored approach. With no consensus on how to manage patients with NLUTD to detect urological malignancies at an early stage, the need for establishing suitable diagnostic methods is substantial. Ultrasound of the bladder, a non-invasive diagnostic tool, is reported to be inferior to urethro-cystoscopy in identifying bladder cancer in patients with

hematuria [12] and therefore inappropriate to replace urethro-cystoscopy and bladder washing cytology. However, the role of urethro-cystoscopy and bladder washing cytology for surveillance has not yet been clearly determined and a cost-benefit analysis is still up for discussion regarding this challenging patient population.

According to the consortium for spinal cord medicine [13], SCI patients should undergo an extensive annual urological evaluation program including urethro-cystoscopic investigation in addition to urodynamics to achieve a sufficient functional/anatomical assessment of the lower urinary tract system. Navon et al. recommended the performance of annual urethro-cystoscopy in SCI patients beginning 10 years after injury and in patients with recurrent or chronic UTI resulting in the diagnosis of squamous cell bladder cancer at early stages with consecutive increase of long-term survival [14]. Annual urethro-cystoscopy and urine cytology with routine random biopsies every 1 to 2 years in individuals with SCI was advocated by Broecker et al. [10]. In contrast, Hamid et al. did not recommend routine surveillance urethro-cystoscopy and biopsy in patients with a neuropathic bladder and chronic suprapubic indwelling catheters due to high screening costs with low detection rate and associated morbidity [8]. Importantly, there are no generally agreed recommendations regarding follow-up evaluation of the neuro-urological patient and there is a complete lack of high-evidence level studies on this topic [15]. In an attempt to minimize morbidity caused by urethro-cystoscopy, antibiotic prophylaxis might be considered in high-risk patients suffering from NLUTD in order to prevent symptomatic UTI. In patients with SCI being at risk of the development of autonomic dysreflexia due to bladder distension during urethro-cystoscopy might profit from using a less traumatic flexible instead of a rigid cystoscope while being on continuous cardiovascular monitoring at the same time, that is, blood pressure and heart rate [16]. To improve the detection rate of bladder cancer, additional diagnostic tools might be useful. Therefore,

we routinely perform bladder washing cytology during urethro-cystoscopy as Stonehill et al. reported good sensitivity (71%) and high specificity (97%) for diagnosis of bladder cancer in SCI patients [17]. As unspecific multifocal flat lesions of the bladder mucosa can often be found by urethro-cystoscopy in patients suffering from NLUTD, particularly in patients with chronic indwelling catheter due to chronic irritation/inflammation, bladder-washing cytology might be a good indicator for the presence of carcinoma in situ (CIS) leading to subsequent biopsy and treatment. Nevertheless, the limitation of cytology concerning the high rate of false negative results in detecting low-grade tumors [17] must be taken into consideration. In the present study using surveillance urethro-cystoscopy, relevant histological findings varied widely and included bladder melanosis, nephrogenic adenoma, keratinizing squamous metaplasia, intestinal metaplasia, and mucus-producing adenocarcinoma. Considering the fact that relevant histological findings were present even in 15% (2/13) of the patients with NLUTD who voided spontaneously, NLUTD itself might be a risk factor for the development of bladder cancer as suggested by Kalisvaart et al. [18]. Although a relevant high percentage (45%) of patients was exposed to at least one risk factor for bladder cancer, we did not find any dysplastic lesions. Bladder melanosis (Figure 1), a rare entity with less than 20 cases reported up to date, is characterized by abnormal/excessive deposits of melanin in the urothelium without any signs of atypia [19]. In general, bladder melanosis is considered to be a benign lesion but it has been described as a potential premalignant factor associated with primary melanoma of the bladder [20], high grade transitional cell carcinoma of the bladder [21] and the upper urinary tract [19]. Although our patient with bladder melanosis suffered from NLUTD due to cerebral palsy, it is unknown, if there is a causal relationship between NLUTD and the development of bladder melanosis or if it is just a coincidental histological finding. Nephrogenic adenoma (Figure 2) is an uncommon metaplastic lesion of the urothelium found in all

locations of the urinary tract often with multifocal appearance and most commonly seen in the urinary bladder [22, 23]. Etiology and biological potential are still unclear, but it seems to be attributed to urothelial irritation by trauma, previous surgery, urinary calculi, radiation, chronic infections and urinary catheterization [22, 23]. Clinical symptoms, when present, are non-specific such as lower urinary tract symptoms and hematuria [22, 23]. Therapeutic options vary from watchful waiting to cystectomy [23], whereas in most cases a transurethral resection is favored [24], as it was the case in our 3 patients with a nephrogenic adenoma. A high recurrence rate up to 80% has been reported [23]. Although nephrogenic adenoma is usually thought to be a benign entity [22], there might be some potential for malignant transformation as its occurrence has been reported in association with transitional cell carcinoma [23] and moderately differentiated adenocarcinoma of the bladder in a patient with NLUTD [24]. Because of the high recurrence rate of the nephrogenic adenoma and its assumed potential for neoplastic transformation, regular follow-up should be considered. Keratinizing squamous metaplasia (Figure 3) is rare and characterized by a replacement of the urothelial layer with stratified keratinizing squamous epithelium due to chronic inflammation/irritation of the bladder mucosa caused by different predisposing factors such as urinary tract infections, indwelling catheters, fistula, urinary calculi, bladder outlet obstruction, bladder extrophy and neurogenic bladder [25]. Keratinizing squamous metaplasia of the bladder is postulated to be a pre-malignant condition leading to a higher risk of the development of bladder cancer, especially if dysplasia and/or extensive keratinization is present [26]. Most commonly, keratinizing squamous metaplasia of the bladder is associated with co-existing or subsequent squamous cell carcinoma and transitional cell carcinoma [27]. The risk of developing bladder cancer [26] in patients with keratinizing squamous metaplasia of the bladder is high as progression has been reported in 27% with a variable period of latency between 4 and 28

years [27]. In keratinizing squamous metaplasia of the bladder, a complete transurethral resection is considered to be the therapy of choice [26] and a close follow-up using urethro-cystoscopy and bladder washing cytology is highly advocated in order to detect malignant transformation at early stages [27]. Intestinal metaplasia (Figure 4) of the bladder is characterized by the presence of intestinal-type epithelium and/or goblet cells in the bladder urothelium and is suggested to occur as a result of chronic inflammation and irritating stimuli such as urinary tract infections, calculi, neurogenic bladder, bladder extrophy, and long-term catheterization [25, 28]. Clinical symptoms reported by patients contain hematuria, dysuria, urgency and mucusuria as a sign of mucus production/secretion of these lesions [25, 28]. Intestinal metaplasia of the bladder is often associated with adenocarcinoma of the bladder [28] implying pre-malignant characteristics [25] comparable to Barrett's metaplasia of the esophagus [29]. Thus, considering the potential of intestinal metaplasia to progress to a malignant condition such as adenocarcinoma of the bladder, we regularly perform urethro-cystoscopy and bladder washing cytology in such patients to ensure an early detection of malignant transformation. Importantly, patients with SCI have a higher risk to develop bladder cancer compared to the general population [18]. We agree with the existing literature [6], that bladder cancer appears to be a late complication in patients suffering from NLUTD due to SCI, i.e. bladder cancer was detected in our patient 33 years post-injury. In patients with muscle-invasive bladder cancer, bilateral pelvic lymphadenectomy and radical cystectomy combined with urinary diversion is mandatory as in our SCI patient with co-existing intestinal metaplasia and mucus-producing adenocarcinoma of the bladder (Figure 5). Patients with NLUTD require an extensive and specific work-up before embarking on an individualized therapy taking into account the patients' medical and physical condition as well as their expectations [30]. Considering the high incidence of relevant histological findings in the present study, surveillance

urethro-cystoscopy and bladder washing cytology might be warranted. While awaiting the results of well-designed risk-stratification studies, we recommend performing surveillance urethro-cystoscopy and bladder washing cytology on patients suffering from NLUTD for at least 5 years on a regular short-term basis, that is, every 1 to 2 years. This has also been suggested in a very recent retrospective study of surveillance urethro-cystoscopy in SCI patients relying on an indwelling urethral or suprapubic catheter [31]. Although our recommendation is not yet based on high-evidence level studies, this policy seems justified considering the highly relevant, potentially life threatening consequences of not detecting and consequently not treating a pre-malignant and/or malignant lesion. Metaplastic lesions such as nephrogenic adenoma, keratinizing squamous metaplasia and intestinal metaplasia may progress to a malignant condition but the underlying mechanisms involved are unclear and remain to be elucidated. Given the heterogeneous nature and management of neurogenic LUTD, it would be of great interest to know which subgroup of patients is at highest risk to develop relevant histological lesions. In addition, further studies are needed to determine the ideal starting point and frequency of surveillance urethro-cystoscopy. Thus, prospective large-scale multicenter studies are highly warranted to investigate these still unanswered questions and to further improve the management of patients with NLUTD.

CONCLUSIONS

In patients suffering from NLUTD for at least 5 years, we detected suspicious urethro-cystoscopy and/or bladder washing cytology findings in 10% and relevant histological findings in 5%. The relevant histological findings varied widely, that is, from bladder melanosis to muscle-invasive mucus-producing adenocarcinoma of the bladder. Considering the important percentage of clinically relevant findings, surveillance urethro-cystoscopy might be war-

ranted. However, we cannot conclude from our data which sub-group of patients with NLUTD would profit most from surveillance urethro-cystoscopy. In addition, starting point and frequency of surveillance urethro-cystoscopy remain unclear. Thus, further well-designed, adequately powered and sampled prospective studies are needed to investigate these highly relevant issues.

Supporting Information:
S1 Dataset. De-identified patients' data used in this study.
S2 Figure. STROBE checklist part 1.
S3 Figure. STROBE checklist part 2.

Competing Interests:
The authors have declared that no competing interests exist.

Funding:
The authors have no support or funding to report.

Author Contributions:
Conceived and designed the study: US, MW and TMK
Performed investigation: US, MW, SK, UM, BBL, TMK
Analyzed the data: US, MW
Statistical analysis: US, MW
Contributed to the writing of the manuscript: US, MW, TMK
Critical review of the manuscript: SK, UM, BBL, TMK

REFERENCES

1. WHO. International statistical classification of diseases and related health problems - 2010 Edition 1993 [2014/07/22]. Available from: http://www.who.int/classifications/icd/ICD10Volume2_en_2010.pdf?ua=1.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013;49(6):1374-403.
3. Donsky H, Coyle S, Scosyrev E, Messing EM. Sex differences in incidence and mortality of bladder and kidney cancers: national estimates from 49 countries. Urol Oncol. 2013. Epub 08 July 2013
4. Estimated cancer incidence, mortality and prevalence worldwide in 2012 [Internet]. WHO - International Agency for Research of Cancer. 2012 [cited 2014/07/22]. Available from: http://globocan.iarc.fr.
5. Witjes JA, Compérat E, Cowan NC, De Santis M, Gakis G, Lebrét T, et al. EAU guidelines on muscle-invasive and metastatic bladder cancer 2014 [2014/07/22]. Available from: http://www.uroweb.org/gls/pdf/07 Muscle Invasive BC_LR.pdf.
6. Welk B, McIntyre A, Teasell R, Potter P, Loh E. Bladder cancer in individuals with spinal cord injuries. Spinal Cord. 2013. Epub 23.04.2013. doi: 10.1038/sc.2013.33.
7. Groah SL, Weitzenkamp DA, Lammertse DP, Whiteneck GG, Lezotte DC, Hamman RF. Excess risk of bladder cancer in spinal cord injury: evidence for an association between indwelling catheter use and bladder cancer. Arch Phys Med Rehabil. 2002;83(3):346-51. Epub 2002/03/12. PubMed PMID: 11887115.
8. Hamid R, Bycroft J, Arya M, Shah PJ. Screening cystoscopy and biopsy in patients with neuropathic bladder and chronic suprapubic indwelling catheters: is it valid? J Urol. 2003;170(2 Pt 1):425-7. Epub 2003/07/11. doi: 10.1097/01.ju.0000076700.00853.ad. PubMed PMID: 12853791.
9. Hollingsworth JM, Rogers MA, Krein SL, Hickner A, Kuhn L, Cheng A, et al. Determining the noninfectious complications of indwelling urethral catheters: a systematic review and meta-analysis. Ann Intern Med. 2013;159(6):401-10. Epub 2013/09/18. doi: 10.7326/0003-4819-159-6-201309170-00006. PubMed PMID: 24042368.
10. Broecker BH, Klein FA, Hackler RH. Cancer of the bladder in spinal cord injury patients. The Journal of urology. 1981;125(2):196-7. Epub 1981/02/01. PubMed PMID: 7206053.
11. Planz B, Jochims E, Deix T, Caspers HP, Jakse G, Boecking A. The role of urinary cytology for detection of bladder cancer. Eur J Surg Oncol. 2005;31(3):304-8. Epub 2005/03/23. doi: 10.1016/j.ejso.2004.12.008. PubMed PMID: 15780568.
12. Stamatiou K, Papadoliopoulos I, Dahanis S, Zafiropoulos G, Polizois K. The accuracy of ultrasonography in the diagnosis of superficial bladder tumors in patients presenting with hematuria. Annals of Saudi Medicine. 2009;29(2):134-7. PubMed Central PMCID: PMCPMC2813642.
13. Consortium for Spinal Cord Medicine. Bladder management for adults with spinal cord injury: a clinical practice guideline for health-care providers. The journal of spinal cord medicine. 2006;29(5):527-73. Epub 2007/02/06. PubMed PMID: 17274492; PubMed Central PMCID: PMC1949036.
14. Navon JD, Soliman H, Khonsari F, Ahlering T. Screening cystoscopy and survival of spinal cord injured patients with squamous cell cancer of the bladder. J Urol. 1997;157(6):2109-11. Epub 1997/06/01. PubMed PMID: 9146592.
15. Averbeck MA, Madersbacher H. Follow-up of the neuro-urological patient: a systematic review. BJU Int. 2015;115 Suppl 6:39-46. Epub 2015/04/22. doi: 10.1111/bju.13084. PubMed PMID: 25891319.
16. Rivas DA, Chancellor MB. Flexible cystoscopy in spinal cord injury. Review article. Paraplegia. 1994;32(7):454-62. doi: 10.1038/sc.1994.72.
17. Stonehill WH, Goldman HB, Dmochowski RR. The use of urine cytology for diagnosing bladder cancer in spinal cord injured patients. J Urol. 1997;157(6):2112-4. Epub 1997/06/01. PubMed PMID: 9146593.
18. Kalisvaart JF, Katsumi HK, Ronningen LD, Hovey RM. Bladder cancer in spinal cord injury patients. Spinal Cord. 2009;48(3):257-61. doi: 10.1038/sc.2009.118.
19. Harikrishnan JA, Chilka S, Jain S. Urinary tract transitional cell carcinoma and melanosis of the bladder: a case report and review of the literature. Ann R Coll Surg Engl. 2012;94(4):e152-4. Epub 2012/05/23. doi: 10.1308/003588412x13171221589135. PubMed PMID: 22613284.

20. Kerley SW, Blute ML, Keeney GL. Multifocal malignant melanoma arising in vesicovaginal melanosis. Arch Pathol Lab Med. 1991;115(9):950-2. Epub 1991/09/01. PubMed PMID: 1929794.
21. Sanborn SL, MacLennan G, Cooney MM, Zhou M, Ponsky LE. High-grade transitional cell carcinoma and melanosis of urinary bladder: case report and review of the literature. Urology. 2009;73(4):928 e13-5. Epub 2008/12/06. doi: 10.1016/j.urology.2008.05.039. PubMed PMID: 19056107.
22. Chen C-S, Cheng C-L. Nephrogenic adenoma of the urinary bladder: clinical experience and review of the literature. J Chin Med Assoc. 2006;69(4):166-8. doi: http://dx.doi.org/10.1016/S1726-4901(09)70199-6.
23. Zougkas K, Kalafatis M, Kalafatis P. Nephrogenic adenoma of the urinary bladder. Int Urol Nephrol. 2004;36(4):513-7. Epub 2005/03/25. PubMed PMID: 15787327.
24. Hungerhuber E, Bach E, Hartmann A, Frimberger D, Stief C, Zaak D. Adenocarcinoma of the bladder following nephrogenic adenoma: a case report. J Med Case Rep. 2008;2(1):164. doi: 10.1186/1752-1947-2-164.
25. Samaratunga H, Martignoni G, Egevad L, Delahunt B. Premalignant lesions of the urinary bladder. Pathology. 2013;45(3):243-50. Epub 2013/03/02. doi: 10.1097/PAT.0b013e32835f6169. PubMed PMID: 23448810.
26. Ahmad I, Barnetson RJ, Krishna NS. Keratinizing squamous metaplasia of the bladder: a review. Urol Int. 2008;81(3):247-51. doi: 10.1159/000151398.
27. Khan MS, Thornhill JA, Gaffney E, Loftus B, Butler MR. Keratinising squamous metaplasia of the bladder: natural history and rationalization of management based on review of 54 years experience. European urology. 2002;42(5):469-74. Epub 2002/11/14. PubMed PMID: 12429156.
28. Clouston D, Lawrentschuk N. Metaplastic conditions of the bladder. BJU Int. 2013;112:27-31. doi: 10.1111/bju.12378.
29. Bryan RT, Nicholls JH, Harrison RF, Jankowski JA, Wallace DM. The role of beta-catenin signaling in the malignant potential of cystitis glandularis. J Urol. 2003;170(5):1892-6. Epub 2003/10/09. doi: 10.1097/01.ju.0000092740.51330.39. PubMed PMID: 14532801.
30. Pannek J, Blok B, Castro-Diaz D, Del Popolo G, Groen J, Karsenty G, et al. EAU guidelines on neuro-urology 2014 [2014/07/22]. Available from: http://www.uroweb.org/gls/pdf/21 Neuro-Urology_LR.pdf.
31. El Masri y WS, Patil S, Prasanna KV, Chowdhury JR. To cystoscope or not to cystoscope patients with traumatic spinal cord injuries managed with indwelling urethral or suprapubic catheters? That is the question! Spinal Cord. 2014;52(1):49-53. Epub 2013/11/28. doi: 10.1038/sc.2013.119. PubMed PMID: 24276418.

CHAPTER 3

Autonomic dysreflexia and repeatability of cardiovascular changes during same session repeat urodynamic investigation in women with spinal cord injury

Walter M*, Knüpfer SC*, Leitner L, Mehnert U, Schubert M, Curt A, Kessler TM
World J Urol. 2016 Mar;34(3):391-7. (*Equal contribution, i.e. shared-first authorship)

ABSTRACT

Purpose

To investigate autonomic dysreflexia (AD) and repeatability of cardiovascular changes during same session repeat urodynamic investigation (UDI) in women with spinal cord injury (SCI).

Methods

Prospective investigation of 33 consecutive women with suprasacral SCI suffering from neurogenic lower urinary tract dysfunction (NLUTD) undergoing same session repeat UDI and synchronous continuous cardiovascular monitoring (systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR)). UDIs were performed according to the International Continence Society guidelines. AD was defined according to the International Standards to document remaining Autonomic Function after SCI. Neurological level of SCI was determined using the American Spinal Injury Association impairment scale.

Results

Mean age and duration since SCI of the 33 women were 58±19 and 6±11 years, respectively. Overall AD incidence was 73% (24/33) and 19 of the 33 women (58%) showed AD in both UDIs. The repeatability of detecting AD between the 2 same session UDIs was good (k=0.67, 95% CI 0.4 to 0.94). When applying the Bland and Altman method, wide 95% limits of agreement for differences in same session SBP, DBP and HR indicated poor repeatability. There was a significant increase in SBP ($p<0.001$) and DBP ($p<0.001$) and a significant decrease in HR ($p=0.007$) in patients with compared to those without AD.

Conclusions

In all women with NLUTD due to suprasacral SCI, we strongly recommend continuous cardiovascular monitoring during UDI and repeat measurements considering the high incidence of AD, the relevant risks involved with sudden hypertension and the poor repeatability of cardiovascular monitoring.

INTRODUCTION

Spinal cord injury (SCI) is a life-changing and sometime life-threatening event with an incidence rate between 40 to 80 new cases per million people per year, that is, between 250'000 to 500'000 people become injured worldwide per year [1]. Primary consequence of SCI is the impairment or complete loss of motor and sensory function [2]. Patients also suffer from secondary complications, such as disorders of mental health [3], metabolic [4] and cardiovascular system [5], which is particularly influenced by the autonomic nervous system (ANS) often affected by SCI [5]. Dysfunction of the ANS, especially autonomic dysreflexia (AD) [6], has become a very intriguing field of interest for SCI research over last two decades [7]. AD occurs in subjects with SCI as a response to a stimulus from below the level of lesion leading to an imbalanced reflex sympathetic discharge and is characterized as a sudden increase of the systolic blood pressure (SBP) [8]. AD can be elicited by noxious (e.g., pain) and non-noxious stimuli (e.g., bladder filling) from below the level of injury [9]. AD may be accompanied by heart rate changes and/or a variety of symptoms: (1) in general (throbbing headache, blurred vision and a feeling of anxiety), (2) above the lesion level (profound perspiration, piloerection, warm skin and flushing due to vasodilation), and/or (3) below the lesion level (pale and cold skin due to vasoconstriction) [10, 11]. However, patients might not be aware of being at risk [12] as AD can occur asymptomatically, also known as silent AD, i.e. without any clinical symptoms. The extent of SBP elevation as a result from a stimulus is gradual [13]. Therefore, AD can range from a mild reaction to life-threatening situation [13]. Hence, many patients might experience multiple episodes of AD each day, since bladder filling takes place naturally several times a day. Neurogenic lower urinary tract dysfunction (NLUTD) is a common consequence after SCI [14]. Urodynamic investigation (UDI) is the gold standard to evaluate lower urinary tract function and same session repeat measurement is crucial for clinical decision-making [15,

16]. However, UDI may elicit AD. Episodic blood pressure measurement has often been used during UDI to record AD [13, 17-19] but although continuous cardiovascular monitoring seems more accurate since it may reveal even short episodes of AD, only one retrospective study [9] is available. Moreover, repeatability of cardiovascular changes during same session repeat UDI is unknown and there is a complete lack of women-specific AD data. Therefore, we aimed to investigate AD and cardiovascular changes during same session repeat UDI in women with suprasacral SCI using continuous cardiovascular monitoring.

PATIENTS AND METHODS

Patients

From June 2011 and April 2013, a consecutive series of 33 women with suprasacral SCI suffering from NLUTD who underwent same session repeat UDI and synchronous continuous cardiovascular monitoring was prospectively investigated at our institution. Study exclusion criteria were pregnancy, breastfeeding, age less than 18 years and urinary tract infection. Patients were asked to empty their bowels prior to UDI. Patients did not have any pressure ulcer at time when UDI was performed. This study was approved by the local ethics committee (Kantonale Ethikkommision Zürich). All patients gave written informed consent according to the Helsinki II declaration.

Measurements

The neurological level and completeness of SCI, i.e. motor and sensory impairment, was determined using the American Spinal Injury Association impairment scale (AIS) according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) [20]. UDI was performed according to Good Urodynamic Practices recommended by the International Continence Society (ICS) [21, 22]. Patients were investigated in a sitting position,

whenever possible. A 7 French transurethral latex free single use air-charged catheter (TDOC-7FD, T-DOC Company, Wilmington, DE, USA) was used. The bladder was filled retrograde with a 37 degrees centigrade mixture of 0.9% sodium chloride solution and contrast medium. UDI was performed using a multichannel urodynamic system (Sedia®, Givisiez, Switzerland). Several parameters were assessed during filling cystometry including infusion speed, maximum cystometric capacity (MCC) and presence or absence of detrusor overactivity (DO). SBP, diastolic blood pressure (DBP) and heart rate (HR) were continuously recorded 'beat to beat' (Finometer® PRO, Finapres Medical Systems (FMS), Amsterdam, The Netherlands) synchronized to the ongoing UDI. AD was defined according to the International Standards to document remaining Autonomic Function after SCI (ISAFSCI) as an increase in SBP ≥ 20mmHg from baseline [23]. In case of clinical signs of AD, UDI was stopped immediately and the bladder was emptied.

Outcome measures

The outcome measures were AD and cardiovascular changes during same session repeat UDI.

Statistical analysis

Data distribution was tested by Q-Q plots. Data were approximately normally distributed and are presented as mean ± standard deviation (SD). Mean values and frequencies were compared between patients with and without AD using the unpaired t test (age, duration since SCI, infusion speed, MCC, SBP, DBP and HR) and the Fisher's exact test (bladder management, presence of DO, stage of injury, type of plegia, completeness of injury and lesion). The k statistic was used to investigate agreement of the presence or absence of AD between the 2 UDIs. Agreement between quantitative cardiovascular parameters (SBP, DBP and HR), was assessed applying the Bland and Altman 95%

limits of agreement, as estimated by the mean difference ± 1.96 SD of the differences, which provided an interval within which 95% of the differences between the 2 UDIs were expected to lie [24]. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL., USA) with $p<0.05$ considered statistically significant.

RESULTS

Of the 33 women with suprasacral SCI enrolled, mean age and mean duration since SCI were 58±19 and 6±11 years, respectively. Overall incidence of AD was 73% (24/33). Nineteen of the 33 women (58%) showed AD in both UDIs, two and three only in UDI 1 and 2, respectively. Thus, AD was found in 21 (64%) versus 22 (67%) of the 33 patients at UDI 1 versus 2. The repeatability of detecting AD between the 2 same session UDIs was good (k=0.67, 95% CI 0.4-0.94). When applying the Bland and Altman method, there were wide 95% limits of agreement for differences in same session SBP, DBP and HR indicating poor repeatability (Figure 1). Comparing women with and without AD (Table 1), there were no significant differences regarding age, duration after SCI, stage of injury (acute vs. chronic), type of plegia (tetraplegic vs. paraplegic), completeness of injury (motor and sensory complete SCI, i.e. AIS A vs. incomplete SCI, i.e. AIS B-D), lesion level (at or above T6 vs. below T6), infusion speed, MCC or presence of DO. In addition, we found a significant increase in SBP ($p<0.001$) and DBP ($p<0.001$) and a significant decrease in HR ($p=0.007$) in patients with AD compared to those without AD. Comparisons within the group of women with AD regarding the stage of injury, type of plegia, completeness of injury and lesion level are shown in Table 2. Symptomatic AD (8 of 24 patients (33%)) was more frequent in women with complete SCI (AIS A: 3/7 (42%) vs. AIS B-D: 5/17 (27%), $p=0.65$) but the differences were statistically not significant. In addition, the distribution of the lesion

level, i.e. ≤T6 or >T6, was almost equal in women with complete or incomplete SCI (AIS A: 4/7 (57%) vs. AIS B-D: 10/17 (59%), p=0.99).

Table 1 – Differences between patients with and without AD - Baseline characteristics, urodynamic investigation and cardiovascular changes.

Baseline characteristics	AD (n=24)	no AD (n=9)	p Value
Age in years (± SD)	58 (18)	56 (22)	0.71
Time after SCI in years (± SD)	5 (10)	10 (14)	0.24
*Stage of SCI**			
Acute / chronic	11/13	4/5	0.99
Type of plegia			
Tetraplegic / paraplegic	10/14	2/7	0.43
Completeness of lesion			
AIS A (complete) / AIS B-D (incomplete)	7/17	2/7	0.99
AIS B	2	2	
AIS C	4	1	
AIS D	11	4	
Level of lesion			
≤T6 / >T6	14/10	3/6	0.26
Cervical	10	2	
Thoracic	13	5	
T1-T6	4	1	
T7-T12	9	4	
Lumbar (L1)	1	2	
Bladder management			0.99
Spontaneous micturition	3	4	
ISC	6	1	
Indwelling catheters	15	4	
Transurethral	9	3	
Suprapubic	6	1	
Urodynamic investigation			
Infusion speed in mL/min (± SD)	31 (12)	32 (13)	0.81
MCC in mL (± SD)	575 (240)	465 (255)	0.25
DO (presence / absence)	19/5	6/3	0.65
Cardiovascular changes (Δ)			
SBP in mmHg (± SD)	43 (15)	-1 (6)	<0.001
DBP in mmHg (± SD)	17 (12)	-3 (8)	<0.001
HR in beats/min (± SD)	-7 (9)	3 (8)	0.007

* SCI defined as 'acute' upon 300 days since injury and 'chronic' after 300 days according to the European Mulitcenter Study about Spinal Cord injury (EMSCI, www.emsci.org). AD, Autonomic dysreflexia; AIS, American spinal injury association impairment scale; DBP, Diastolic blood pressure; DO, Detrusor overactivity; HR, Heart rate; ISC, Intermittent self-catheterisation; MCC, Maximum cystometric capacity; SBP, Systolic blood pressure; SCI, Spinal cord injury.

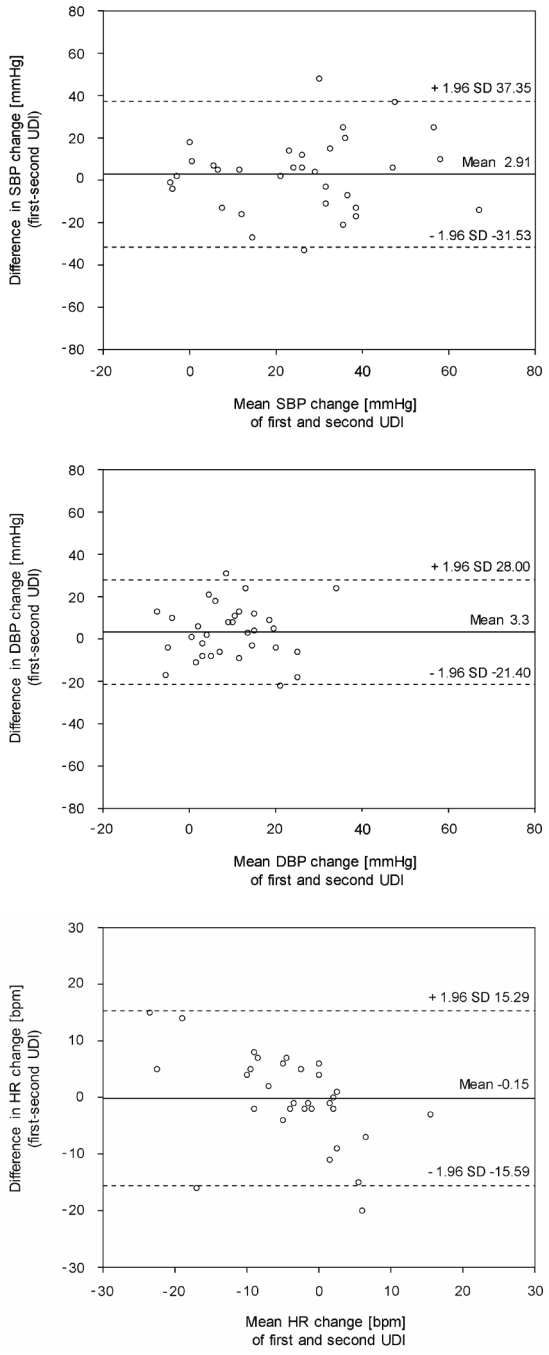

Figure 1 – Bland & Altman for changes in SBP (top), DBP (middle) and HR (bottom).
DBP, Diastolic blood pressure; HR, Heart rate; SBP, Systolic blood pressure; UDI, Urodynamic investigation.

Table 2 – Distribution of the duration of SCI, type of plegia, completeness and level of lesion on baseline characteristics, urodynamic investigation and cardiovascular changes in patients with AD.

	Stage of injury			Type of plegia			Completeness of SCI			Level of lesion		
	Acute (n= 11)	Chronic (n= 13)	p Value	tSCI (n= 10)	pSCI (n = 14)	p Value	AIS A (n =7)	AIS B-D (n = 17)	p Value	≤T6 (n =14)	>T6 (n = 10)	p Value
Baseline characteristics												
Age in years	66 (15)	52 (20)	0.08	63 (15)	55 (21)	0.3	43 (14)	65 (16)	0.006	60 (16)	56 (22)	0.65
Time after SCI in years	0	9 (12)	0.016	3 (7)	7 (11)	0.33	9 (14)	3 (7)	0.29	7 (12)	3 (3)	0.28
Urodynamic investiagtion												
Infusion speed in mL/min	30 (9)	31 (14)	0.71	29 (10)	32 (13)	0.55	35 (16)	29 (10)	0.24	31 (10)	30 (14)	0.85
MCC in mL	515 (215)	630 (250)	0.25	545 (275)	600 (215)	0.58	695 (205)	525 (240)	0.11	605 (265)	535 (200)	0.52
Cardiovascular changes (Δ)												
SBP in mmHg	46 (15	40 (15)	0.3	42 (11)	43 (17)	0.94	48 (17)	40 (14)	0.28	44 (16)	41 (14)	0.64
DBP in mmHg	17 (11)	17 (14)	0.96	16 (14)	18 (11)	0.67	24 (13)	14 (11)	0.07	16 (13)	18 (11)	0.81
HR in beats/min	-4 (4)	-10 (11)	0.12	-4 (6)	-9 (10)	0.19	-16 (7)	-3 (7)	0.001	-6 (8)	-9 (10)	0.44

All values are presented as mean and standard deviation (± SD)
AIS, American spinal injury association impairment scale; DBP, Diastolic blood pressure; HR, Heart rate; MCC, Maximum cystometric capacity; pSCI, Paraplegic SCI; SBP, Systolic blood pressure; SCI, Spinal cord injury; tSCI, Tetraplegic SCI.

DISCUSSION

In women with NLUTD due to suprasacral SCI, in accordance to literature we found a high incidence of AD of more than 70%. The repeatability of AD in same session UDIs was good but cardiovascular monitoring parameters showed insufficient agreement. Thus, we strongly recommend continuous cardiovascular monitoring during UDI and repeat measurements considering the high incidence of AD, the relevant risks involved with sudden hypertension and the poor repeatability of cardiovascular monitoring. UDI is crucial to assess NLUTD [16] but it might elicit AD constituting a potential risk for the patient [25-27]. Therefore, patients with AD have to be identified to avoid a severe and potentially life-threatening situation during UDI. Cardiovascular changes during UDI such as in SBP, leading to AD, have been reported before [9, 13, 17-19]. Retrospective studies [9, 19] presented higher AD incidence rates (57-63% versus 37-42%) than observational or case control studies [13, 17, 18]. In contrast to all other studies [9, 13, 17-19], we performed same session repeat UDI instead of only one UDI. Although the repeatability of AD in same session repeat UDI was good, 15% (5/33) of our patients showed AD in one UDI only so that the overall incidence of AD is generally underestimated. In addition, we included patients with lesion levels at L1 and above, similar to Huang et al. [17] while others only included those with a lesion level at T6 and above [9, 13, 18, 19]. In fact, 40% (10/24) of all women experiencing AD during UDI had a lesion below T6. Crucially, a noxious or non-noxious triggering factor from below the lesion level can lead to AD. This reaction is mediated by arising afferent synaptic input to sympathetic pre-ganglionic neurons [28], leading to a vasoconstriction below the lesion level (skin, muscular, splanchnic vessels) [8]. The pre-ganglionic sympathetic neurons originate from T1 to L2 [29] and supply various regions. This includes the heart (T1-T5) and stomach, liver, small and large intestines and abdominal blood vessels through the celiac (T5-T11) and superior mesenteric ganglion

(T10-T12). Furthermore, the descending colon, sigmoid colon, rectum, urinary bladder, reproductive organs and associated blood vessel through the inferior mesenteric ganglion (L1-L2) [30, 31]. That is why even in patients with a lower lesion level, that is, between L2 to T6, an AD might occur. Indeed, in the present study 42% (10/24) of the patients with AD had a lesion below T6, although the magnitude of cardiovascular changes has been reported to be smaller than in patients with a lesion at or above T6 [32] what is also in line with our findings. In contrast to other studies [13, 17-19], we used continuous cardiovascular monitoring likewise to Liu et al. [9]. This technique allows stepless documentation of temporal progression of SBP, DBP and HR by 'beat to beat' recordings during the full course of bladder filling providing a higher precision to detect AD during UDI and the patients are not bothered by inflating the cuff for episodic cardiovascular monitoring what might irritate the patient and thereby indirectly influence bladder function. When comparing within the group of women with AD, patients with complete SCI had a significantly greater decrease in HR than those with an incomplete lesion. The baroreflex is responsible for maintaining the blood pressure to supply the brain with oxygen sufficiently. When AD is elicited, the sudden rise in SBP is detected by baroreceptors. Thereafter, the ANS tries to respond in two ways, i.e. via sympathetic and parasympathetic pathways [33]. The sympathetic efferent input from the lower brainstem to the heart is abolished in complete cervical lesion, only partially preserved in a high thoracic lesion (T1-T5) and unrestricted in a lesion below T6 [33, 34]. In incomplete cervical and high thoracic lesions, inhibitory sympathetic efferent efficacy is limited depending on the disruption of its pathways, i.e. magnitude of SCI. In this way, the control of blood vessels, by means dissolving the vasoconstriction below the lesion level, is reduced [33]. Therefore, the increase in SBP persists or is barely reduced. With an insufficient sympathetic inhibition, the ANS relies on the parasympathetic pathway to reduce the SBP. The parasympathetic outflow to the

heart is maintained after SCI since it runs through the vagal nerve (cranial nerve X) and not through the spinal cord [34]. Due to the sympathetically induced vasoconstriction below the lesion, the only way to counterattack the resulting SBP increase is to slow down the heart rate. However, this response could result in a vicious circle, i.e. brain hypoxia due to a severe bradycardia or even cardiac arrest, if the triggering stimulus is not removed, i.e. emptying of the bladder. When such deterioration takes place, a patient is most likely to show symptoms. This is in line with our observation as in the group with a complete lesion a higher percentage of patients had a symptomatic AD. The distribution of the lesion level, i.e. ≤T6 or >T6, was almost equal between both groups. Subsequently, the greater heart rate decrease in our AD patients with AIS A is most likely caused by parasympathetic input as inhibitory descending sympathetic signals are reduced or even abolished, therefore insufficient to reduce the SBP elevation. Good repeatability of detecting AD in same session repeat UDIs might imply that one UDI would be sufficient. However, wide Bland and Altman 95% limits of agreement indicate poor repeatability for continuous cardiovascular monitoring parameters, i.e. SBP, DBP and HR. The wide range of cardiovascular monitoring parameters is a reflection of the intra-subject variability of responses to bladder filling, rather than technical factors. This is highly relevant in daily clinical practice. For instance, 95% limits of agreement for differences between same session repeat UDIs in SPB of -32 to 37 mmHg is not acceptable. UDI 1 may reveal a SBP change of 5 mmHg, which would be within the safe limit not requiring any treatment. However, based on our findings UDI 2 may lead to completely different results, i.e. to a SBP change of 70 mmHg. This would indicate severe AD and puts the patient at risk for a cerebrovascular accident so that appropriate treatment to decrease the SBP is mandatory. Importantly, AD can also be silent and might occur in an asymptomatic patient. Although to our knowledge this is

the first prospective study investigating AD and repeatability of cardiovascular changes during same session UDI in women with suprasacral SCI suffering from NLUTD, some limitations should be addressed. Same session repeat UDI may limit the outcome as physiological changes could be biased in the second UDI when performed immediately after the first UDI [35]. However, common conditions such as urinary tract infection, neurological status fluctuations and medication can influence lower urinary tract function and consequently UDI and cardiovascular monitoring parameters so that longer intervals between investigations could provide misleading results due to these confounding factors. Considering that we only studied women with NLUTD it is unclear whether the results could also be extrapolated to SCI patients without NLTUD. Although reporting about the largest cohort of women with NLUTD suffering from suprasacral SCI, the influence of the duration of injury, completeness of SCI, lesion level and type of plegia on cardiovascular changes might be more distinctive in larger patient cohorts or in those with a more balanced distribution between patients with and without AD. Moreover, it would be of great interest to continuously monitor cardiovascular parameters during long-term ambulatory urodynamics to further investigate the cardiovascular risk profile in patients with SCI and to compare these findings with same session repeat UDIs.

CONCLUSSION

With an overall incidence of AD in more than 70% of our patients and considering the risks involved with sudden hypertension such as seizures, strokes [27] and even death due to myocardial infarction [26] or intracerebral hemorrhage [25], we strongly recommend to search for (symptomatic and asymptomatic) AD using continuous cardiovascular monitoring during UDI in all women with suprasacral SCI suffering from NLUTD and to perform same session repeat UDI to capture the extent of cardiovascular changes. If AD occurs during examination, stopping UDI and immediate emptying of the bladder is mandatory to avoid a life-threatening situation in daily clinical practice. In patients with AD, optimizing NLUTD management is essential and if AD persists, continuous pharmacological treatment might be considered to abort AD but high-evidence level studies are lacking and well-designed prospective investigations on this topic are highly warranted.

Conflict of interest:
None.

Ethical statement:
This study was approved by the local ethics committee (Kantonale Ethikkommision Zürich) and all patients gave written informed consent according to the Helsinki II declaration.

Author Contributions:
Conceived and designed the study: MW, SCK, MS, AC, TMK
Performed investigation: MW, SCK, LL, UM
Analyzed the data: MW, SCK
Statistical analysis: MW, SCK
Contributed to the writing of the manuscript: MW, SCK, TMK
Critical review of the manuscript: LL, UM, MS, AC, TMK

REFERENCES

1. ISCOS, W. International perspectives on spinal cord injury. 2013 2014/07/28]; Available from: http://apps.who.int/iris/bitstream/10665/94190/1/9789241564663_eng.pdf.
2. Furlan, J.C., et al., Assessment of impairment in patients with acute traumatic spinal cord injury: a systematic review of the literature. J Neurotrauma, 2011. 28(8): p. 1445-77.
3. Migliorini, C., L. Callaway, and P. New, Preliminary investigation into subjective well-being, mental health, resilience, and spinal cord injury. J Spinal Cord Med, 2013. 36(6): p. 660-5.
4. Giangregorio, L. and N. McCartney, Bone loss and muscle atrophy in spinal cord injury: epidemiology, fracture prediction, and rehabilitation strategies. J Spinal Cord Med, 2006. 29(5): p. 489-500.
5. Furlan, J.C. and M.G. Fehlings, Cardiovascular complications after acute spinal cord injury: pathophysiology, diagnosis, and management. Neurosurg Focus, 2008. 25(5): p. E13.
6. Krassioukov, A.V., et al., Assessment of autonomic dysfunction following spinal cord injury: rationale for additions to International Standards for Neurological Assessment. J Rehabil Res Dev, 2007. 44(1): p. 103-12.
7. Weaver, L.C., et al., Disordered cardiovascular control after spinal cord injury. Handb Clin Neurol, 2012. 109: p. 213-33.
8. Karlsson, A.K., Autonomic dysreflexia. Spinal Cord, 1999. 37(6): p. 383-91.
9. Liu, N., et al., Autonomic dysreflexia severity during urodynamics and cystoscopy in individuals with spinal cord injury. Spinal Cord, 2013. 51(11): p. 863-7.
10. Furusawa, K., et al., Incidence of symptomatic autonomic dysreflexia varies according to the bowel and bladder management techniques in patients with spinal cord injury. Spinal Cord, 2011. 49(1): p. 49-54.
11. Karlsson, A.K., Autonomic dysfunction in spinal cord injury: clinical presentation of symptoms and signs. Prog Brain Res, 2006. 152: p. 1-8.
12. Linsenmeyer, T.A., D.I. Campagnolo, and I.H. Chou, Silent Autonomic Dysreflexia During Voiding in Men with Spinal Cord Injuries. The Journal of Urology, 1996. 155(2): p. 519-522.
13. Giannantoni, A., et al., Autonomic dysreflexia during urodynamics. Spinal Cord, 1998. 36(11): p. 756-60.
14. Ruffion, A., et al., Systematic Review of the Epidemiology of Urinary Incontinence and Detrusor Overactivity among Patients with Neurogenic Overactive Bladder. Neuroepidemiology, 2013. 41(3-4): p. 146-155.
15. Linsenmeyer, T.A. and M.A. Linsenmeyer, Impact of annual urodynamic evaluations on guiding bladder management in individuals with spinal cord injuries. J Spinal Cord Med, 2013. 36(5): p. 420-6.
16. Pannek, J., et al. EAU guidelines on neuro-urology. 2014 2014/07/22]; Available from: http://www.uroweb.org/gls/pdf/21 Neuro-Urology_LR.pdf.
17. Huang, Y.H., et al., Autonomic dysreflexia during urodynamic examinations in patients with suprasacral spinal cord injury. Arch Phys Med Rehabil, 2011. 92(9): p. 1450-4.
18. Huang, Y.H., et al., Blood pressure and age associated with silent autonomic dysreflexia during urodynamic examinations in patients with spinal cord injury. Spinal Cord, 2013. 51(5): p. 401-5.
19. Sayilir, S., M. Ersoz, and S. Yalcin, Comparison of urodynamic findings in patients with upper and lower cervical spinal cord injury. Spinal Cord, 2013. 51(10): p. 780-3.
20. Kirshblum, S. and W. Waring, 3rd, Updates for the International Standards for Neurological Classification of Spinal Cord Injury. Phys Med Rehabil Clin N Am, 2014. 25(3): p. 505-17, vii.
21. Abrams, P., et al., The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn, 2002. 21(2): p. 167-78.
22. Schafer, W., et al., Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn, 2002. 21(3): p. 261-74.
23. Krassioukov, A., et al., International standards to document remaining autonomic function after spinal cord injury. J Spinal Cord Med, 2012. 35(4): p. 201-10.
24. Linsenmeyer, T.A., D.I. Campagnolo, and I.H. Chou, Silent autonomic dysreflexia during voiding in men with spinal cord injuries. The Journal of Urology, 1996. 155(2): p. 519-22.

25. Dolinak, D. and E. Balraj, Autonomic dysreflexia and sudden death in people with traumatic spinal cord injury. Am J Forensic Med Pathol, 2007. 28(2): p. 95-8.
26. Ho, C.P. and A.V. Krassioukov, Autonomic dysreflexia and myocardial ischemia. Spinal Cord, 2010. 48(9): p. 714-5.
27. Wan, D. and A.V. Krassioukov, Life-threatening outcomes associated with autonomic dysreflexia: a clinical review. J Spinal Cord Med, 2014. 37(1): p. 2-10.
28. Llewellyn-Smith, I.J., L.C. Weaver, and J.R. Keast, Effects of spinal cord injury on synaptic inputs to sympathetic preganglionic neurons. Prog Brain Res, 2006. 152: p. 11-26.
29. FitzGerald, M.J.T., G. Gruener, and E. Mtui, Clinical Neuroanatomy and Neuroscience. 6th Edition ed. 2012: Saunders Elsevier.
30. Trepel, M., Neuroanatomie. 5. Auflage ed. 2012: Urban & Fischer, Elsevier.
31. Schünke, M., et al., Atlas of anatomy: neck and internal organs. 1st ed. 2010: Thieme.
32. Moeller, B.A., Jr. and D. Scheinberg, Autonomic dysreflexia in injuries below the sixth thoracic segment. JAMA, 1973. 224(9): p. 1295.
33. Phillips, A.A., et al., Baroreflex function after spinal cord injury. J Neurotrauma, 2012. 29(15): p. 2431-45.
34. Krassioukov, A., Autonomic function following cervical spinal cord injury. Respir Physiol Neurobiol, 2009. 169(2): p. 157-64.
35. Rahmanou, P., et al., Reliability testing of urodynamics, pressure flow studies and cough leak point pressure in women with urodynamic stress incontinence with and without detrusor overactivity. Int Urogynecol J Pelvic Floor Dysfunct, 2008. 19(7): p. 933-8.

CHAPTER 4

Urodynamic Investigation: A Valid Tool to Define Normal Lower Urinary Tract Function?

Leitner L*, **Walter M***, Sammer U, Knüpfer SC, Mehnert U, Kessler TM
PLoS One. 2016 Oct 13;11(10):e0163847. (*Equal contribution, i.e. shared-first authorship)

ABSTRACT

Objectives

To evaluate whether urodynamic investigation (UDI), the gold standard to assess refractory lower urinary tract symptoms (LUTS), is appropriate to select healthy volunteers with apparent normal lower urinary tract function as control subjects for comparative studies.

Subjects and Methods

42 healthy subjects (22 women, mean age 32±10 years; 20 men, mean age 37±12 years) without LUTS were included into this prospective single-centre cohort study. All subjects recorded a 3-day bladder diary, completed validated questionnaires regarding LUTS, and underwent neuro-urological assessment as well as free uroflowmetry. UDI was performed according to "Good Urodynamic Practice" recommended by the International Continence Society, but using an air-charged instead of a water-filled catheter, and evaluated by a blinded investigator.

Results

All 3-day bladder diaries, LUTS questionnaires, neuro-urological assessments and free uroflowmetries were within normal limits. Overall, UDI revealed pathological findings in 71% (30/42): Detrusor overactivity was detected in 14% (3/22) and 30% (6/20), post void residual >100mL in 14% (3/22) and 25% (5/20), bladder outlet obstruction in 9% (2/22) and 20% (4/20) and detrusor sphincter dyssynergia in 77% (17/22) and 65% (13/20) of our women and men, respectively.

Repeatability of detrusor overactivity (κ=0.78, 95% CI: 0.54-1.02) and detrusor sphincter dyssynergia (κ=0.77, 95% CI: 0.55-0.98) showed substantial agreement between both UDIs. All other assessed urodynamic parameters had wide 95% limits of agreement for differences in the parameters indicating poor repeatability.

Conclusions

More than 70% of our healthy subjects showed pathological urodynamic findings. Although UDI is the gold standard to assess refractory LUTS, it seems not to be applicable in healthy subjects to define normal lower urinary tract function. Therefore, we do not recommend using UDI to select healthy control subjects.

INTRODUCTION

Urodynamic investigation (UDI) is the gold standard to assess refractory lower urinary tract symptoms (LUTS), i.e. to detect lower urinary tract dysfunction (LUTD) [1]. However, patients with LUTS do not necessarily show pathological urodynamic findings [2]. In general, knowledge of what constitutes a normal organ function is based on investigation of healthy subjects. However, previous UDIs in symptom-free healthy subjects showed a wide range of urodynamic findings including pathological results [3-6]. Thus, the value of UDIs in asymptomatic subjects, i.e. subjects without LUTS, is largely unknown. The aim of the present study was to assess whether UDI (filling cystometry and pressure-flow study (PFS)), is appropriate to select healthy volunteers, with apparent normal lower urinary tract function, as control subjects for comparative studies with patients suffering from LUTD.

SUBJECTS AND METHODS

Ethics

This prospective cohort study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346), is registered at ClinicalTrials.gov (NCT01768910) and was performed at a single university spinal cord injury centre.

Subjects

Healthy subjects (22 women, mean age 32±10 years; 20 men, mean age 37±12 years) were recruited by public advertisement. Inclusion criteria were: (1) age between 18 and 55 years, (2) no LUTS, (3) no history of previous lower urinary tract surgery, (4) no history of previous or current neurological diseases, including diabetic neuropathy, and (5) no current medication. The flowchart of all visits can be found in Figure 1. During screening visit, healthy

subjects were informed about the study details, i.e. aims, methods, possible risks, and side effects. After obtaining written informed consent, the following data was acquired: 3-day bladder diary and validated, standardised questionnaires (in German language) regarding LUTS, quality of life (QoL), mental status and depression, i.e. International Consultation on Incontinence Modular Questionnaire modules (ICIQ-FUTS, ICIQ-MLUTS) [7], the Overactive Bladder Questionnaire short-form (OAB-q SF) [8], the Mini Mental Status Examination [9], and the Hospital Anxiety and Depression Scale [10]. The scores for ICIQ-FLUTS were derived according to the publication by Brookes et al. [11] and subdivided in filling, voiding, and incontinence symptoms. For ICIQ-MLUTS, the recommendation by the ICS was followed subdividing the scores in voiding and incontinence symptoms [12]. Neuro-urological assessment [13] included medical history, examination of urogenital sensation, bulbocavernosus reflex (performed by squeezing the clitoris or glans during digito-rectal examination and pelvic floor electromyography (EMG)), anal reflex, anal sphincter tone, and anal squeeze response. Free uroflowmetry was performed (uroflowmetry has been repeated in case of voided volume <200 mL) and post void residual (PVR) was measured by ultrasound. Prior to invasive UDI, urinary tract infection (UTI) and pregnancy have been excluded. Subjects were instructed to reduce their fluid intake two hours before the UDI. As a requirement of the local ethics committee, healthy subjects obtained a minimal financial compensation.

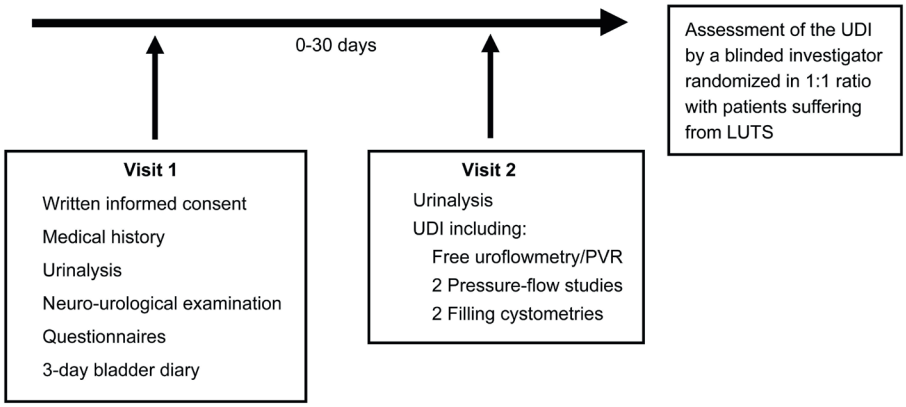

Figure 1 – Flow chart of all visits
On subjects' request UDI could be performed at the first visit, if the 3-day bladder diary was already available and all further inclusion criteria were met.
LUTS = Lower urinary tract symptoms, PVR = post void residual, UDI = Urodynamic investigation.

Urodynamic investigation

UDIs were performed by two examiners (LL and MW) and comprised same session repeat filling cystometry and PFS. UDI was performed according to "Good Urodynamic Practice" recommended by the ICS [1] but using an air-charged instead of a water-filled catheter. No minimal amplitude threshold to define detrusor overactivity was set. Bladder outlet obstruction was defined according to Defreitas et al. [14] as maximum flow rate (Qmax) <12mL/s and detrusor pressure (pDet) Qmax >25cmH2O or according to the Abrams-Griffiths nomogram [15] for women and men, respectively. According to the recommendation of the ICS [16] involuntary contraction of the urethral and/or periurethral striated muscle, i.e. elevated EMG signal, during detrusor contraction, was defined as detrusor sphincter dyssynergia. An air-charged, three-way 7 Fr transurethral catheter (T-DOC-7FD, Laborie Medical Technologies, Ontario, Canada) and a common rectal catheter for simultaneous measurements of vesical and abdominal pressure were used. Both catheters were attached to the body surface, using tape to assure correct placement during the entire UDI. The vesical catheter was attached close to the urethral meatus

to avoid expelling during UDI. Surface electrodes (Neotrode II, ConMed Cooperation, New York, USA) were placed bilaterally around the external anal sphincter to record activity of the pelvic floor, i.e. EMG. For comparison reasons between women and men, free uroflowmetry, filling cystometry, and PFS were performed in sitting position in all subjects, even if standing was the indicated preferred voiding position in some male volunteers. During invasive UDI intravesical pressure, intraabdominal pressure, pDet, pelvic floor EMG as well as urinary flow (Q), and voided volume in the voiding phase, using Laborie Goby and Laborie Urocap IV system (Laborie Medical Technologies, Ontario, Canada), were continuously recorded. Prior to each measurement, proper placement and function of catheters and electrodes were assessed by cough, pelvic floor contraction and elicitation of the bulbocavernosus reflex. In addition, subjects were asked to cough during cystometry, i.e. after every 100 mL, to verify correct placement and function of catheters. Moreover, plausibility and quality control of clinical and urodynamic data was performed: Healthy volunteers reported their sensation during investigations and free uroflowmetry and flow patterns during PFS has been compared. Continuous bladder filling was carried out using body warm (37 °C) sterile saline at a filling speed of 30 mL per minute for filling cystometry. Subjects were asked to report the following sensations: first sensation of bladder filling (FSF), first desire to void (FDV) and strong desire to void (SDV) during bladder filling when perceived, the infused volume at each time point was acquired and correction for permanent urine self-production was made for maximum cystometric capacity (MCC). Permission to void was given, when SDV was reported and PFS was performed in privacy (i.e. no investigator in the room). After PFS, PVR was measured following bladder emptying by a single use 10 Fr transurethral catheter (LoFric Origo/Sense, Wellspect HealthCare, Mölndal, Sweden). MCC was defined as the sum of voided volume and PVR. If no micturition could be initiated, the whole catheterised volume was classified as PVR. Subjects were

blinded to UDI parameters and findings. UDIs were assessed by a blinded investigator (US), i.e. an experienced consultant in neuro-urology. The investigator had no knowledge of subjects' history and clinical examination. Gender only was unmasked to address the different definitions of bladder outlet obstruction. UDIs were presented to the investigator in a randomised order mixed with patients suffering from LUTS in a 1:1 ratio.

Statistical analysis

Data distribution was tested by Q-Q plots. Approximately normally distributed data (FSF, FDV, SDV, MCC, compliance, pDet max during voiding, pDet Qmax, and voided volume) were presented as mean ± standard deviation (SD), skewed data (pDet max during storage and PVR) as median and interquartile range. Comparing unrelated samples, i.e. women vs. men, the unpaired t test was used for approximately normally distributed data and the Mann-Whitney U test for skewed data, respectively. For comparison of unrelated binary data Fisher's exact test was used. Repeatability between quantitative urodynamic parameters was assessed by applying the Bland and Altman 95% limits of agreement [17]. The κ statistic was used to investigate agreement of the presence or absence of detrusor overactivity and detrusor sphincter dyssynergia between both UDIs. Statistical analyses were performed using IBM's Statistical Package for the Social Sciences (SPSS) V22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA) with $p<0.05$ considered statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics of the 42 subjects (22 women and 20 men, all Caucasians) are shown in Table 1. Medical history, 3-day bladder diaries, questionnaires regarding LUTS and QoL, neuro-urological assessment, free

uroflowmetry, PVR, and urine samples, were without pathological findings in all subjects.

Same session repeat urodynamic investigations

Urodynamic findings are shown in Table 2. Significant differences were found between genders for volumes at FSF, pDet Qmax and Qmax within first and second UDI as well as for maximum pDet during the first PFS within first UDI. Overall, UDI revealed pathological findings (Table 3, Figures 2 and 3) in 71% (30/42): Detrusor overactivity (p=0.18) was detected in 14% (3/22, 1 reported synchronous urgency) and 30% (6/20, 3 reported synchronous urgency), bladder outlet obstruction (p=0.29) in 9% (2/22) and 20% (4/20), detrusor sphincter dyssynergia (p=0.3) in 77% (17/22) and 65% (13/20), and PVR >100mL (p=0.29) in 14% (3/22) and 25% (5/20) of our women and men, respectively. Pathological findings were similar during both UDIs (p>0.05). Using the Bland and Altman method, there were wide 95% limits of agreement for differences in same session UDI parameters indicating poor repeatability (Figures 4 and 5). Detrusor overactivity and detrusor sphincter dyssynergia were found in 9 (21%) versus 8 (19%) and in 30 (71%) versus 30 (71%) of the 42 subjects in UDI 1 versus 2, respectively. The repeatability of detecting detrusor overactivity (κ=0.78, 95% CI 0.54-1.02) and detrusor sphincter dyssynergia (κ=0.77, 95% CI 0.55-0.98) showed substantial agreement between both UDIs.

Adverse events

An adverse event, as defined by the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines (E6) [18] and International Organization for Standardization (ISO, 14155) [19], did not occur.

Table 1 - Baseline characteristics.

Baseline characteristics	Women (n=22)	Men (n=20)	p Value
Age [year]	32 ±10	37 ±12	0.13
18-39 years	15/22 (68%)	12/20 (60%)	
40-55 years	7/22 (32%)	8/20 (40%)	
Weight [kg]	61 ± 7	76 ± 6	<0.01**/†
3-day bladder diary			
Micturition frequency per 24 hours	5.7 ± 1.2	5.9 ± 1.2	0.44†
Micturition volume per micturition [mL]	305 ± 110	305 ± 65	0.95†
Fluid intake per 24 hours [mL]	1920 ± 790	2230 ± 775	0.21†
Questionnaires			
ICIQ-FLUTS/MLUTS***			
Filling symptoms	1.1 ± 0.9	-	
Voiding symptoms	0.2 ± 0.4	3 ± 2.8	
Incontinence symptoms	0.3 ± 0.8	1.4 ± 2.5	
OAB-q SF			
Symptoms	6.9 ± 1.2	7.6 ± 1.6	0.18†
QoL	14.1 ± 0.9	13.9 ± 0.9	0.64†
HADS			
Anxiety	2.8 ± 2.2	1.4 ± 1.5	0.01**/†
Depression	1 ± 1.5	1.1 ± 1.5	0.92†
MMSE	29.6 ± 0.5	29.7 ± 0.6	0.45†
Neuro-urological examination			
Urogenital sensation (intact/impaired)	22/0	20/0	
Bulbocavernosus reflex (intact/impaired)	22/0	20/0	
Anal reflex (intact/impaired)	22/0	20/0	
Anal sphincter tone (intact/impaired)	22/0	22/0	
Anal squeeze response (intact/impaired)	22/0	20/0	
Free uroflowmetry (Voided volume > 200 mL)			
Maximum flow rate [mL/s]	29 ± 11	25 ± 6	0.12†
Post void residual [mL]*	0 (0-0)	0 (0-0)	0.18††

* Parameter with a skewed distribution (presented as median and interquartile range); all other parameters are approximately normally distributed (presented as mean ± standard deviation). ** Significant difference between gender using an unpaired t test. *** Due to the different scoring systems, female and male subjects have not been compared. † unpaired t test; †† Mann-Whitney test. ICIQ, International Consultation on Incontinence Modular Questionnaire; FLUTS, Female lower urinary tract symptoms; MLUTS, Male lower urinary tract symptoms; OAB-q SF, The Overactive Bladder Questionnaire short-form; QoL, Quality of life; HADS, Hospital Anxiety and Depression Scale; MMSE, Mini-Mental State Examination.

Table 2 - Urodynamic findings.

	First investigation			Second investigation		
Urodynamic parameter	Women	Men	p Value	Women	Men	p Value
Filling cystometry						
FSF [mL]	75 ± 75	130 ± 85	0.04**	70 ± 85	145 ± 95	0.01**
FDV [mL]	235 ± 110	215 ± 105	0.53	265 ± 115	235 ± 145	0.49
SDV [mL]	465 ± 155	435 ± 140	0.49	530 ± 165	450 ± 150	0.11
MCC [mL]	560 ± 185	500 ± 165	0.27	550 ± 160	500 ± 160	0.33
Compliance [mL/cmH2O]	268 ± 204	241 ± 183	0.66	300 ± 211	193 ± 195	0.1
pDet max during storage [cmH2O]*	4 (3-7)	6 (2-12)	0.46	5 (2-8)	6 (3-11)	0.26
Pressure flow study						
pDet max during voiding [cmH2O]	46 ± 28	65 ± 25	<0.03**	48 ± 24	62 ± 33	0.11
pDet Qmax [cmH2O]	29 ± 11	51 ± 16	<0.01**	32 ± 16	47 ± 20	<0.01**
Qmax [mL/s]	29 ± 17	19 ± 8	0.02**	28 ± 12	20 ± 8	0.03**
Voided volume [mL]	510 ± 255	445 ± 190	0.36	505 ± 185	450 ± 195	0.36
PVR [mL]*	0 (0-15)	15 (0-105)	0.14	0 (0-5)	0 (0-60)	0.21

FSF, First sensation of filling; FDV, First desire to void; SDV, Strong desire to void; MCC, Maximum cystometric capacity; pDet max, Maximum detrusor pressure; pDet Qmax, Detrusor pressure at maximum flow rate; Qmax, Maximum flow rate; PVR, Post void residual.
* Parameter with a skewed distribution (presented as median and interquartile range); all other parameters are approximately normally distributed (presented as mean ± standard deviation).
** Significant difference between gender using an unpaired t test.

Table 3 - Pathological findings during urodynamic investigations.

	First investigation		Second investigation	
Urodynamic parameter	Women	Men	Women	Men
Filling cystometry				
Detrusor overactivity	14% (3/22)	30% (6/20)	14% (3/22)	25% (5/20)
Detrusor overactivity incontinence	14% (3/22)	5% (1/20)	0	0
Compliance < 20 [mL/cmH2O]	5% (1/22)	0	0	0
Pressure flow study				
Micturition not possible	9% (2/22)	5% (1/20)	9% (2/22)	5% (1/20)
Obstructed*	9% (2/22)	10% (2/20)	9% (2/22)	20% (4/20)
Equivocal*	-	20% (4/20)	-	5% (1/20)
Detrusor sphincter dyssynergia	77% (17/22)	65% (13/20)	77% (17/22)	65% (13/20)
PVR >100 mL	14% (3/22)	25% (5/20)	5% (1/22)	20% (4/20)

No significant difference for appearance of pathological findings between gender was seen
* According to Defreitas et al. and the Abrams-Griffiths nomogram for women and men, respectively (due to the different definitions, female and male subjects have not been compared).

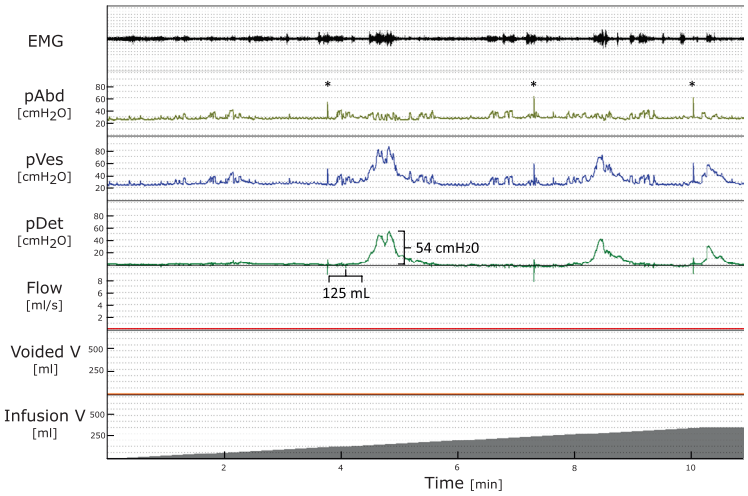

Figure 2 - Pathological findings during filling cystometry.
Filling cystometry (30 mL/min) of a 51 years old healthy man: A first detrusor overactivity occurs at 125 mL with maximum pDet of 54 cmH20 but no detrusor overactivity incontinence, the maximum cystometric capacity is 345 mL.

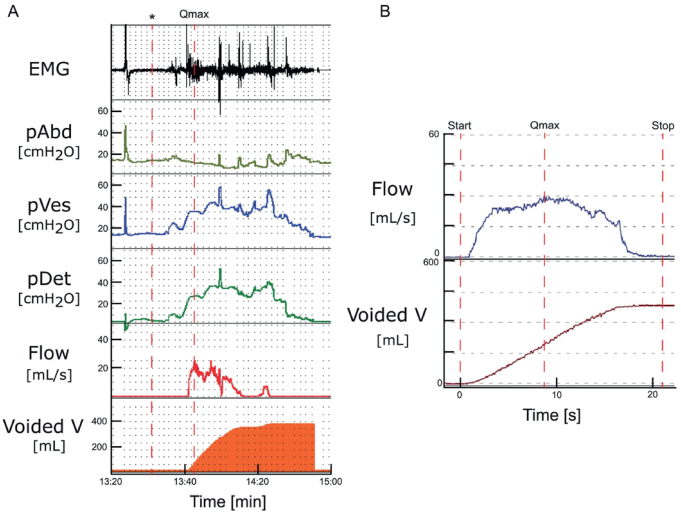

Figure 3 - Pathological findings during filling cystometry.
a) Pressure flow study of a 24 years old healthy woman: The first spike indicates a cough to evaluate correct catheter placement, thereafter permission to void (*) is obtained. The EMG signal during pressure flow study is elevated, the flow is interrupted; consequent spikes in the vesical and detrusor pressure can be seen. Maximum cystometric capacity/voided volume 395 mL, maximum pDet voiding 31 cmH20 at maximum flow rate 25 mL/s, no post void residual. b) The free uroflowmetry in the same subject reveals a normal flow-curve. Maximum flow rate 30 mL/s, voided volume 390 mL, no post void redidual.
EMG, Electromyography; pAbd, intraabdominal pressure; pVes, intravesical pressure; pDet, detrusor pressure; Qmax, maximum flow rate; V, volume. * Permission to void.

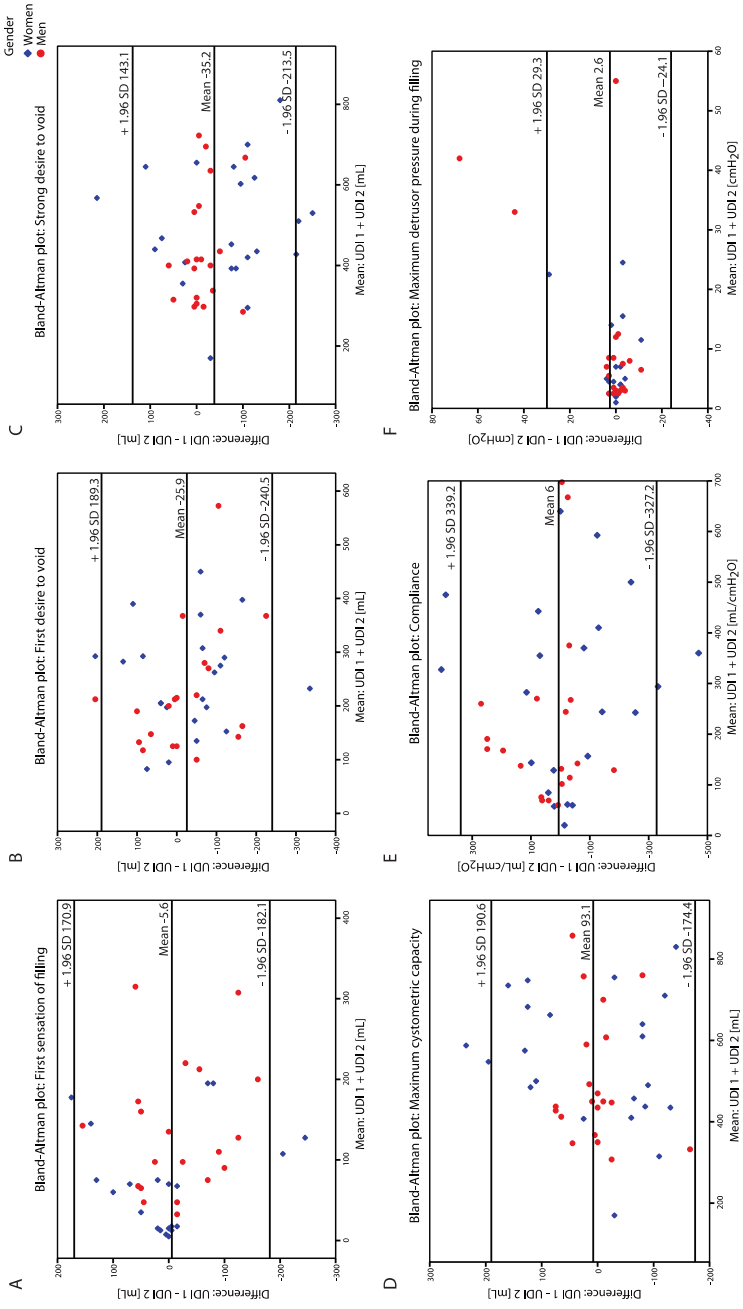

Figure 4 - Difference against mean plot filling cystometry.
Difference against mean plot for a) first sensation of filling, b) first desire to void, c) strong desire to void, d) maximum cystometric capacity, e) compliance, and f) maximum detrusor pressure during filling for UDI 1 vs. 2. Wide 95% limits of agreement reflect poor repeatability with unacceptable discrepancies between same session repeat UDIs. UDI, Urodynamic investigation.

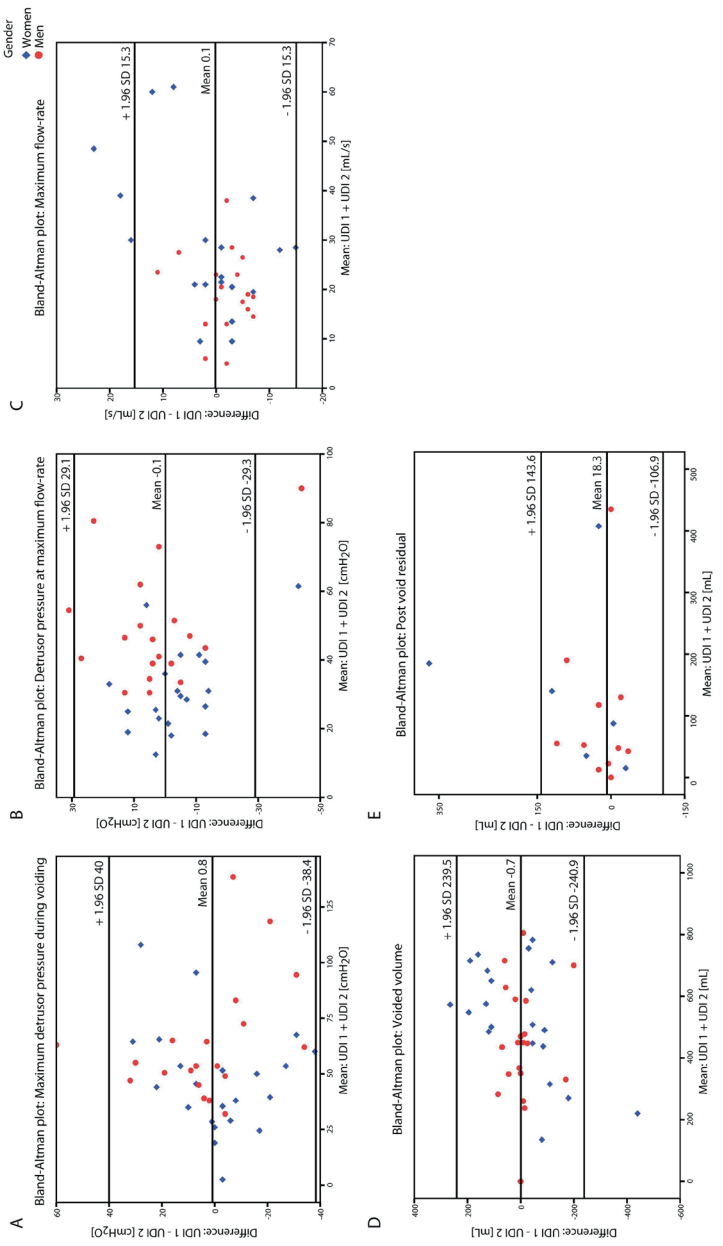

Figure 5 - Difference against mean plot pressure flow study.
Difference against mean plot for a) maximum detrusor pressure during voiding, b) detrusor pressure at maximum flow-rate, c) maximum flow rate, d) voided volume, and e) post void residual for UDI 1 vs. 2. Wide 95% limits of agreement reflect poor repeatability with unacceptable discrepancies between same session repeat UDIs. UDI, Urodynamic investigation.

DISCUSSION

Main findings

In more than 70% of our healthy subjects UDI revealed pathological findings, most commonly detrusor sphincter dyssynergia defined as a detrusor contraction concurrent with an involuntary contraction of the urethral and/or periurethral striated muscle [16]. Although UDI is the gold standard to assess refractory LUTS, it seems not to be applicable in apparently healthy subjects to define normal lower urinary tract function. Therefore, we do not recommend using UDI to select healthy subjects as controls for comparative studies.

Findings in the context of existing evidence

The literature on UDI findings in healthy subjects is very limited. In line with previous studies [5, 6, 20], we found significantly lower bladder volumes at FSF in women compared to men. In contrast to other studies, the volumes at FSF were relatively low, what could be instruction related, e.g. filling sensation versus awareness of the urethral catheter irritating the bladder wall, as subjects were instructed to report the first bladder perception during filling cystometry. However, as FSF is a very subjective sensation wide variability seems plausible. We also detected significantly lower pDet during PFS and a higher Qmax in women compared to men, i.e. phenomena well known from UDI in patients with LUTS [14, 15]. In healthy volunteers, involuntary detrusor contractions would be expected, but earlier studies revealed detrusor overactivity in 4-18% during conventional and up to 69% during ambulatory urodynamics in asymptomatic subjects [5, 21, 22]. It could be shown that the method of instruction, i.e. neither try to void nor to inhibit micturition during bladder filling versus simply report sensation to the examiner, affects the incidence of involuntary detrusor contractions [23]. Overall, detrusor overactivity was detected in 21% of our healthy subjects. It was found more frequently in men

(30%) than in women (14%). The reason for this finding is unclear but anatomical gender differences may be relevant as the catheter could irritate the urethral mucosa causing detrusor overactivity during UDI. Lower bladder capacity in bladder diary compared to UDI might be explained by the fact that in everyday life SDV is not always awaited before voiding. Moreover, bladder volume effects on detrusor overactivity episodes cannot be ruled out completely. Regarding parameters from the PFS, our values are in accordance with the very limited available literature [3, 21]. In our cohort, detrusor sphincter dyssynergia was the most commonly detected pathological finding. It might be argued, that detrusor sphincter dyscoordination or dysfunctional voiding would be more appropriate terms than detrusor sphincter dyssynergia to describe our findings. However, according to ICS terminology, detrusor sphincter dyssynergia is a pure urodynamic diagnosis defined as detrusor contraction concurrent with an involuntary contraction of the urethral and/or periurethral striated muscle and typically occurs in patients with a supra-sacral lesion [16], but an underlying neurological disorder has not been a prerequisite for the definition. Indeed, a percentage of 71% (30/42) undetected underlying neurological disorders in presumably healthy volunteers is very unlikely. Considering the normal flow pattern and lack of PVR during free uroflowmetry, the high rate of detrusor sphincter dyssynergia is most likely a phenomenon provoked by the examination itself, i.e. stiff irrigating catheter and the unselective recording of the surface EMG. Although detrusor sphincter dyscoordination is commonly used in German speaking countries for detrusor sphincter dyssynergia in neurologically normal individuals, it is not according to the ICS terminology. Moreover, dysfunctional voiding is not a very specific term defined as intermittent and/or fluctuating flow rate due to involuntary intermittent contractions of the periurethral striated muscle during voiding [16]. Importantly, detrusor contraction is not considered by this definition. Combined pelvic floor EMG and videocystourethrography during UDI

are the most accepted and widely agreed methods for diagnosing detrusor sphincter dyssynergia [24]. In the present study, however, we refrained from using videocystourethrography to avoid radiation exposure to our healthy volunteers. Therefore, we only used pelvic floor EMG to record detrusor sphincter dyssynergia. In line with our findings, an increased EMG signal during voiding has been reported in more than half of 321 neurologically normal female patients with predominant stress urinary incontinence [25], not demonstrating the typically expected relaxation during normal voiding. To distinguish between a solitary EMG activation of the intraurethral striated sphincter and a general muscle activation of the pelvic floor, an intramural sphincter needle electrode EMG would have been needed. However, surface electrodes are used in daily clinical practice and placing needle electrodes in healthy subjects would cause ethical concerns. Repeatability of detrusor overactivity and detrusor sphincter dyssynergia showed substantial agreement between both UDIs. For all other urodynamic parameters assessed, however, there were wide 95% limits of agreement for differences in the parameters, indicating poor repeatability. These findings are very similar to the results in patients with neurogenic LUTD [24, 26] and highlight the importance of same session repeat UDI for clinical decision making.

Implications for practice

The principal aim of a UDI is to detect and specify LUTD related to the patients' symptoms helping to select the most appropriate treatment. However, Katz et al. [2] reported discordance between clinical and UDI findings in 45% of patients with storage, 25% with voiding and 54% with combined storage and voiding problems, respectively. Although UDI did change clinical decision making and therapy in a relevant percentage of men with voiding dysfunction, it remained unclear whether this also leads to reduced symptoms after treatment [27]. Based on our study, identifying pathological findings in more than

70%, UDI seems not be appropriate in healthy subjects to define normal lower urinary tract function. In patients complaining about LUTS, a urodynamic pattern such as in Figure 2 would lead to the initiation of a treatment. However, pathological urodynamic patterns may be iatrogenic, simply induced by the UDI itself and not by an underlying disease. UDI is always a non-physiological attempt to mimic storage and voiding phase and as such it is subject to many errors. In fact, in clinical practice we compare the Q shape of the free uroflowmetry with the PFS and if the free flow Q trace is normal we would disregard an eventual pathological flow pattern of the PFS and attribute it may be secondary to the test situation. Further, social inhibition can be a reason for patients not being able to void. According to the ICS, findings of PVR during invasive UDI should be considered as an artefact caused by UDI, if no PVR is demonstrated after free uroflowmetry [16]. If voiding symptoms are not the urodynamic question the UDI is being performed to answer, such findings may be irrelevant in daily clinical practice. Thus, for clinical decision making, it is crucial that UDI findings reflect the situation in daily life, i.e. the patients' symptoms. Validated questionnaires and bladder diaries are of great value since they provide an objective patient-reported measure of LUTS [28].

Implications for research

In contrast to patients with LUTS, there is no consensus or standardisation regarding normal UDI parameters and/or nomograms in healthy subjects. When applying generally agreed definitions [16], one will encounter a very high percentage of pathological findings. Therefore, we do not recommend using UDI to select healthy subjects as controls for comparative studies but rather rely on bladder diaries, validated questionnaires and neuro-urological assessment [13]. Intermittent involuntary contraction of the urethral and/or periurethral striated muscle due to a contact with the transurethral urodynamic catheter could have contributed to these findings. This may also be

relevant in patients with preserved sensory function [29]. Thus, many important questions remain to be answered highly warranting further investigations in healthy subjects.

Limitations of the study

Although to the best of our knowledge, this is the first prospective study to assess the value of UDI to appropriately select healthy volunteers with apparent normal lower urinary tract function as control subjects, some limitations should be addressed. Since we used an air-charged catheter for UDI, it is unclear whether the results can be extrapolated to UDI with a water-filled catheter, especially considering that for urethral pressure measurement urodynamic catheters cannot be used interchangeably because of insufficient agreement [30] and that this may also be the case for cystometry and PFS. The response to pressure changes and the frequency rate of attenuated signals are different between the air-charged and water-filled systems [31, 32]. Although there are no randomised controlled trials, higher pressures were consistently found with the air-charged compared with the water-filled catheters [32, 33]. The Abrams-Griffiths nomogram to define bladder outlet obstruction in men [15] has only been validated for water-filled systems, but in lack of alternatives we applied it also in our study, so that bladder outlet obstruction may have been overestimated. Considering the relative young age of the studied cohort, a selection bias cannot be completely excluded. Based on age-related urodynamic changes [34], it may be hypothesised that older subjects are more vulnerable for UDI-induced pathological findings. Subsequently, an older population of healthy subjects could even show a higher percentage of pathological results. Moreover, we used a constant filling rate of 30 mL/min and did not explore a potential impact by changing the infusion speed between UDIs and/or subjects. Taking into account that a physiological filling rate (mL/min) has been defined as less than body weight in kg divided

by 4 [16], we cannot exclude that a supra-physiological infusion speed might have provoked detrusor overactivity contributing to the high incidence of detrusor overactivity found in our healthy volunteers. In addition, different bladder volumes in free uroflowmetry and invasive UDI might be relevant and complicate comparability of these parameters.

CONCLUSIONS

UDI is the gold standard to assess refractory LUTS, i.e. to detect LUTD. However, UDI is a non-physiological examination and may result in investigation-induced pathological findings. Indeed, in more than 70% of our healthy subjects, UDI revealed pathological findings, most commonly detrusor sphincter dyssynergia. UDI findings should not be used standalone but be considered in the context of clinical data such as validated questionnaires, bladder diaries, free uroflowmetry, PVR, medical history and examination. Thus, UDI alone may result in exclusion of many potential candidates due to false positive pathological findings and seems not to be a sensible tool in healthy subjects to define normal lower urinary tract function. Based on the current study, we do not recommend using UDI to select healthy control subjects for comparative studies.

Competing interests:
The authors declare that they have no competing interests.

Funding:
We would like to acknowledge Wings for Life, Swiss National Science Foundation, Emily Dorothy Lagemann Stiftung, and Swiss Continence Foundation for financial support. The supporters had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions:
Conceived and designed the study: LL, MW, US, SCK, UM and TMK
Performed investigation: LL, MW and TMK
Analyzed the data: LL, MW, US
Statistical analysis: LL, MW
Contributed to the writing of the manuscript: LL, MW, US, SCK, UM and TMK

REFERENCES

1. Schafer W, Abrams P, Liao L, Mattiasson A, Pesce F, Spangberg A, et al. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies. Neurourol Urodyn. 2002;21(3):261-74. Epub 2002/04/12. PubMed PMID: 11948720.
2. Katz GP, Blaivas JG. A diagnostic dilemma: when urodynamic findings differ from the clinical impression. J Urol. 1983;129(6):1170-4. Epub 1983/06/01. PubMed PMID: 6854793.
3. Mahfouz W, Al Afraa T, Campeau L, Corcos J. Normal urodynamic parameters in women: part II--invasive urodynamics. Int Urogynecol J. 2012;23(3):269-77. Epub 2011/10/21. doi: 10.1007/s00192-011-1585-y. PubMed PMID: 22011933.
4. Pfisterer MH, Griffiths DJ, Rosenberg L, Schaefer W, Resnick NM. Parameters of bladder function in pre-, peri-, and postmenopausal continent women without detrusor overactivity. Neurourol Urodyn. 2007;26(3):356-61. Epub 2007/02/08. doi: 10.1002/nau.20381. PubMed PMID: 17285577.
5. Wyndaele JJ. Normality in urodynamics studied in healthy adults. J Urol. 1999;161(3):899-902. Epub 1999/02/18. PubMed PMID: 10022710.
6. Wyndaele JJ, De Wachter S. Cystometrical sensory data from a normal population: comparison of two groups of young healthy volunteers examined with 5 years interval. Eur Urol. 2002;42(1):34-8. Epub 2002/07/18. PubMed PMID: 12121727.
7. Abrams P, Avery K, Gardener N, Donovan J. The International Consultation on Incontinence Modular Questionnaire: www.iciq.net. J Urol. 2006;175(3 Pt 1):1063-6; discussion 6. Epub 2006/02/14. doi: 10.1016/S0022-5347(05)00348-4. PubMed PMID: 16469618.
8. Coyne KS, Thompson CL, Lai JS, Sexton CC. An overactive bladder symptom and health-related quality of life short-form: Validation of the OAB-q SF. Neurourol Urodyn. 2015;34(3):255-63. Epub 2015/03/19. doi: 10.1002/nau.22559. PubMed PMID: 25783168.
9. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98. PubMed PMID: 1202204.
10. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-70. PubMed PMID: 6880820.
11. Brookes ST, Donovan JL, Wright M, Jackson S, Abrams P. A scored form of the Bristol Female Lower Urinary Tract Symptoms questionnaire: data from a randomized controlled trial of surgery for women with stress incontinence. Am J Obstet Gynecol. 2004;191(1):73-82. doi: 10.1016/j.ajog.2003.12.027. PubMed PMID: 15295345.
12. Donovan JL, Peters TJ, Abrams P, Brookes ST, de aa Rosette JJ, Schafer W. Scoring the short form ICSmaleSF questionnaire. International Continence Society. J Urol. 2000;164(6):1948-55. PubMed PMID: 11061889.
13. Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. Lancet Neurol. 2015;14(7):720-32. Epub 2015/06/13. doi: 10.1016/S1474-4422(15)00070-8. PubMed PMID: 26067125.
14. Defreitas GA, Zimmern PE, Lemack GE, Shariat SF. Refining diagnosis of anatomic female bladder outlet obstruction: comparison of pressure-flow study parameters in clinically obstructed women with those of normal controls. Urology. 2004;64(4):675-9; discussion 9-81. Epub 2004/10/20. doi: 10.1016/j.urology.2004.04.089. PubMed PMID: 15491697.
15. Lim CS, Abrams P. The Abrams-Griffiths nomogram. World J Urol. 1995;13(1):34-9. Epub 1995/01/01. PubMed PMID: 7539679.
16. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn. 2002;21(2):167-78. Epub 2002/02/22. PubMed PMID: 11857671.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10. Epub 1986/02/08. PubMed PMID: 2868172.
18. International conference on harmonisation. Good clinical practice guideline 1996. Available from: http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html.

19. International organization for standardization. ISO 14155 2011. Available from: http://www.iso.org/iso/catalogue_detail?csnumber=45557.
20. Wyndaele JJ. The normal pattern of perception of bladder filling during cystometry studied in 38 young healthy volunteers. J Urol. 1998;160(2):479-81. Epub 1998/07/29. PubMed PMID: 9679902.
21. Rosario DJ, Woo HH, Chapple CR. Definition of normality of pressure-flow parameters based on observations in asymptomatic men. Neurourol Urodyn. 2008;27(5):388-94. Epub 2007/12/08. doi: 10.1002/nau.20537. PubMed PMID: 18064668.
22. van Waalwijk van Doorn ES, Remmers A, Janknegt RA. Conventional and extramural ambulatory urodynamic testing of the lower urinary tract in female volunteers. J Urol. 1992;147(5):1319-25; discussion 26. Epub 1992/05/01. PubMed PMID: 1569676.
23. Blaivas JG, Groutz A, Verhaaren M. Does the method of cystometry affect the incidence of involuntary detrusor contractions? A prospective randomized urodynamic study. Neurourol Urodyn. 2001;20(2):141-5. Epub 2001/02/15. PubMed PMID: 11170188.
24. Bellucci CH, Wollner J, Gregorini F, Birnbock D, Kozomara M, Mehnert U, et al. Neurogenic lower urinary tract dysfunction--do we need same session repeat urodynamic investigations? J Urol. 2012;187(4):1318-23. Epub 2012/02/22. doi: 10.1016/j.juro.2011.11.107. PubMed PMID: 22341264.
25. Kirby AC, Nager CW, Litman HJ, Fitzgerald MP, Kraus S, Norton P, et al. Perineal surface electromyography does not typically demonstrate expected relaxation during normal voiding. Neurourol Urodyn. 2011;30(8):1591-6. Epub 2011/05/12. doi: 10.1002/nau.21080. PubMed PMID: 21560157.
26. Walter M, Knupfer SC, Leitner L, Mehnert U, Schubert M, Curt A, et al. Autonomic dysreflexia and repeatability of cardiovascular changes during same session repeat urodynamic investigation in women with spinal cord injury. World J Urol. 2015. Epub 2015/06/10. doi: 10.1007/s00345-015-1589-1. PubMed PMID: 26055644.
27. Clement KD, Burden H, Warren K, Lapitan MC, Omar MI, Drake MJ. Invasive urodynamic studies for the management of lower urinary tract symptoms (LUTS) in men with voiding dysfunction. Cochrane Database Syst Rev. 2015;4:CD011179. Epub 2015/04/29. doi: 10.1002/14651858.CD011179.pub2. PubMed PMID: 25918922.
28. Kessler TM. Diagnosis of urinary incontinence. JAMA. 2008;300(3):283; author reply Epub 2008/07/18. doi: 10.1001/jama.2008.30. PubMed PMID: 18632541.
29. Suskind AM, Clemens JQ, Kaufman SR, Stoffel JT, Oldendorf A, Malaeb BS, et al. Patient perceptions of physical and emotional discomfort related to urodynamic testing: a questionnaire-based study in men and women with and without neurologic conditions. Urology. 2015;85(3):547-51. Epub 2015/03/04. doi: 10.1016/j.urology.2014.11.001. PubMed PMID: 25733264; PubMed Central PMCID: PMC4349219.
30. Zehnder P, Roth B, Burkhard FC, Kessler TM. Air charged and microtip catheters cannot be used interchangeably for urethral pressure measurement: a prospective, single-blind, randomized trial. J Urol. 2008;180(3):1013-7. Epub 2008/07/22. doi: 10.1016/j.juro.2008.05.028. PubMed PMID: 18639301.
31. Cooper MA, Fletter PC, Zaszczurynski PJ, Damaser MS. Comparison of air-charged and water-filled urodynamic pressure measurement catheters. Neurourol Urodyn. 2011;30(3):329-34. Epub 2011/02/10. doi: 10.1002/nau.20991. PubMed PMID: 21305591.
32. Digesu GA, Derpapas A, Robshaw P, Vijaya G, Hendricken C, Khullar V. Are the measurements of water-filled and air-charged catheters the same in urodynamics? Int Urogynecol J. 2014;25(1):123-30. Epub 2013/08/02. doi: 10.1007/s00192-013-2182-z. PubMed PMID: 23903819.
33. Gammie A, Abrams P, Bevan W, Ellis-Jones J, Gray J, Hassine A, et al. Simultaneous in vivo comparison of water-filled and air-filled pressure measurement catheters: Implications for good urodynamic practice. Neurourol Urodyn. 2015. doi: 10.1002/nau.22827. PubMed PMID: 26250500.
34. Kessler TM, Madersbacher H. [Urodynamic phenomena in the aging bladder]. Urologe A. 2004;43(5):542-6. Epub 2004/03/27. doi: 10.1007/s00120-004-0564-z. PubMed PMID: 15045179.

CHAPTER 5

Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms

Walter M, Michels L, Kollias S, van Kerrebroeck PE, Kessler TM, Mehnert U
BMJ Open. 2014 May 21;4(5):e004357.

ABSTRACT

Introduction

Lower urinary tract symptoms (LUTS) are highly prevalent, cause an enormous economic burden on healthcare systems and significantly impair the quality of life (QoL) of affected patients. The dependence of the LUT on complex central neuronal circuits makes it unique in comparison to other visceral functions, such as the gastrointestinal tract, but also more vulnerable to neurological diseases.

Methods and Analysis

This is a prospective neuroimaging study investigating the supraspinal control of LUT function in healthy controls and in patients with non-neurogenic LUTS.

The clinical assessment will include medical history, neuro-urological examination, bladder diary, urine analysis, urodynamic investigations, as well as standardised questionnaires regarding LUTS and QoL.

The acquisition of neuroimaging data will include structural assessments (T1-weighted imaging and diffusion tensor imaging) as well as functional investigations using blood-oxygen-level dependent (BOLD) sensitive functional fMRI in a 3 T MR scanner. The fMRI will be performed during four different bladder tasks using an automated MR-compatible and MR-synchronised pump system. The first three task-related fMRIs will consist of automated, repetitive filling of 100mL warm (37°C) saline starting with (1) an empty bladder, (2) a low prefilled bladder volume (100mL) and (3) a high prefilled bladder volume (persistent desire to void). The fourth task-related fMRI will comprise of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder.

Ethics and Dissemination

The local ethics committee approved this study (KEK-ZH-Nr. 2011-0346). The findings of the study will be published in peer-reviewed journals and presented at national and international scientific meetings.

Trial registration number: This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

INTRODUCTION

Lower urinary tract symptoms (LUTS) are highly prevalent, that is, about 11% in the worldwide population in 2008, and forecasted to increase up to 20% until 2018 [1, 2]. Moreover, LUTS cause an enormous economic burden on each healthcare system [3, 4], which is comparable to diabetes mellitus [5], and significantly impair the quality of life (QoL) of affected patients [6, 7]. For a proper functioning, LUT structures, that is, bladder, bladder neck, urethra, and urethral sphincter, rely on intact neuronal innervations that are under the control of a complex supraspinal network [8 – 10]. The dependence of the LUT on such complex central neuronal circuits makes it unique in comparison with other visceral functions, for example gastrointestinal tract, but also more vulnerable to neurological diseases [10]. Recent neuroimaging studies have shown that patients with neurological disorders such as Parkinson's disease [11 – 13] and spinal cord injury [14] demonstrate different supraspinal activity patterns compared with healthy controls in response to LUT stimulation tasks, which might represent a neural correlate to their LUTS. Although there are several concepts regarding the human LUT function and neuronal control in normal and pathological conditions, the exact pathophysiological mechanisms involved remain largely unknown [9]. Despite the popularity of resting-state functional MRI (RS-fMRI) [15 – 20] and diffusion tensor imaging (DTI) [21] in other fields in neuroscience, these techniques have not been applied in the context of supraspinal LUT control. There are only two DTI studies published in regard to LUT control in general: (1) a case report by Théaudin et al [22] studying spinal cord infarctions and clinical symptoms and (2) a prospective study by van der Jagt et al [23] investigating architectural configuration and microstructural properties of the sacral plexus. As cortical and subcortical (eg, brainstem) brain regions are crucial for voluntary LUT control [10, 24, 25], investigation of the supraspinal regions with high-resolution imaging

techniques, such as fMRI, can significantly contribute to increase our understanding of the effects of supraspinal lesions and alterations related to LUTS [10, 26]. In this study, we are aiming to identify supraspinal areas associated with LUT control in healthy controls and in patients with non-neurogenic LUTS. Task-related blood-oxygen-level dependent (BOLD) and RS-fMRI will be used along with structural MRI (T1-weighted MRI and DTI). Hence, we will examine whether bladder processing is already altered on the structural level and on baseline (RS-fMRI) functional connectivity (FC). For example, multiple repetition of the RS-fMRI will help to understand whether manipulation of sensory perception (induced by infusion and withdrawal) will alter the default mode network [20] of the brain. Furthermore, we can examine volumetric parameters (eg, grey matter concentration) by voxel-based morphometry (VBM) [27], structural integrity and connectivity of white matter tracts (DTI) as well as FC. This unique and detailed multimodal imaging protocol should pinpoint to structural and functional processing units involved during supraspinal LUT control and should identify all dysfunctional neuronal components in patients with disturbed LUT control. Importantly, we will investigate the reliability [28] of BOLD signals in task-related fMRI and RS-fMRI in healthy controls and patients with non-neurogenic LUTS. The test–retest validation, that is, the intraclass correlation coefficient (ICC) for absolute or consistent agreement of subject activations from visit to visit, has not been evaluated in regard to the supraspinal LUT control yet.

Strengths and limitations of this study

- This will be the first study to identify brain networks of supraspinal LUT control in healthy subjects and abnormalities within such brain networks in patients with non-neurogenic LUTS using structural and functional MRI techniques in correlation with clinical measurements.
- Investigation of test–retest reliability has not yet been performed for neuroimaging of LUT tasks. However, this is important for the interpretation of participants' activations in regard to the validity of these activations, i.e. absolute and consistent agreement (ICC) from visit to visit.
- Comparison of clinical correlates of treatment efficacy in patients with non-neurogenic LUTS with the associated changes in brain activity and connectivity.

METHODS

Study design

This prospective research study will be conducted at the University of Zürich, Zürich, Switzerland.

Study population and recruitment

According to the inclusion and exclusion criteria (Table 1), we will investigate patients with non-neurogenic LUTS and healthy controls with an unimpaired LUT function. Participants of both groups will be matched according to age and gender. Patients with non-neurogenic LUTS will be recruited from our own department (Neuro-Urology, Balgrist University Hospital, Zürich, Switzerland) and through our partners at the University Hospital Zürich and the Triemli Hospital Zürich. Eligible patients with non-neurogenic LUTS and healthy controls will be invited to a first visit (screening) during which detailed information

about the study, in particular the aims, methods, possible risks and side effects, will be given. After obtaining written informed consent, the following data will be collected: medical history, a 3-day bladder diary, urine sample to exclude urinary tract infection (UTI) and pregnancy in female participants, urodynamic parameters and postvoid residual measured by ultrasound as well as standardised questionnaires regarding LUTS and QoL. The validated German versions of these questionnaires will be used with permission of the International Consultation on Incontinence Modular Questionnaire ((ICIQ), Bristol Urological Institute, Southmead Hospital Bristol, UK) and will address the LUTS (ICIQ-LUTS) in women (ICIQ-FLUTS) and men (ICIQ-MLUTS), whereas the ICIQ-LUTSQoL will display the QoL in regard to LUTS.

Table 1 – Inclusion and exclusion criteria for all participants.

Groups	Inclusion criteria	Exclusion criteria
All subjects	▪ Right-handed ▪ Gender (female and male) ▪ Age limits: 18-55 years ▪ MR suitability ▪ Written informed consent	▪ Pregnancy or breast feeding ▪ Any craniocerebral injury or surgery ▪ Any permanent ferromagnetic implant ▪ Any previous surgery of LUT/genitalia ▪ Any anatomical anomaly of LUT/genitalia ▪ Any LUT malignancy ▪ PVR > 150mL ▪ UTI

Healthy controls	▪ Unimpaired LUT function
	▪ No LUTS (3-day bladder diary)
	  ▪ No episode of urinary urgency/week
	  ▪ Urinary frequency < 8/24h
		▪ Impaired LUT function
		▪ Any LUTS (3-day bladder diary)
		  ▪ Any number of episodes of urinary urgency/week
		  ▪ Urinary frequency > 8/24h

Patients with non-neurogenic LUTS	▪ LUTS > 6 months (3-day bladder diary) ▪ ≥ 2 episodes of urinary urgency/week ▪ Urinary frequency > 8/24h	▪ Any neurological, psychological, metabolic, or cardiovascular disease ▪ Any concomitant treatment for the LUT (e.g. neuromodulation) ▪ SUI ▪ Indwelling catheters or necessity to perform ISC

ISC, intermittent self-catheterisation; LUTS, lower urinary tract symptoms; PVR, postvoid residual; SUI, stress urinary incontinence; MR, magnet resonance, UTI, urinary tract infection.

Determination of sample size

A power analysis was conducted using G*Power (www.gpower.hhu.de). In order to have sufficient power (0.80) to detect a large effect size (0.80) between

healthy controls and patients with non-neurogenic LUTS (significance level 0.05), at least 21 participants per group need to be recruited. To demonstrate post-treatment effects in patients with non-neurogenic LUTS compared with their baseline using the same power, effect size and significance level, at least 12 participants for each treatment option are necessary. These sample sizes are in line with earlier studies [22, 23] including our own [14, 29], which provided statistical evidence using small collectives, that is, between 12 and 21 participants.

Study location

- Neuro-Urology, Spinal Cord Injury Centre and Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland.
- MR-Centre, University Hospital Zürich, Zürich, Switzerland.

Partners

- Institute of Neuro-Radiology, University of Zürich, University Hospital Zürich, Zürich, Switzerland.
- Departments of Urology and Gynaecology, University Hospital Zürich, Zürich, Switzerland.
- Department of Urology and Gynaecology, Triemli Hospital, Zürich, Switzerland.

Investigations

Following screening and study inclusion, all participants will be scheduled for the second and third visit (first and second MRI measurements) at the MR-Centre. Patients with non-neurogenic LUTS will return for a fourth visit (third MRI measurement), either after receiving treatment for LUTS or without treatment acting as a direct control group within the patient cohort (Figure 1).

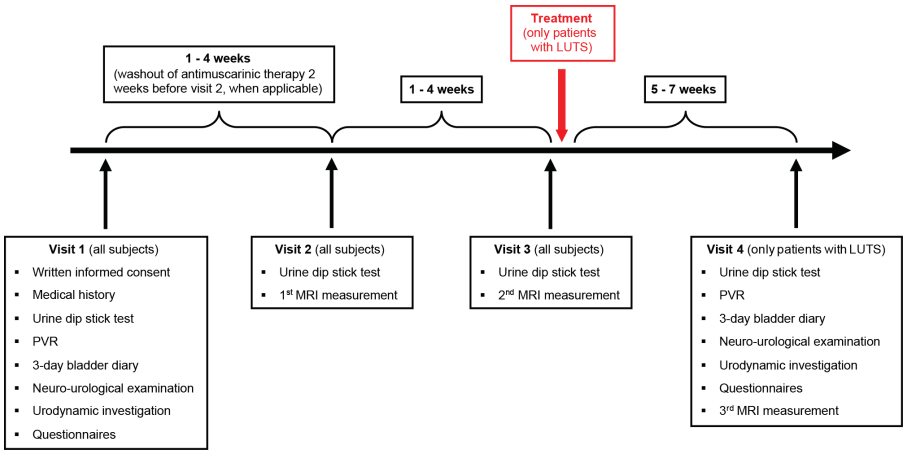

Figure 1 – Timetable and characteristics of all four visits.
LUTS, lower urinary tract symptoms; MRI, magnetic resonance imaging; PVR, postvoid residual; QoL, quality of life.

All MRI measurements (Figure 2) will be performed using a Philips Ingenia 3 TMR scanner (Philips Medical Systems, Best, The Netherlands) with a 16-channel head coil. During the second visit, we will acquire the following neuroimaging data. Structural measurements will contain T1-weighted MRI and DTI. Functional measurements will comprise RS-fMRIs and task-related fMRIs. Four different RS-fMRIs will be applied, that is, (1) at baseline with an empty bladder, (2) with an empty bladder plus transurethral catheter, (3) with a low prefilled bladder (100mL saline, body warm) prior to task-related fMRI and (4) after a task-related fMRI, to understand whether manipulation of sensory perception (induced by catheter or prefilling) will alter the default mode network [20]. The task-related fMRI will be acquired during four different bladder tasks (Figure 3). In order to precisely fill and drain the bladder (ie, specific volume and duration of time), we designed an automated MR-compatible and MR-synchronised pump system. In the first three task-related fMRIs, we will examine the effect of visceral bladder sensation by automated, repetitive filling with 100mL body warm saline starting with (1) an empty bladder, (2) a low prefilled bladder and (3) a high prefilled bladder (persistent

desire to void). The fourth task-related fMRI will consist of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder to investigate the neural correlates of cold bladder sensation (Figure 3). Participants will rate their desire to void and their level of pain using a displayed visual analogue scale and an fMRI-compatible handheld response system [30]. During the second MRI measurement (third visit, 1–4 weeks later), we will utilise a selection of MRI measurements for the purpose of reliability analysis [28], that is, RS-fMRI (baseline with an empty bladder plus transurethral catheter) and task-related fMRI to compare within and between groups. The third MRI measurement will be identical to the first MRI measurement to evaluate post-treatment versus pretreatment effects in patients with non-neurogenic LUTS. The time from the start of treatment to the fourth visit, that is, 5–7 weeks (Figure 1), is necessary to let clinical improvements develop [31, 32].

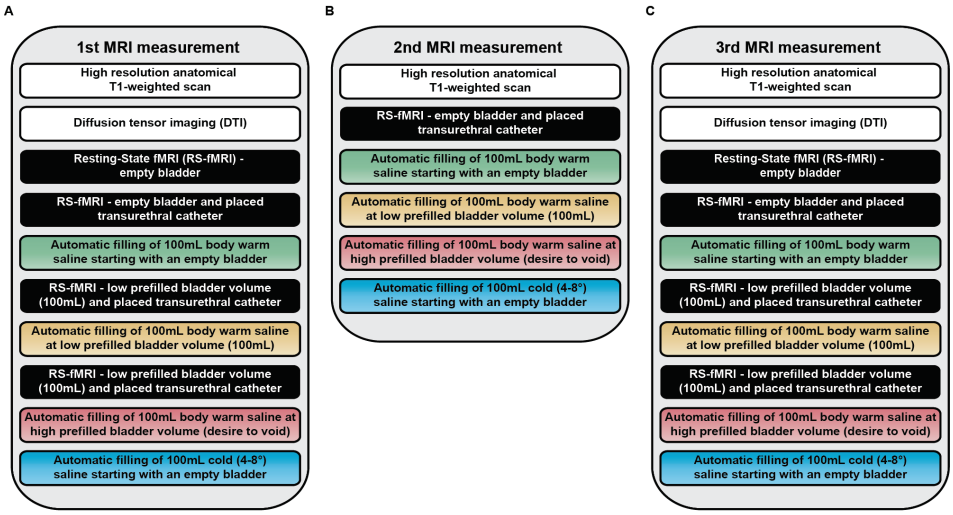

Figure 2 – Schematic protocol of operational sequences of magnetic resonance imaging (MRI) measurements including functional MRI (fMRI).
A) first MRI measurement, B) second MRI measurement and C) third MRI measurement.

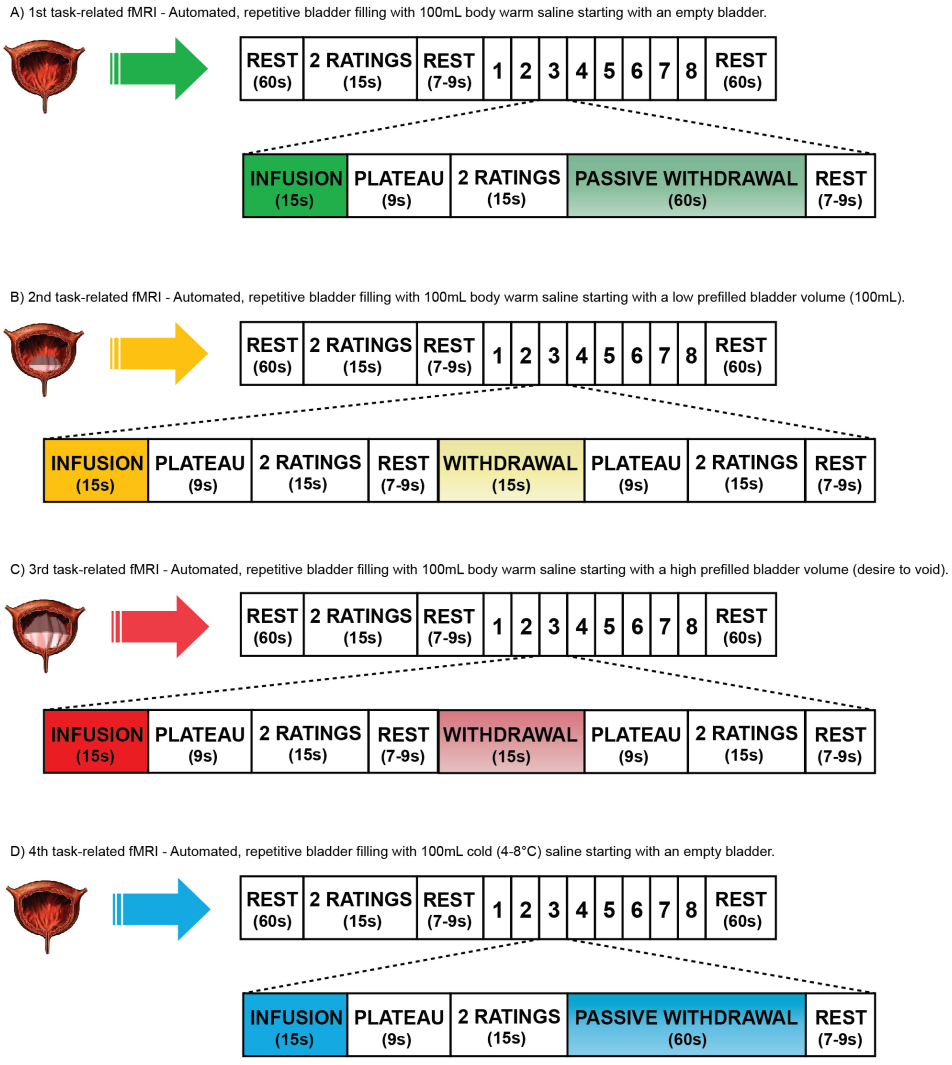

Figure 3 – Schematic diagram of the scan paradigm of four different task-related functional MRIs (fMRIs) at visits 2, 3 and 4.
All task-related fMRIs identically start with a 'baseline' rest (60 s, no specific stimulus or task is performed), a 'baseline' rating of desire to void and level of pain, a short rest jittered between 7 and 9 s in which blood-oxygen-level dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition and conclude with a 'last' rest (60 s, no specific stimulus or task is performed). All task-related fMRIs consist of eight repetitive blocks, each with either five (first and fourth fMRIs) or eight (second and third fMRIs) conditions. (A) Conditions of the first task-related fMRI: (1) automated infusion of 100 mL body warm saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) passive withdrawal to empty the bladder completely and (5) short rest jittered between 7 and 9 s. This task-related fMRI starts with an empty bladder and will be performed in patients with non-neurogenic LUTS in visits 2, 3 and 4, while in healthy controls only at visit 3 (second MRI measurement). (B and C) Conditions of the second and third task-related fMRIs: (1) automated infusion of 100 mL warm saline, (2) plateau phase (bladder

distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) short rest jittered between 7 and 9 s in which BOLD activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, (5) automated withdrawal of 100 mL, (6) plateau phase (bladder distention after withdrawal is perceived), (7) rating of desire to void and level of pain and (8) short rest jittered between 7 and 9 s in which BOLD activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition. The second task-related fMRI (B) starts with a low prefilled bladder volume (100 mL) and will be performed only in healthy controls at visits 2 and 3 (first and second MRI measurements). The third task-related fMRI (C) starts with a high prefilled bladder volume (persistent desire to void) and will be performed in all participants (patients with non-neurogenic LUTS and healthy controls) during visits 2 and 3 (first and second MRI measurements). Additionally, this task-related fMRI will be carried out in patients with non-neurogenic LUTS at visit 4 (third MRI measurement). (D) Conditions of the fourth task-related fMRI task: (1) automated infusion of 100 mL cold (4–8°C) saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) passive withdrawal to empty the bladder completely and (5) short rest jittered between 7 and 9 s. This task-related fMRI starts with an empty bladder and will be performed in all participants (patient with non-neurogenic LUTS and healthy controls) during visit 2 and 3 (first and second MRI measurement). Additionally, this task-related fMRI will be executed in patients with non-neurogenic LUTS at visit 4 (third MRI measurement).

Safety

The staff involved in this study will be instructed and trained according to the safety regulations of the MR-Centre of the University Hospital Zürich. All participants will be asked to remove any ferromagnetic items, for example, bra, earrings, chains, rings, and piercings prior to entering the scanner room. All participants will be provided with standardised clinical scrubs instead of wearing their own clothes. Before every MRI measurement (visits 2, 3 and 4), urine samples will be analysed from every participant in order to exclude UTI or pregnancy. In case of pregnancy, the participant will be excluded from the study and referred to a gynaecologist. In case of UTI, the participant will not undergo the experiment, but will receive immediate antibiotic treatment if the UTI is symptomatic or be treated depending on further microbiological urine analysis in the absence of UTI symptoms. The participant can be reassigned to the study if the microbiological urine analysis shows no evidence of a UTI or the UTI has been successfully treated. In the situation of an adverse event (AE) or a severe AE (SAE), as defined by the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines (E6) [33] and International Organization for Standardization (ISO, 14155) [34], appropriate actions will be executed and the according body (principle investigator, ethics committee) will be informed. All AEs and SAEs will be followed as long as

medically indicated.

Study outcome measures

Primary

(A) BOLD signal intensity changes during task-related fMRI in relation to the specific condition, that is, infusion or to a contrast, that is, low versus full bladder volume, during two (healthy controls) or three (patients with non-neurogenic LUTS) visits. Investigation of these changes will focus on supraspinal regions of interest (ROI) that are known from the existing literature, for example, pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area and prefrontal cortex. However, the precise selection of ROIs will be based on the coordinates of the peak activations during task-related fMRI taken from the Montreal Neurological Institute (MNI) space.

(B) Reliability of BOLD signal changes during RS-fMRI and task-related fMRI across visits (eg, second and third visits) in healthy controls and patients with non-neurogenic LUTS.

(C) BOLD signal changes in supraspinal ROIs during task-related fMRI in patients with non-neurogenic LUTS before and after treatment to quantify the link between BOLD signal changes and treatment efficacy.

Secondary

(A) Structural differences, i.e. between healthy controls and patients with non-neurogenic LUTS, and changes in the post-treatment versus pre-treatment state of grey matter concentration using VBM.

(B) Structural connectivity (SC) and FC between supraspinal ROIs to identify specific alterations with DTI [35] between healthy controls and

patients with non-neurogenic LUTS (including post-treatment versus pretreatment changes). This will include whole-brain fractional anisotropy (FA) and mean diffusivity (MD) comparison as well as probabilistic tractography between ROIs (white matter fibre structure).

(C) Differences in BOLD signals during RS-fMRI between healthy controls and patients with non-neurogenic LUTS (including post-treatment versus pretreatment changes), in regard to intrahemisheric and interhemispheric connectivity [33]. Investigation will focus on whether these signals differ (1) already at baseline or are influenced by the (2) presence of a catheter, (3) by prefilling and/or (4) by task-related fMRI.

(D) Clinical scores (eg, bladder volume, urodynamic parameters and level of desire to void during fMRI) will be correlated to BOLD signal changes as well as to structural markers (eg, grey matter volume or number of white matter tracts between ROIs) using regression analyses.

Data analysis

Clinical data, for example, urodynamic parameters, 3-day bladder diary outcome and questionnaire scores, will be statistically analysed and compared between groups using IBM's Statistical Package for the Social Sciences (SPSS) version 19.0 or newer (Armonk, New York, USA) and will be presented with means and SDs or with medians and IQRs as appropriate. The neuroimaging data will be analysed using statistical parametric mapping (SPM) V.8 or newer (Wellcome Department of Imaging Neuroscience, University College London, UK). Preprocessing of functional data from each task-related fMRI will be carried out for each participant individually. The images will be realigned to the first scan, unwarped to control for movement-induced and susceptibility-induced image distortions [37], spatially coregistered to the T1-weighted image and normalised to the MNI anatomical standard space. At

last, the functional data will be smoothed spatially with an isotropic Gaussian kernel. Thereafter, first-level analysis using the general linear model will be performed to create contrasts of interest, for example, low versus full bladder or infusion versus withdrawal [38]. The six movement parameters will be modelled as additional regressors to control for potential head motion. Second-level factorial design will include at least (1) one-sample t tests to compute a mean for each group, (2) two-sample t tests to compare healthy controls and patients with non-neurogenic LUTS and (3) paired t tests to evaluate post-treatment versus pretreatment effects in patients with non-neurogenic LUTS. The ICC for task-related and RS-fMRI reliability will be analysed using the SPM-compatible ICC toolbox (http://www.kcl.ac.uk/iop/depts/neuroimaging/research/imaginganalysis/Software/ICC-Toolbox.aspx). Association of individual (clinical) variables with BOLD signal changes will be assessed by whole-brain and ROI-based correlation analyses (correlation coefficients will be reported). DTI data will be analysed using TBSS (http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/tbss/index) and BrainVoyager (http://www.brainvoyager.com/downloads/downloads.html) with the following established DTI analyses: whole-brain FA and MD comparison between groups as well as probabilistic tractography between ROIs. Seed and target regions, that is, ROIs will be defined (1) a priori using anatomic coordinates, for example, from the SPM toolbox WFU_PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas) and (2) from peak activations acquired from task-related fMRI and RS-fMRI on the standard MNI space. Volumetric changes in grey and white matter will be analysed using voxel-based morphometry (VBM), for example, the VBM toolbox (http://dbm.neuro.uni-jena.de/vbm) in SPM.

ETHICS AND DISSEMINATION

This cohort study will be performed in accordance with the World Medical Association Declaration of Helsinki [38] and the guidelines of the Swiss Academy of Medical Sciences [40]. Furthermore, handling of all personal data will strictly comply with the federal law of data protection in Switzerland [41]. This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

DISCUSSION

This study will investigate supraspinal LUT control in healthy controls and patients with non-neurogenic LUTS using a multimodal imaging protocol, that is, structural (T1-weighted and DTI) and fMRI (RS-fMRI and task-related fMRI), to examine haemodynamic responses to LUT stimulation. From the acquired neuroimaging data, SC and FC, and structural integrity evaluation of white matter tracts and grey matter concentration (using VBM) will be analysed to identify specific alterations of the supraspinal LUT control. In addition, effects on the supraspinal LUT control after treatment for LUTS (eg, antimuscarinergics or botulinum toxin) will be investigated. It is expected that this study will provide new insights into the supraspinal neuronal mechanisms and networks responsible for LUT control. The findings will help to verify, amend or adjust neuronal circuitry models established from findings in healthy controls, now in the context of patients with non-neurogenic LUTS. The use of newer imaging and evaluation techniques has the potential to serve as quantifiable outcome measures for therapy success and provide evidence for non-responders of LUTS treatment.

Author affiliations:
1 Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist
University Hospital, Zürich, Switzerland
2 Institute of Neuro-Radiology, University of Zürich, University Hospital Zürich, Zürich, Switzerland
3 Department of Urology, University Hospital Maastricht, Maastricht, The Netherlands

Acknowledgements:
The authors would like to acknowledge the Swiss National Science Foundation, SwissLife Jubiläumsstiftung, and Swiss Continence Foundation for financial support. Furthermore, we would like to thank our partners contributing to this study: Departments of Urology and Gynaecology of the University Hospital Zürich and Departments of Urology and Gynaecology, Triemli Hospital, Zürich, Switzerland. They also thank Behnaz Jarrahi for granting figure contents.

Contributors:
All authors participated in creating the study design. MW and UM drafted the manuscript. LM, SK, PEvK and TMK critically reviewed the manuscript. UM, SK and TMK obtained the funding of this study. All the authors read and approved the final manuscript.

Funding:
Swiss National Science Foundation (grant number: 135774), SwissLife Jubiläumsstiftung, Swiss Continence Foundation

Competing interests:
The authors declare that they have no competing interests.

Ethics approval:
This study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346).

Provenance and peer review:
Not commissioned; externally peer reviewed.

REFERENCES

1. 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 obstruc-tion. BJU Int 2011;108(7):1132-8.
2. Irwin DE, Milsom I, Hunskaar S, Reilly K, Kopp Z, Herschorn S, et al. Population-based sur-vey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. Eur Urol 2006;50(6):1306-14; discussion 14-5.
3. Ganz ML, Smalarz AM, Krupski TL, Anger JT, Hu JC, Wittrup-Jensen KU, et al. Economic costs of over-active bladder in the United States. Urology 2010;75(3):526-32, 32 e1-18.
4. Klotz T, Bruggenjurgen B, Burkart M, Resch A. The economic costs of overactive bladder in Germany. Eur Urol 2007;51(6):1654-62; discussion 62-3.
5. Hampel C, Gillitzer R, Pahernik S, Hohenfellner M, Thuroff JW. Epidemiology and etiology of overactive bladder. Urologe A 2003;42(6):776-86.
6. 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 pro-ductivity, sexuality and emotional well-being in men and women: results from the EPIC study. BJU Int 2008;101(11):1388-95.
7. Irwin DE, Milsom I, Kopp Z, Abrams P, Cardozo L. Impact of overactive bladder symptoms on employ-ment, social interactions and emotional well-being in six European countries. BJU Int 2006;97(1):96-100.
8. Blok BF. Brain control of the lower urinary tract. Scand J Urol Nephrol Suppl 2002(210):11-5.
9. Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. Nat Rev Neurosci 2008;9(6):453-66.
10. Fowler CJ, Griffiths DJ. A decade of functional brain imaging applied to bladder control. Neurourol Urodyn 2010;29(1):49-55.
11. Herzog J, Weiss PH, Assmus A, Wefer B, Seif C, Braun PM, et al. Subthalamic stimulation modulates cortical control of urinary bladder in Parkinson's disease. Brain 2006;129(Pt 12):3366-75.
12. Herzog J, Weiss PH, Assmus A, Wefer B, Seif C, Braun PM, et al. Improved sensory gating of urinary bladder afferents in Parkinson's disease following subthalamic stimulation. Brain 2008;131(Pt 1):132-45.
13. Kitta T, Kakizaki H, Furuno T, Moriya K, Tanaka H, Shiga T, et al. Brain activation during detrusor overactivity in patients with Parkinson's disease: a positron emission tomography study. J Urol 2006;175(3 Pt 1):994-8.
14. Mehnert U, Michels L, Zempleni MZ, Schurch B, Kollias S. The supraspinal neural correlate of bladder cold sensation–an fMRI study. Hum Brain Mapp 2011;32(6):835-45.
15. Biswal BB, Van Kylen J, Hyde JS. Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps. NMR Biomed 1997;10(4-5):165-70.
16. Cauda F, Costa T, Torta DM, Sacco K, D'Agata F, Duca S, et al. Meta-analytic clustering of the insular cortex: characterizing the meta-analytic connectivity of the insula when involved in active tasks. Neu-roimage 2012;62(1):343-55.
17. Greicius MD, Supekar K, Menon V, Dougherty RF. Resting-state functional connectivity reflects struc-tural connectivity in the default mode network. Cereb Cortex 2009;19(1):72-8.
18. Gusnard DA, Akbudak E, Shulman GL, Raichle ME. Medial prefrontal cortex and self-referential men-tal activity: relation to a default mode of brain function. Proc Natl Acad Sci USA 2001;98(7):4259-64.
19. Gusnard DA, Raichle ME. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2001;2(10):685-94.
20. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001;98(2):676-82.
21. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. Biophys J 1994;66(1):259-67.
22. Théaudin M, Saliou G, Denier C, Adams D, Ducreux D. A correlation between fractional anisotropy variations and clinical recovery in spinal cord infarctions. J Neuroimaging 2013;23(2):256-8.

23. van der Jagt PK, Dik P, Froeling M, Kwee TC, Nievelstein RA, ten Haken B, et al. Architec-tural configuration and microstructural properties of the sacral plexus: a diffusion tensor MRI and fiber tractography study. Neuroimage 2012;62(3):1792-9.
24. Andrew J, Nathan PW. Lesions on the Anterior Frontal Lobes and Disturbances of Micturi-tion and Defaecation. Brain 1964;87:233-62.
25. Holstege G. Micturition and the soul. J Comp Neurol 2005;493(1):15-20.
26. de Groat WC. A neurologic basis for the overactive bladder. Urology 1997;50(6A Suppl):36-52; discussion 53-6.
27. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000;11(6 Pt 1):805-21.
28. Caceres A, Hall DL, Zelaya FO, Williams SC, Mehta MA. Measuring fMRI reliability with the intra-class correlation coefficient. Neuroimage 2009;45(3):758-68.
29. Michels L, Mehnert U, Boy S, Schurch B, Kollias S. The somatosensory representation of the human clitoris: an fMRI study. Neuroimage 2010;49(1):177-84.
30. Jarrahi B, Wanek J, Mehnert U, Kollias S. An fMRI-compatible multi-configurable handheld response system using an intensity-modulated fiber-optic sensor. Conf Proc IEEE Eng Med Biol Soc 2013;2013:6349-52.
31. Agency for Healthcare Research and Quality. Treatment of Overactive Bladder in Women, 2009.
32. Gormley EA, Lightner DJ, Burgio KL, Chai TC, Clemens JQ, Culkin DJ, et al. Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline. J Urol 2012;188(6 Suppl):2455-63.
33. International conference on harmonisation. Good clinical practice guideline, 1996.
34. International organization for standardization. ISO 14155, 2011.
35. Kucyi A, Moayedi M, Weissman-Fogel I, Hodaie M, Davis KD. Hemispheric asymmetry in white matter connectivity of the temporoparietal junction with the insula and prefrontal cor-tex. PLoS One 2012;7(4):e35589.
36. Maldjian JA, Davenport EM, Whitlow CT. Graph Theoretical Analysis of Resting-State MEG data: Identifying Interhemispheric Connectivity and the Default Mode. Neuroimage 2014;96:88-94.
37. Andersson JL, Hutton C, Ashburner J, Turner R, Friston K. Modeling geometric deformations in EPI time series. Neuroimage 2001;13(5):903-19.
38. Friston KJ, Holmes AP, Poline JB, Grasby PJ, Williams SC, Frackowiak RS, et al. Analysis of fMRI time-series revisited. Neuroimage 1995;2(1):45-53.
39. World Medical Association. Declaration of Helsinki - Ethical principles for medical research involving human subjects, 1964.
40. Swiss Academy of Medical Sciences. Guideline - Concerning scientific research involving human beings, 2009.
41. The Federal Authorities of the Swiss Confederation. Bundesgesetz über den Datenschutz (DSG) vom 19. Juni 1992, Stand. 01.01.2011, 1992.

CHAPTER 6

Protocol for a prospective magnetic resonance imaging study on supraspinal lower urinary tract control in healthy subjects and spinal cord injury patients undergoing intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity

Leitner L*, **Walter M***, Freund P, Mehnert U, Michels L, Kollias S, Kessler TM
BMC Urol. 2014 Aug 18;14(1):68. (*Equal contribution, i.e. shared-first authorship)

ABSTRACT

Background

The control of the lower urinary tract is a complex, multilevel process involving both the peripheral and central nervous system. Due to lesions of the neuraxis, most spinal cord injury patients suffer from neurogenic lower urinary tract dysfunction, which may jeopardise upper urinary tract function and has a negative impact on health-related quality of life. However, the alterations to the nervous system following spinal cord injury causing neurogenic lower urinary tract dysfunction and potential effects of treatments such as intradetrusor onabotulinumtoxinA injections on lower urinary tract control are poorly understood.

Methods/Design

This is a prospective structural and functional magnetic resonance imaging study investigating the supraspinal lower urinary tract control in healthy subjects and spinal cord injury patients undergoing intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity. Neuroimaging data will include structural magnetic resonance imaging (T1-weighted imaging and diffusion tensor imaging) as well as functional, i.e. blood oxygen level-dependent sensitive magnetic resonance imaging using a 3 T magnetic resonance scanner. The functional magnetic resonance imaging will be performed simultaneously to three different bladder stimulation paradigms using an automated magnetic resonance compatible and synchronised pump system. All subjects will undergo two consecutive and identical magnetic resonance imaging measurements. Healthy subjects will not undergo any intervention between measurements but spinal cord injury patients will receive intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity. Parameters of the clinical assessment including bladder diary, urinalysis, medical history, neuro-urological examination, urodynamic investigation as well as standardised questionnaires regarding lower urinary tract function and quality of life will serve as co-variates in the magnetic resonance imaging analysis.

Ethics and Dissemination

This study will identify structural and functional alterations in supraspinal networks of lower urinary tract control in spinal cord injury patients with neurogenic detrusor overactivity compared to healthy controls. Post-treatment magnetic resonance imaging measurements in spinal cord injury patients will provide further insights into the mechanism of action of treatments such as intradetrusor onabotulinumtoxinA injections and the effect on supraspinal lower urinary tract control.

Trial registration: ClinicalTrials.gov NCT01768910.

BACKGROUND

Spinal cord injury (SCI) is a devastating event with far-reaching consequences for the individual's health and the economic and social future. In the past, renal failure due to neurogenic lower urinary tract dysfunction (NLUTD) was a leading cause of death after SCI [63]. Furthermore, NLUTD has a highly negative impact on patients' quality of life (QoL). Acute SCI initially causes "spinal shock", characterised by an acontractile/hypocontractile detrusor and urinary retention, which in case of a suprasacral lesion (today the vast majority of SCI) is followed by development of detrusor overactivity mostly combined with detrusor sphincter dyssynergia [64]. Antimuscarinics are the pharmacological first-line treatment for detrusor overactivity although the effectiveness is limited [65]. In addition, many patients discontinue antimuscarinics due to bothersome side-effects [66]. Thus, intradetrusor onabotulinumtoxinA injections have become an established, highly effective, minimally invasive, and generally well-tolerated therapy for refractory detrusor overactivity [67] to improve patients' health and QoL [68]. Despite the popularity of intradetrusor onabotulinumtoxinA injections, the exact mechanisms of action remain to be elucidated. Nevertheless, it seems highly probable that, in addition to a direct efferent effect by blocking the presynaptic release of acetylcholine from the parasympathetic innervation resulting in temporary chemodenervation of the detrusor, onabotulinumtoxinA also modulates afferent pathways [69]. It is, however, not known whether this treatment can normalise alterations in supraspinal areas and whether supraspinal modulation correlates with clinical improvements. The control of the lower urinary tract (LUT) is a complex, multilevel process that involves both the peripheral and central nervous system but the exact mechanisms involved in humans are still incompletely understood [70]. Neuroimaging studies over the last decade have consistently pointed to a complex supraspinal network that controls LUT function [71]. These studies tremendously increased our understanding of how human LUT

function is coordinated and how it can be affected by neurological disorders. Recent neuroimaging studies demonstrated reorganisation of supraspinal activity in response to LUT stimulation tasks in patients with disorders such as Parkinson's disease [72-74] and SCI [75] as compared to healthy controls, which might represent the neural correlate of their NLUTD. Cortical and subcortical (for example, brainstem) brain regions are essential for voluntary LUT control [71, 76, 77]. Investigation of the supraspinal regions with high-resolution imaging techniques, for example, structural magnetic resonance imaging (MRI) and functional MRI (fMRI), can significantly increase our knowledge on the effects of supraspinal lesions and alterations related to NLUTD [71, 78]. Although diffusion tensor imaging (DTI) [79] is popular in other fields in neuroscience, it has only been applied in the context of supraspinal LUT control in one prospective study [80] in patients with non-neurogenic LUT symptoms. In this study, we will first identify supraspinal areas associated with LUT control in SCI patients with neurogenic detrusor overactivity and healthy controls. Subsequently, we will investigate the effects of intradetrusor onabotulinumtoxinA injections on supraspinal areas. Task-related blood oxygen level-dependent (BOLD) fMRI will be used along structural MRI (T1-weighted and DTI). Moreover, we will examine volumetric parameters (for example, grey and white matter concentration) by voxel- (VBM) [81] and tensor-based morphometry (TBM) [82], structural integrity and connectivity of white matter tracts (DTI) as well as structural (SC) and functional connectivity (FC). This unique and detailed multimodal imaging and clinical approach will distinguish the different structural and functional processing units involved during supraspinal LUT control and will identify dysfunctional neuronal components in SCI patients responsible for neurogenic detrusor overactivity. For a test-retest validation, we will additionally investigate the reliability [83] of BOLD signals in task-related fMRI in healthy controls by the intra-class correlation coefficient (ICC) for absolute or consistent agreement of participants activations

over two visits.

METHODS/DESIGN

Study design

This prospective research study will be conducted at the University of Zürich, Zürich, Switzerland in cooperation with our partner, the institute of Neuro-Radiology, University of Zürich, University Hospital Zürich, Zürich, Switzerland.

Study location

The study has two study locations, that is, the department of Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland (first and third visit, see below) and the MR-Centre, University Hospital Zürich, Zürich, Switzerland (second and third visit, see below).

Study population and recruitment

In line with the inclusion and exclusion criteria (Table 1), we will investigate SCI patients with neurogenic detrusor overactivity and healthy controls with an unimpaired LUT function. Participants of both groups will be similar according to age and gender. 20-24 SCI patients with neurogenic detrusor overactivity refractory to antimuscarinics and scheduled for study independent intradetrusor onabotulinum-toxinA injections and 12-24 healthy controls will be recruited.

Table 1 – Inclusion and exclusion criteria for all participants.

Groups	Inclusion criteria	Exclusion criteria
All participants	▪ MR suitability ▪ Written informed consent	▪ Pregnancy or breast feeding ▪ Any anatomical anomaly of LUT/genitalia ▪ Any LUT malignancy ▪ Claustrophobia
SCI patients	▪ Age limit: > 18 years ▪ Neurogenic detrusor overactivity ▪ Refractory to antimuscarinic treatment ▪ Scheduled for intradetrusor onabotulinumtoxinA injections	▪ Symptomatic UTI
Healthy controls	▪ Age limit: > 18 years ▪ Unimpaired LUT function ▪ No LUTS (3-day bladder diary) ▪ No episode of urinary urgency/week ▪ Urinary frequency < 8/24h	▪ Impaired LUT function ▪ Any LUTS (3-day bladder diary) ▪ Any number of episodes of urinary urgency/week ▪ Urinary frequency > 8/24h ▪ Any craniocerebral injury or surgery ▪ Any permanent ferromagnetic implant ▪ Any previous surgery of LUT/genitalia ▪ UTI ▪ PVR > 150mL

PVR, postvoid residual; LUT, lower urinary tract; LUTS, lower urinary tract symptoms; UTI, urinary tract infection; MR, magnet resonance, SCI, spinal cord injury.

Interventions

Subjects providing written informed consent will be invited for the following visits (Figure 1):

Screening (Visit 1):

Evaluation for study eligibility will be based on medical history, urinalysis to exclude urinary tract infection (UTI) and pregnancy in female participants, 3-day bladder diary, post void residual, urodynamic parameters as well as on standardised validated questionnaires such as Qualiveen [84] and International Consultation on Incontinence modular questionnaire [(ICIQ), Bristol Urological Institute, Southmead Hospital Bristol, UK] assessing lower urinary tract symptoms (LUTS) in both woman (ICIQ-FLUTS) and men (ICIQ-MLUTS).

MRI measurements (Visit 2 and 3, Figure 1):

Two MRI measurements will be performed in identically manner within a 5 to 7 week interval. Both MRI measurements will be performed using a Philips Ingenia 3 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with a 16-channel head coil to acquire the following sequences (Figure 2):

- Structural sequences will comprise T1-weighted and DTI.
- Functional sequences will comprise three different task-related fMRI paradigms (Figure 3).
    - In the first two task-related fMRI paradigms, we will examine the effect of visceral bladder sensation by automated, repetitive bladder filling with 100mL body warm (37 °C) saline starting with an empty bladder (first paradigm) and a bladder volume eliciting desire to void (second paradigm).
    - The third task-related fMRI paradigm will contain automated, repetitive filling of 100mL cold (4-8 °C) saline starting with an empty bladder.

Repetitive filling will be performed using an automated MR-compatible and MR-synchronised pump system [80] to precisely fill and drain the bladder. During the MRI measurements, all participants will use an MR-compatible handheld response system [85] to rate their desire to void and level of pain on a displayed visual analogue scale.

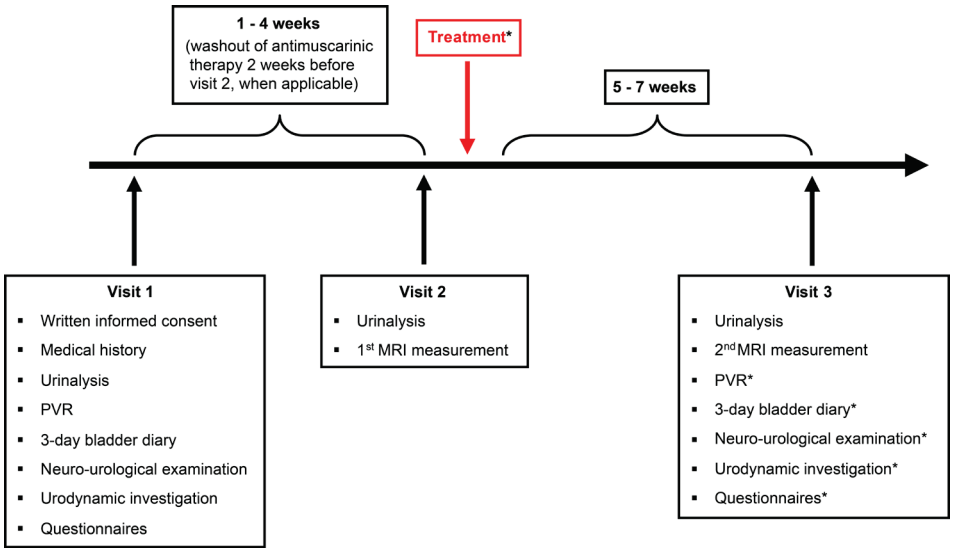

Figure 1 – Timetable and characteristics of all visits.
* that is intradetrusor onabotulinumtoxinA injections (patients only), PVR, post void residual; MRI, magnetic resonance imaging.

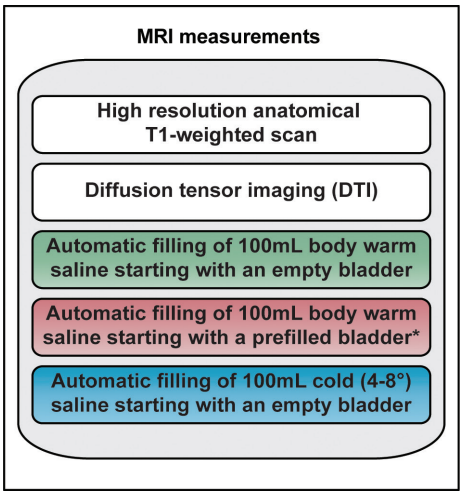

Figure 2 – Sequences of magnetic resonance imaging (MRI) measurements.
* Bladder will be filled with body warm saline until a persistent desire to void is present.

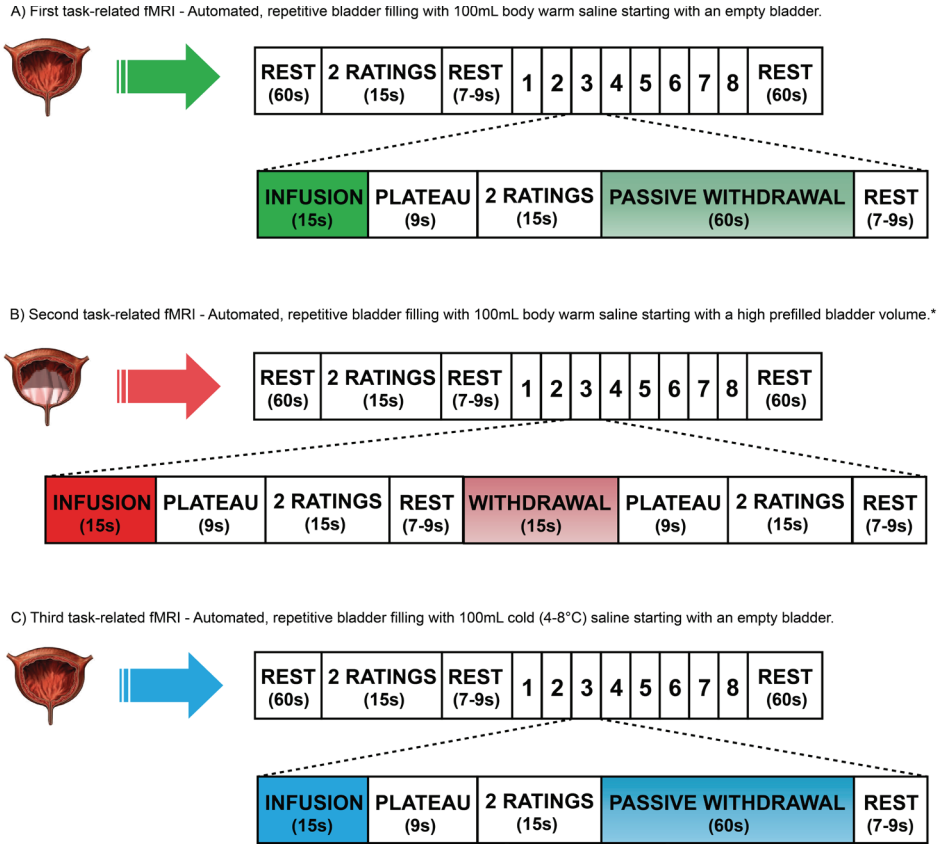

Figure 3 – Scan paradigm of three different task-related functional MRIs (fMRIs).
All task-related fMRIs identically start with a 'baseline' rest (60 s, no specific stimulus or task is performed), a 'baseline' rating (desire to void and level of pain), a short rest (jittered between 7 and 9 s in which blood oxygen level-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition) and conclude with a 'last' rest (60 s, no specific stimulus or task is performed).
All task-related fMRIs consist of eight repetitive blocks, each with either five (first and third fMRIs) or eight (second fMRI) conditions.
(A) Conditions of the first task-related fMRIs: (1) automated infusion of 100 mL body warm saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating, (4) passive withdrawal to empty the bladder completely and (5) short rest. This task-related fMRI starts with an empty bladder.
(B) Conditions of the second task-related fMRIs: (1) automated infusion of 100 mL warm saline, (2) plateau phase, (3) rating, (4) short rest, (5) automated withdrawal of 100 mL, (6) plateau phase (bladder distention after withdrawal is perceived), (7) rating and (8) short rest. *This task-related fMRI (B) starts with a high prefilled bladder volume, that is, the bladder will be filled with body warm saline until a persistent desire to void is present
(C) Conditions of the third task-related fMRIs: (1) automated infusion of 100 mL cold (4–8°C) saline, (2) plateau phase, (3) rating, (4) passive withdrawal to empty the bladder completely and (5) short rest. This task-related fMRI starts with an empty bladder.

Study outcome measures

Primary

(A) Task-related BOLD signal intensity in supraspinal regions of interest (ROI) in SCI patients with neurogenic detrusor overactivity compared to healthy controls.

(B) Supraspinal morphometry of ROIs in SCI patients with neurogenic detrusor overactivity compared to healthy controls.

(C) SC and FC between ROIs of the supraspinal LUT controlling circuitries in SCI patients with neurogenic detrusor overactivity and healthy controls.

(D) Changes of task-related BOLD signal intensity, SC and FC between ROIs in SCI patients with neurogenic detrusor overactivity before and after study independent intradetrusor onabotulinumtoxinA injections.

Secondary

(E) Reliability of BOLD signal changes between first and second MRI measurement in healthy controls.

(F) Correlations between clinical co-variates that are bladder diary parameters, urodynamic parameters, level of desire to void during fMRI and task-related BOLD signal intensity in supraspinal ROIs as well as structural parameters, that is, grey matter volume and number of white matter tracts between ROIs.

Data analysis

Clinical data, for example, questionnaires scores, urodynamic parameters and 3-day bladder diary outcomes will be statistically analysed and compared between groups using IBM's Statistical Package for the Social Sciences (SPSS) version 19.0 or newer (Armonk, New York, U.S.). The statistical analysis will be presented with means and standard deviations or with medians and interquartile ranges as appropriate. For the analysis of the neuroimaging

data, we will use statistical parametric mapping (SPM) V.8 or newer (Wellcome Department of Imaging Neuroscience, University College London, UK) and toolboxes as appropriate.

Regulatory issues

Ethical approval

This study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346) and will be performed in accordance to the World Medical Association Declaration of Helsinki [86], the guidelines for Good Clinical Practice (GCP) [87], and the guidelines of the Swiss Academy of Medical Sciences [88]. Handling of all personal data will strictly comply with the federal law of data protection in Switzerland [89].

Safety

According to the safety regulations of the MR-Centre of the University Hospital Zürich, the staff involved in this study will be instructed and trained. Prior to entering the scanner room, all participants will be asked to remove any ferromagnetic items (for example, bra, chains, earrings, rings, and piercings). All participants will be provided with standardised clinical scrubs to prevent incidental import of ferromagnetic items into the MR room. To exclude UTI or pregnancy, urinalysis will be performed on every participant before urodynamic investigations and MR measurements. In case of pregnancy, the participant will be excluded from the study and referred to a gynaecologist. In case of UTI, the participant will not undergo the experiment but will be treated appropriately. After successful treatment, a reassignment to the study is possible. In case of an adverse event (AE) or a severe adverse event (SAE), as defined by the International Organization for Standardization (ISO, 14155) [90] and the International Conference on Harmonisation (ICH) GCP guidelines (E6) [87], responsible authorities, that is, the principle investigator and the

ethics committee will be informed. Appropriate actions will be executed. All AEs and SAEs will be followed as long as medically indicated.

Funding

The Swiss National Science Foundation (grant number: 135774), Wings for Life, the Emily Dorothy Lagemann Stiftung and the Swiss Continence Foundation are funding this study.

DISCUSSION

This study will investigate structural and functional abnormalities and specific alterations in the brain networks of supraspinal LUT control in SCI patients with neurogenic detrusor overactivity compared to healthy controls using a multimodal imaging protocol. Importantly, effects on the supraspinal LUT control after treatment for neurogenic detrusor overactivity with intradetrusor onabotulinumtoxinA injections in SCI patients will be explored. The findings will help to verify, amend, or adjust neuronal circuitry models established from findings in healthy controls, now in the context of SCI patients with neurogenic detrusor overactivity. Furthermore, it will show whether neurogenic detrusor overactivity specific treatment such as intradetrusor onabotulinumtoxinA injections induce structural or functional reorganisation in supraspinal areas related to LUT control and in how far such changes correlate with improvements of clinical outcome parameters. These investigations will help us to understand how onabotulinumtoxinA modulates afferent pathways. Advanced neuroimaging and evaluation techniques have the potential to serve as quantifiable outcome measures for therapy success and for improving our treatment strategies in this patient population.

Trial status

The trial is in the recruiting phase at the time of manuscript submission.

List of abbreviations

AE: adverse event; BOLD: blood oxygen level-dependent; DTI: diffusion tensor imaging; FA: fractional anisotropy; FC: functional connectivity; fMRI: functional magnetic resonance imaging; GLM: general linear model; GCP: good clinical practice; ICC: intra-class correlation coefficient; ICH: International Conference on Harmonisation; ISO: International Organization for Standardization; LUT: lower urinary tract; LUTS: lower urinary tract symptoms; MR: magnetic resonance; MRI: magnetic resonance imaging; MD: mean diffusivity; MNI: Montreal Neurologic Institute; NLUTD: neurogenic lower urinary tract dysfunction; PVR: post void residual QoL: quality of life; ROI: regions of interest; SAE: severe adverse event; SPM: statistical parametric mapping; SC: structural connectivity; SCI: spinal cord injury; TBM: tensor-based morphometry; UTI: urinary tract infection; VBM: voxel-based morphometry

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in creating the study design. LL, MW, and TMK drafted the manuscript. PF, UM, LM, and SK provided a critical revision of the manuscript. PF, UM, SK and TMK obtained the funding of this study. All the authors read and approved the final manuscript.

Acknowledgements

We would like to acknowledge Wings for Life, Swiss National Science Foundation, Emily Dorothy Lagemann Stiftung, and Swiss Continence Foundation for financial support.

Author details

[1] Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland

[2] Institute of Neuro-Radiology, University of Zürich, University Hospital Zürich, Zürich, Switzerland

REFERENCES

1. Hackler RH: A 25-year prospective mortality study in the spinal cord injured patient: comparison with the long-term living paraplegic. *J Urol* 1977, 117:486-488.
2. Pannek J, Blok B, Castro-Diaz D, del Popolo G, Groen J, Karsenty G, Kessler TM, Kramer G, Stöhrer M: Guidelines on Neuro-Urology, European Association of Urology 2014. [www.uroweb.org/gls/pdf/21%20Neuro-Urology_LR.pdf]
3. Buser N, Ivic S, Kessler TM, Kessels AG, Bachmann LM: Efficacy and adverse events of antimuscarinics for treating overactive bladder: network meta-analyses. *Eur Urol* 2012, 62:1040-1060.
4. Kessler TM, Bachmann LM, Minder C, Lohrer D, Umbehr M, Schunemann HJ, Kessels AG: Adverse event assessment of antimuscarinics for treating overactive bladder: a network meta-analytic approach. *PLoS One* 2011, 6:e16718.
5. Wollner J, Kessler TM: Botulinum toxin injections into the detrusor. *BJU Int* 2011, 108:1528-1537.
6. Chancellor MB, Patel V, Leng WW, Shenot PJ, Lam W, Globe DR, Loeb AL, Chapple CR: OnabotulinumtoxinA improves quality of life in patients with neurogenic detrusor overactivity. *Neurology* 2013, 81:841-848.
7. 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, 49:644-650.
8. Fowler CJ, Griffiths D, de Groat WC: The neural control of micturition. *Nat Rev Neurosci* 2008, 9:453-466.
9. Fowler CJ, Griffiths DJ: A decade of functional brain imaging applied to bladder control. *Neurourol Urodyn* 2010, 29:49-55.
10. Herzog J, Weiss PH, Assmus A, Wefer B, Seif C, Braun PM, Herzog H, Volkmann J, Deuschl G, Fink GR: Subthalamic stimulation modulates cortical control of urinary bladder in Parkinson's disease. *Brain* 2006, 129:3366-3375.
11. Herzog J, Weiss PH, Assmus A, Wefer B, Seif C, Braun PM, Pinsker MO, Herzog H, Volkmann J, Deuschl G, Fink GR: Improved sensory gating of urinary bladder afferents in Parkinson's disease following subthalamic stimulation. *Brain* 2008, 131:132-145.
12. Kitta T, Kakizaki H, Furuno T, Moriya K, Tanaka H, Shiga T, Tamaki N, Yabe I, Sasaki H, Nonomura K: Brain activation during detrusor overactivity in patients with Parkinson's disease: a positron emission tomography study. *J Urol* 2006, 175:994-998.
13. Mehnert U, Michels L, Zempleni MZ, Schurch B, Kollias S: The supraspinal neural correlate of bladder cold sensation–an fMRI study. *Hum Brain Mapp* 2011, 32:835-845.
14. Andrew J, Nathan PW: Lesions on the Anterior Frontal Lobes and Disturbances of Micturition and Defaecation. *Brain* 1964, 87:233-262.
15. Holstege G: Micturition and the soul. *J Comp Neurol* 2005, 493:15-20.
16. de Groat WC: A neurologic basis for the overactive bladder. *Urology* 1997, 50:36-52; discussion 53-36.
17. Basser PJ, Mattiello J, LeBihan D: MR diffusion tensor spectroscopy and imaging. *Biophys J* 1994, 66:259-267.
18. Walter M, Michels L, Kollias S, van Kerrebroeck PE, Kessler TM, Mehnert U: Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms. *BMJ Open* 2014, 4:e004357.
19. Ashburner J, Friston KJ: Voxel-based morphometry–the methods. *Neuroimage* 2000, 11:805-821.
20. Ashburner J, Friston KJ: Diffeomorphic registration using geodesic shooting and Gauss-Newton optimisation. *Neuroimage* 2011, 55:954-967.
21. Caceres A, Hall DL, Zelaya FO, Williams SC, Mehta MA: Measuring fMRI reliability with the intra-class correlation coefficient. *Neuroimage* 2009, 45:758-768.
22. Costa P, Perrouin-Verbe B, Colvez A, Didier J, Marquis P, Marrel A, Amarenco G, Espirac B, Leriche A: Quality of life in spinal cord injury patients with urinary difficulties. Development and validation of qualiveen. *Eur Urol* 2001, 39:107-113.

23. Jarrahi B, Wanek J, Mehnert U, Kollias S: An fMRI-compatible multi-configurable handheld response system using an intensity-modulated fiber-optic sensor. *Conf Proc IEEE Eng Med Biol Soc* 2013, 2013:6349-6352.
24. World Medical Association: Declaration of Helsinki - Ethical principles for medical research involving human subjects, 1964 [http://www.wma.net/en/30publications/10policies/b3/]
25. International conference on harmonisation: Good clinical practice guideline [http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html]
26. Swiss Academy of Medical Sciences: Guideline - Concerning scientific research involving human beings. 2009.
27. The Federal Authorities of the Swiss Confederation: Bundesgesetz über den Datenschutz (DSG) vom 19. Juni 1992, Stand. 01.01.2011. 1992.
28. International organization for standardization, ISO 14155 [http://www.iso.org/iso/catalogue_detail?csnumber=45557]

CHAPTER 7

A novel infusion-drainage device to assess lower urinary tract function in neuro-imaging

Leitner L*, **Walter M***, Jarrahi B, Wanek J, Diefenbacher J, Michels L, Liechti MD, Kollias SS, Kessler TM, Mehnert U
BJU Int. 2017 Feb;119(2):305-316. (*Equal contribution, i.e. shared-first authorship)

ABSTRACT

Objectives

To evaluate the applicability and precision of a novel infusion-drainage device (IDD) for standardized filling paradigms in neuro-urology and functional magnetic resonance imaging (fMRI) studies of lower urinary tract (LUT) function/dysfunction.

Subjects/Patients and Methods

The IDD is based on electrohydrostatic actuation which was previously proven feasible in a prototype setup. The current design includes hydraulic cylinders and a motorized slider to provide force and motion. Methodological aspects have been assessed in a technical application laboratory as well as in healthy subjects (n=33) and patients with LUT dysfunction (n=3) undergoing fMRI during bladder stimulation. After catheterization, the bladder was pre-filled until a persistent desire to void was reported by each subject. The scan paradigm comprised automated, repetitive bladder filling and withdrawal of 100 mL body warm (37 °C) saline, interleaved with rest and sensation rating. Neuroimaging data were analysed using Statistical Parametric Mapping (SPM) 12.

Results

Volume delivery accuracy was between 99.1±1.2% and 99.9±0.2%, for different flow rates and volumes. Magnetic resonance (MR) compatibility was demonstrated by a small decrease in signal-to-noise ratio (SNR), i.e. 1.13% for anatomical and 0.54% for functional scans, and a decrease of 1.76% for time-variant SNR. Automated, repetitive bladder filling elicited robust (P = 0.05, familiy-wise error corrected) brain activity in areas previously reported to be involved in supraspinal LUT control. There was a high synchronism between the LUT stimulation and the blood oxygenation level-dependent (BOLD) signal changes in such areas.

Conclusions

We were able to develop an MR-compatible and MR-synchronized IDD to routinely stimulate the LUT during fMRI in a standardized manner. The device provides LUT stimulation at high system accuracy resulting in significant supraspinal BOLD signal changes in interoceptive and LUT control areas in synchronicity to the applied stimuli. The IDD is commercially available, portable and multi-configurable. Such a device may help to improve precision and standardization of LUT tasks in neuro-imaging studies on supraspinal LUT control, and may therefore facilitate multisite studies and comparability between different LUT investigations in the future.

INTRODUCTION

Over the last decades neuro-imaging studies have improved and expanded our knowledge of supraspinal lower urinary tract (LUT) function [1]. Earlier, pioneering studies used single-photon emission CT [2] and positron emission tomography [3]. It was not until 2005 that functional magnetic resonance imaging (fMRI) was first used to examine supraspinal LUT control [4–6]. Since then, fMRI has emerged as the most popular approach in this field, as it can assess correlates of functional processes involved in LUT control in the human brain non-invasively, with a high spatial and acceptable temporal resolution [7]. Nevertheless, performing LUT stimulation tasks within a magnetic resonance (MR) environment is still a challenge, as the low signal-to-noise ratio (SNR) in echo-planar imaging (EPI) sequences as used in fMRI requires repeated captures of task-related data [8], i.e. repeated cycles of bladder filling and emptying [1]. Yet, most currently available devices that can be used for bladder filling are neither MR compatible, nor adapted to MR synchronization. Furthermore, they have only limited speed and accuracy of filling, and/or are not capable of also performing fluid withdrawal. Hence, recent fMRI studies used different approaches, i.e. manual and semiautomatic bladder filling protocols, to assess supraspinal network response to bladder filling and distention. Not surprisingly, the significance of supraspinal network activation and localization varies largely among studies [1,9–11]; however, to analyse and interpret neuroimaging findings reliably based on visceral stimulation tasks, it is necessary to correlate task performance precisely with the neuroimaging signal acquisition [12]. To facilitate standardization and precision of task-related LUT neuroimaging studies we developed, based on our prototype [13], a commercially available, fully automated, flexibly programmable, MR-compatible and MR-synchronized infusion-drainage device (IDD).

SUBJECTS/PATIENTS AND METHODS

Infusion-Drainage Device

The MR-compatible IDD is based on an electrohydrostatic actuation principle [13]. All components inside the MR scanner room are non-ferromagnetic and operated from the MR control room using a personal computer. A schematic overview of the arrangement of the IDD within the MR scanner facility is shown in Figure 1.

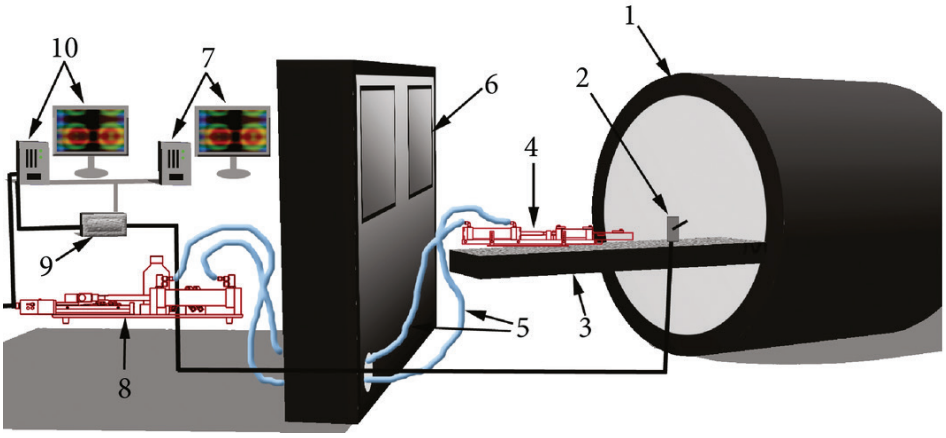

Figure 1 - Schematic overview of the experimental setup and arrangement in the magnetic resonance (MR) facility.
1, MR scanner; 2, handheld response system (HRS); 3, moveable examination table; 4, slave cylinder assembly group, which connects the transurethral catheter with the bladder filling syringe and is placed between the legs of the subject/patient; 5, polyurethane tubing; 6, partition wall with electromagnetic shielding between MR scanner and MR control room; 7, MR control workstation; 8, master cylinder assembly group; 9, relay box with circuit board; 10, complementary PC to run the infusion-drainage device and HRS operational software. PC, personal computer

The key components of the IDD (Figure 2) consist of two polypropylene hydraulic cylinders (Typ 1108 DN40; PSK Ingenieurgesellschaft GmbH, Erfurt, Germany), i.e. a master cylinder with a stainless-steel piston rod, a slave cylinder with a plastic piston rod, and a bipolar stepper motor controlling a linear slider with a moving platform (T-LSR 150 B; Zaber Technologies Inc., Vancouver, Canada). The cylinders are bidirectly coupled through a 10-m flexible polyurethane tubing with an outer diameter of 5 mm and a wall thickness of

1.5mm (TFU0805B-2; SMC Corp., Tokyo, Japan). The system operates with distilled water. The piston rod of the master cylinder (Figures 2C and D) is fixed to the moving platform of the computer-controlled motorized linear slider system (travel range of 150 mm at a 0.5-lm resolution; speed range 0.00465mm/s to 20 mm/s). Movement of the linear slider causes motion in the master piston rod, and a fluid flow, and pressure change in the hydraulic actuator. As a result of the pressure changes, fluid transmits the energy through the tube causing a controllable bi-directional (positive or negative pressure) movement of the slave piston. The slave piston (Figures 2E and F) is connected to a commercially available, standard single-use 100-mL polypropylene syringe (Ominifix; B Braun Melsungen AG, Melsungen, Germany; movement of the syringe piston of 1mm corresponds to 1 mL). The subject's bladder can be filled and drained through a single-use transurethral catheter attached to the syringe (Figure 2E). To aerate the IDD and prevent a vacuum, a water-filled reservoir (Figures 2C and D) is integrated parallel to the cylinders and connected over check valves (AKH08A; SMC Corp., Tokyo, Japan). Pressure transducers (PAA-21 Y; Keller AG für Druckmesstechnik, Winterthur, Switzerland) allow a real-time tracing of the pressures in the IDD to monitor whether the system works appropriately or not. Similarly to our prototype setup [13], we included a 12-bit multifunction USB data acquisition device (USB-6008; National Instruments, TX, USA) as an interface between the IDD and MRI scanner, and also to sample signals from the IDD. During fMRI, the data acquisition device also detects the transistor–transistor logic signals outputted by the MR scanner to synchronize the system with the image acquisition. Using the data acquisition device, multiple feedback devices, such as a fibre optic-based multi-configurable handheld response system (HRS) [14] to assess real-time sensory feedback (described later), or a transducer for measuring intravesical pressure, can be implemented.

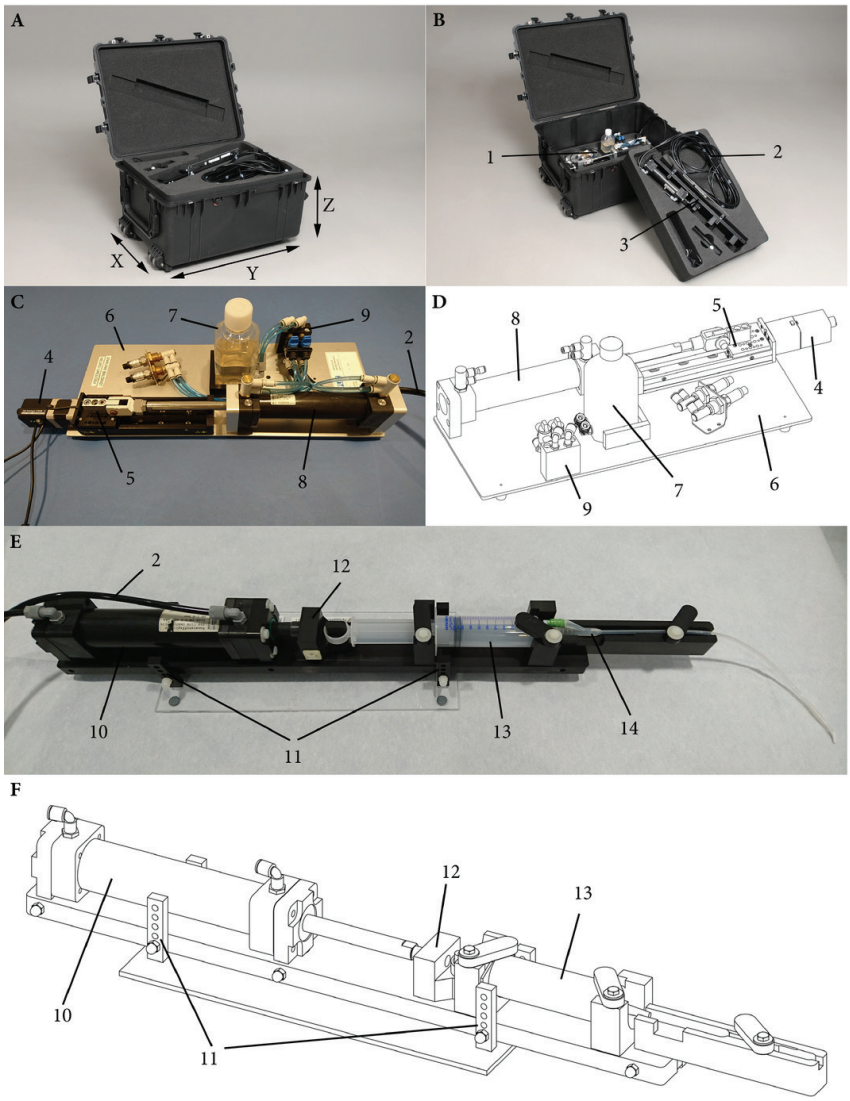

Figure 2 - The transport case and technical components of the infusion-drainage device (IDD).
For easy transportation and safe storage, the IDD can be placed into a wheeled hard-top case molded with foam x = 62 cm, y = 80 cm, z = 45 cm (A,B). The three main components of the IDD are the hydraulic master cylinder assembly group (C,D), a bipolar stepper motor driving the piston of the master cylinder (C), and a hydraulic slave cylinder assembly group (E,F). 1, master cylinder assembly group stored in the hard-top case; 2, polyurethane tubing (10 m) that connects the master and slave cylinders; 3, slave cylinder assembly group; 4, bipolar stepper motor (T-LSR 150 B, Zaber Technologies Inc., Vancouver, Canada); 5, linear slider with moving platform and fixation to the piston rod of the master cylinder; 6, base plate of the master cylinder assembly group; 7, water reservoir for fast and easy deairing; 8, hydraulic master cylinder made of polypropylene; 9, distribution board with check valves; 10, hydraulic slave cylinder made of polypropylene; 11, fixation rack of the slave cylinder with the possibility to adapt the height to the individual subject/patient; 12, slider, connecting the piston of the slave cylinder with the piston of the syringe containing the bladder filling fluid; 13, standard single-use 100-mL polypropylene syringe; 14, Foley catheter for transurethral placement.

Operational Software

The real-time custom-made operational control software for this device is based on C/C++ using Visual Studio 2008 (Microsoft, Redmond, WA, USA) [13]. The National Instruments LabViewTM software package (National Instruments, TX, USA) as well as the built-in motion controller provided by Zaber Technologies (Vancouver, Canada) are implemented. The software offers an all-in-one control tool to operate the IDD and also additional devices, such as the HRS, to quantitatively measure responses to the stimulus/task generated by the IDD, e.g. degree of desire to void. Adjusting the stepper motor variables, flow rate, time and volume, as well as the numbers of repetitions, can be accomplished by the operational software. Synchronization with the MR scanner is achieved via the transistor–transistor logic signal outputted by the MR scanner at the onset of each EPI volume of functional series. Only the first transistor–transistor logic signal is used to start the computerized and temporal standardized stimulation paradigm. IDD and HRS data are stored in a separate log file.

Infusion-Drainage Device Performance Test

To evaluate the performance quality of the IDD, volume delivery accuracy was tested at different flow rates, i.e. 80, 133, 200 and 400 mL/min, and different infusion times, i.e. 3, 7.5, 15, 30 and 45 s, using an Urocap IV scale (Laborie International, Ontario, Canada). According to the feasibility test in humans (eight blocks, infusion/withdrawal volume of 100 mL at a flow rate of 400 mL/min, see below), eight blocks of repetitive infusion and withdrawal cycles, using a 0.9% sodium chloride solution (Braun Melsungen AG, Melsungen, Germany; for the IDD performance test at room temperature, ~25 °C), were performed.

MRI

We evaluated MR compatibility using a Philips Ingenia 3 Tesla MRI system (Philips Medical Systems, Best, The Netherlands) at the University Hospital Zürich equipped with a standard 15-channel head coil array and a proton sphere phantom filled with 5 mL/L 98% acetate (CH3COOH), 10 mL/L 80% ethanol (CH3CH2OH), 8mL/L 98% phosphorus acid (H3PO4), 1 mL/L 1% Arquad® solution, 120 mg/L anhydrous copper sulphate (CuSO4), and 380 mL of demineralized water. fMRI Images were acquired using an EPI T2*- sensitive sequence (echo time = 30 ms, repetition time = 2000 ms, flip angle = 80°, field of view = 240 mm x 135mm x 240 mm, image matrix = 96 x 96, voxel size = 3 mm x 3mm x 3 mm, number of slices 34). Further, we collected a high-resolution three-dimensional T1-weighted gradient echo sequence (echo time = 3.1 ms, repetition time 6.9 ms, flip angle = 8°, field of view 256 mm x 256 mm x 180 mm, imaging matrix = 256 mm x 256 mm, voxel size = 1 mm x 1 mm x 1 mm, number of slices =180). All images were obtained in an oblique axial orientation covering the entire brain, including the cerebellum and brainstem.

Feasibility Test in Humans

Pilot tests were approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346) and registered at ClinicalTrials.gov (NCT01768910). We recruited 33 right-handed healthy subjects (16 females, 17 males) with unimpaired LUT function (Table 1) and no history of neurological disorder, and three patients with LUT dysfunction/symptoms (Table 2).

Table 1 – Characteristics of healthy subjects.

Gender	**Female (*n*=16)**		**Male (*n*=17)**		***P****
Age	33± 10		36±12		0.33
Prefilling volume [mL]	444±115		469±149		0.59
Ratings		***P*****		***P*****	
Desire to void after infusion	8.38±0.97	0.00	7.45±1.61	0.00	0.53
Desire to void after drainage	5.31±1.32		3.93±1.49		0.01
Discomfort after infusion	0.9±0.73	0.03	1.23±1.56	0.05	0.44
Discomfort after drainage	0.56±0.32		0.54±0.31		0.84

*P value for unpaired t-test to reveal difference in subjects' characteristics between gender. **P value for paired t-test to reveal differences in ratings, i.e. urge and discomfort after infusion and drainage.

Table 2 – Characteristics of patients.

Diagnosis	**NNOAB**		**MS**		**SCI AIS D sub TH11**	
Gender	Female		Male		Male	
Age	29		44		41	
Prefilling volume [mL]	200		120		230	
Ratings		***P***		***P***		***P***
Desire to void after infusion	9±1.1	≤0.01	9.9±0.1	≤0.01	7.5±2.4	≤0.01
Desire to void after drainage	0.5±0.4		0.9±0.9		1±1.5	
Discomfort after infusion	8.2±1.4	≤0.01	9.2±1.2	≤0.01	3.9±1	0.41
Discomfort after drainage	0.4±0.4		1.1±0.5		3.5±1.3	

AIS, American Spinal Injury Association Impairment Scale; MS, multiple sclerosis; NNOAB, non-neurogenic overactive bladder; SCI, Spinal cord injury. P value for paired t-test to reveal differences in ratings, i.e. urge and discomfort after infusion and drainage.

After written informed consent was obtained, subjects were placed in the scanner in a supine position. Thereafter, a soft 14-Fr silicon transurethral catheter (UROMED Kurt Drews GmbH, Oststeinbek, Germany) was introduced using a non-anaesthetic lubricant (Endosgel®, FARCO-PHARMA GmbH, Cologne, Germany). To assess fluctuations in the subjective sensations throughout the entire LUT stimulation task, and therefore to evaluate the effect of fluid infusion and withdrawal by the IDD on the subject's sensory-perceptual level, a custom-designed MR-compatible HRS, described previously [14], was used.

Scan Paradigm

Before the EPI scans, the bladder was pre-filled with sterile body warm (37 °C) saline until subjects reported a persistent desire to void, i.e. six out of 10 on the visual analogue scale (VAS). Thereafter, eight blocks of repetitive infusion and withdrawal of 100 mL saline using the IDD were performed (Figure 3). A total of 450 functional scans were acquired. After an initial phase of 60 s REST (visual fixation) and two baseline RATINGS (rating of desire to void and discomfort on a projected VAS using the HRS, each 7 s), each block comprised the following conditions: INFUSION (automated bladder filling of 100 mL body warm (37 °C) saline, 15 s), PLATEAU (9 s), REST (visual fixation, 7–9 s), two RATINGS (2 x 7 s), WITHDRAWAL (automated bladder draining of 100 mL, 15 s), PLATEAU (15 s), two RATINGS (2 x 7 s), and another REST condition (visual fixation, 7–9 s) [15,16]. The scan was finalized with a REST condition of 60 s. During the RATING conditions subjects rated their desire to void (7 s) and discomfort (7 s) on a projected VAS (0–10) using the HRS. During all other conditions, subjects were instructed to focus on a displayed fixation cross.

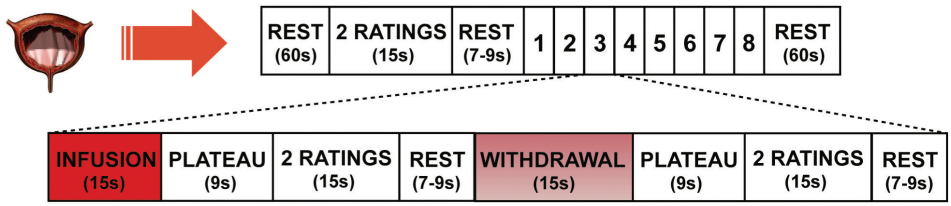

Figure 3 - Scan paradigm of the task-related functional MRI (fMRI).
The fMRI task starts with a pre-filled bladder volume, i.e. the bladder will be filled with body warm (37 °C) saline until a persistent desire to void is present. The fMRI starts with a 'baseline' REST (60 s, no specific stimulus or task is performed), a 'baseline' RATING of desire to void and level of pain, a short REST jittered between 7 and 9 s in which blood oxygen level-dependent (BOLD) activation resulting from motor activity during the previous RATING will return to baseline to avoid contamination of the following condition and conclude with a 'last' REST (60 s, no specific stimulus or task is performed). The eight specific task-related blocks consist of: (1) automated INFUSION of 100 mL warm saline; (2) plateau phase (bladder distention after INFUSION is perceived); (3) RATING of desire to void and level of pain; (4) short REST jittered between 7 and 9 s in which BOLD activation resulting from motor activity during the previous RATING will return to baseline to avoid contamination of the following condition; (5) automated WITHDRAWAL of 100 mL; (6) plateau phase (bladder distention after WITHDRAWAL is perceived); (7) RATING of desire to void and level of pain; and (8) short REST jittered between 7 and 9 s, in which BOLD activation resulting from motor activity during the previous RATING will return to baseline to avoid contamination of the following condition. Reproduced from [Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with nonneurogenic lower urinary tract symptoms, Walter M. et al., BMJ Open 2014, copyright notice 07/2015] with permission from BMJ Publishing Group Ltd.

Data Analysis

Static SNR was evaluated for anatomical and functional images by a signal-background method, i.e. by dividing the mean signal intensity within the phantom over the standard deviation of the background-signal (defined by four smaller regions of interest outside the phantom) corrected with the Rayleigh distribution factor. The time-variant SNR (tSNR) was measured [17] to define the time course stability and degree of signal distortion during the scan under different conditions, i.e. phantom only, phantom with installed and working IDD and HRS, placed 40 cm away from the phantom. fMRI data were analysed using SPM 12 (Wellcome Trust Centre for Neuroimaging, London, UK). The first five (dummy) scans were removed to allow for longitudinal magnetization equilibrium. The pre-processing consisted of realignment, normalization to the Montreal Neurological Institute (MNI) template, temporal filtering (128 s)

and special smoothing (6 mm) [18,19]. Each subject was analysed separately [first (within-subject)-level analysis] using a general linear model convolved with a canonical haemodynamic response function [19]. Head-motion variables (translation and rotation values) were included in the general linear model as regressor of no interest. The design matrix of the general linear model for the first-level analysis consisted of the following three events INFUSION, WITHDRAWAL and REST (60 s), i.e. pre-filled bladder (60 s; Figure 3). The contrast images were then used for a second (between-subject) level analysis. To assess the bladder network in healthy subjects, an anatomical mask was created according to the study by Griffiths et al. [9] containing bilaterally the pons, periaqueductal grey (PAG), frontal and prefrontal cortex (covering the motor/premotor cortex [BA6], as well as as well as the dorsolateral [BA 8, 9 and 46], ventrolateral [BA 44, 45, and 47], orbitofrontal/orbital [BA 47, and 11] and frontopolar [BA 10] subdivisions), cingulate cortex (subdivided in anterior [BA 24, 32, and 33] and posterior [BA 23, 29, 30 and 31]), insula, thalamus, putamen, hypothalamus and the cerebellum, using the WFU PickAtlas (http://fmri.wfubmc.edu/software/pickatlas). For areas that did not show significant activation in the *a priori*-defined mask, i.e. cerebellum, hypothalamus, pons and PAG, we additionally used a small volume correction, only including the specific brain region. Considering the limited literature according to alterations of the known bladder network in patients, only whole-brain analyses were performed in this subgroup. Data are presented as mean ± SD. Comparing unrelated samples, i.e. female vs male subjects, the unpaired t-test, for related samples, i.e. desire to void and level of discomfort (INFUSION vs WITHDRAWAL), the paired t-test was used, respectively. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) V22 (IBM, Armonk, NY, USA). For the fMRI group results, a P value ≤ 0.05 (using the family-wise error [FWE] correction) [20] was considered statistically significant.

RESULTS

Infusion-Drainage Device Performance Test

The results of the volume delivery accuracy of the IDD are shown in Table 3.

Table 3 – Accuracy for volume delivery.

Set flow time at 15 sec modulate flow rate			
Flow rate [mL/min]	80	200	400
Expected volume [mL]	20	50	100
Infusion volume [mL]	19.91±0.27	49.95±0.27	100.14±0.16
Infusion volume block 1 [mL]	20.49	49.83	100.08
Infusion volume block 8 [mL]	19.82	50.37	100.08
Withdrawal volume [mL]	19.71±0.19	49.79±0.33	100.06±0.16
Withdrawal volume block 1 [mL]	19.66	49.78	100.25
Withdrawal volume block 8 [mL]	19.82	50.15	100.08
Overall volume transport accuracy*	99.1±1.2%	99.7±0.6%	99.9±0.2%
Set flow rate at 400 mL/min modulate infusion/drainage time			
Flow time [s]	3	7.5	15
Expected volume [mL]	20	50	100
Infusion volume [mL]	20.03±0.29	50.03±0.34	100.14±0.16
Infusion volume block 1 [mL]	19.48	49.1	100.08
Infusion volume block 8 [mL]	20.14	50.46	100.08
Withdrawal volume [mL]	20.08±0.14	49.9±0.33	100.06±0.16
Withdrawal volume block 1 [mL]	19.84	49.31	100.25
Withdrawal volume block 8 [mL]	20.14	49.89	100.08
Overall volume transport accuracy*	99.7±1.1%	99.9±0.6%	99.9±0.2%
Set infusion/drainage volume at 100 mL modulate flow rate			
Flow time [s]	133	200	400
Expected volume [mL]	100	100	100
Infusion volume [mL]	99.86±0.32	100.18±0.35	100.14±0.16
Infusion volume block 1 [mL]	99.72	100.39	100.08
Infusion volume block 8 [mL]	100.32	100.99	100.08
Withdrawal volume [mL]	99.74±0.42	100.08±0.43	100.06±0.16
Withdrawal volume block 1 [mL]	100.01	100.41	100.25
Withdrawal volume block 8 [mL]	100.27	100.16	100.08
Overall volume transport accuracy*	99.8±0.4%	99.9±0.2%	99.9±0.2%

* Average volume transport accuracy over eight blocks infusion and withdrawal according to the expected volume.

Precision of the delivered volume over eight cycles of infusion and withdrawal was between 99.1±1.2% and 99.9±0.2%, for different flowrates and volumes. Time delay between the movement of the stepper motor and movement of the master as well as the slave piston rod, i.e. fluid transfer, was 1.31±0.21 sec (Figure 4). MR compatibility testing for the device with a phantom showed a decrease of overall SNR of 1.13% and 0.54% for anatomical and functional scans, respectively, and tSNR of 1.76% for functional images (Table 4).

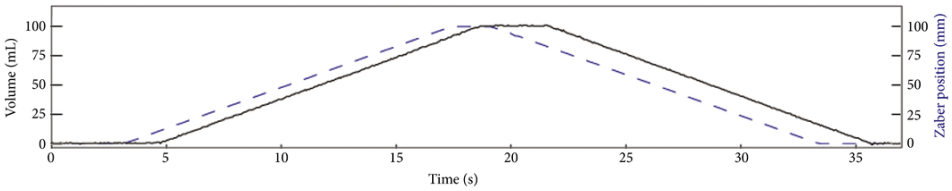

Figure 4 - Sample infusion and drainage.
Volume changes over time (straight black line) and position changes of the motorized linear slider (Zaber, blue dashed lines) over one cycle of infusion and drainage. The offset in the curves correspond to the time delay between movement of the linear slider and fluid transfer movement slave piston rod.

Table 4 – MRI-compatibility tests with a phantom.

Test Condition	Anatomical SNR	Functional SNR	Functional tSNR
Baseline	741.04	783.64	271.95±31.46
Installed IDD HRS	749.54	787.86	276.82±31.59
Equivalence percentage	98.87%	99.46%	98.24%

Baseline = phantom only, installed infusion-drainage device (IDD) and handhold response system (HRS). SNR = Signal-to-noise ratio, tSNR = time variant SNR

Feasibility Test in Healthy Subjects

Subject characteristics, including data on pre-filled bladder volume, desire to void and level of discomfort during bladder stimulation, are shown in Tables 1 and 2, respectively. In summary, over eight blocks, healthy subjects rated

a significantly higher (P≤0.05) desire to void and level of discomfort for infusion versus drainage. Using the *a priori*-defined regions of interest, healthy subjects showed significant activation (P≤0.05, FWE-corrected) in response to INFUSION vs REST in the bilateral frontal and prefrontal cortex, bilateral insula, bilateral thalamus, bilateral premotor cortex, left putamen and the cingulate cortex (detailed anatomical and spatial information: Figure 5, Tables S1A–E). Using a small volume correction for the bilateral cerebellum, bilateral hypothalamus, the PAG, and the pons, significant (P≤0.05, FWE-corrected) activation could also be shown in these areas.

Feasibility Test in Patients

All patients rated a significant different (P<0.001) desire to void for infusion vs drainage. Ratings for the level of discomfort were significantly different (infusion vs. drainage, P<0.001), except for the patient with spinal cord injury. Patients showed a heterogeneous pattern of significant activations (p=0.05 FWE-corrected) in response to INFUSION vs REST in whole brain analyses (Figure 6). Activated brain areas included the bilateral frontal and prefrontal cortex, the cingulate cortex, the insula, the bilateral cerebellum and the somatosensory cortex, as well as the temporal and parietal lobe (Tables S2A–C). For both, healthy subjects and patients task-related adjusted BOLD signal response plotted over time showed changes in activation related to the different conditions of the scan paradigm (Figures 7 A–E).

Safety

All subjects and patients tolerated the investigation well. An adverse event, as defined by the International Conference on Harmonisation Good Clinical Practice Guidelines (E6) [21] and International Organization for Standardization (ISO, 14155) [22], did not occur.

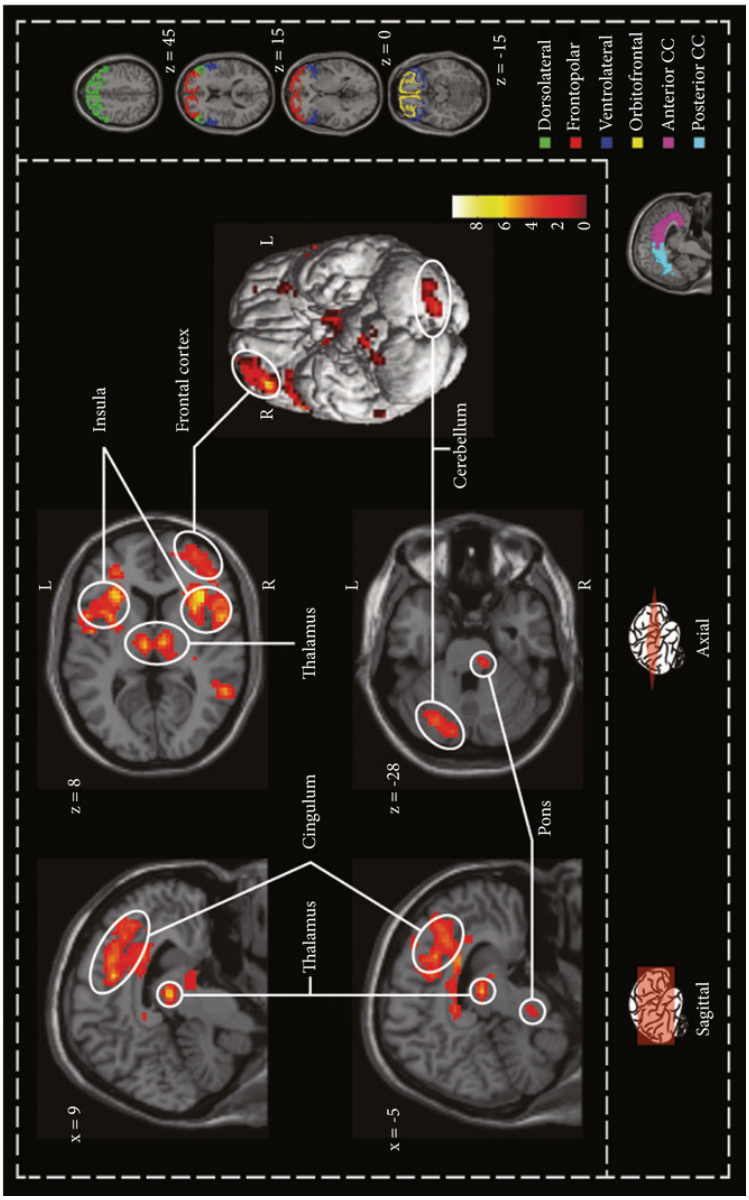

Figure 5 – Second-level analysis of brain activity in response to automated, repetitive bladder filling (INFUSION) vs REST in 33 healthy subjects, using a predefined mask containing bilaterally the pons, periaqueductal grey (PAG), middle and inferior frontal gyrus, cingulate cortex, insula, thalamus, putamen, hypothalamus, premotor cortex, and the cerebellum.

For display reasons, results are shown at a threshold of $P < 0.001$ uncorrected with cluster extend KE 42. The coordinates correspond to the brain template of the Montreal Neurological Institute and Hospital. The colour coding at the image frame indicates the further subdivisions of activated brain areas within the prefrontal (dorsolateral: Brodmann area [BA] 8, 9, 46; ventrolateral: BA 44, 45, 47; orbitofrontal/orbital: BA 47, 11; frontopolar: BA 10) and cingulate (anterior: BA 24, 32, 33; posterior: BA 23, 29, 30, 31) cortex. CC, cingulate cortex.

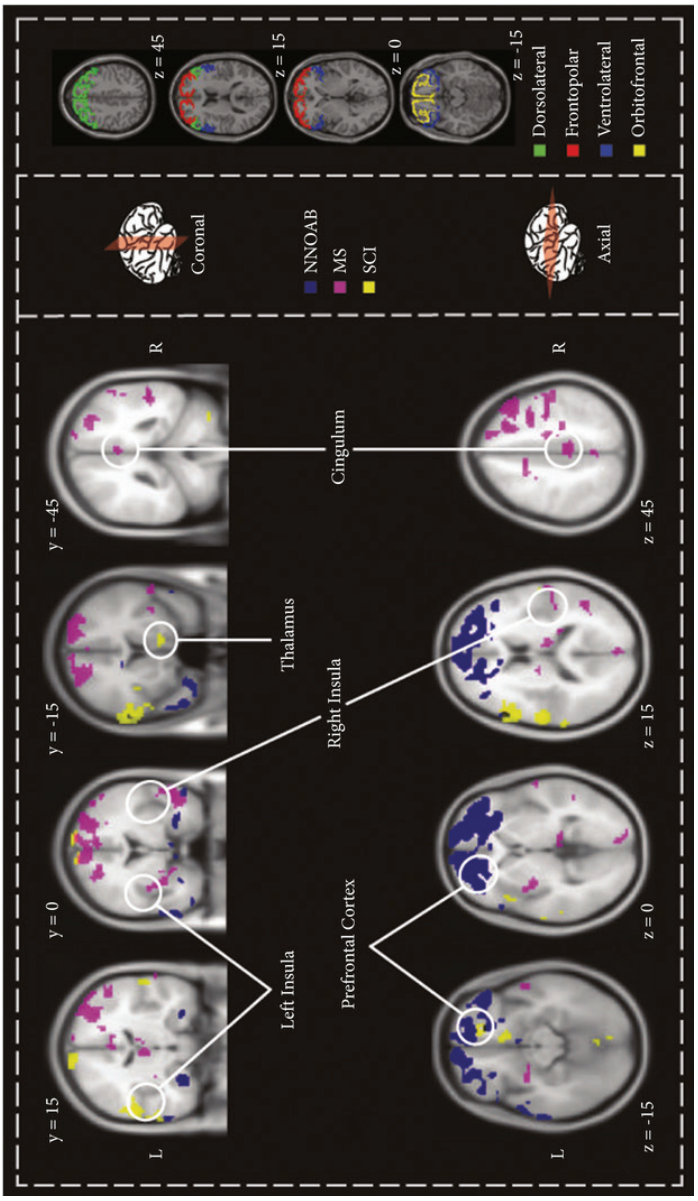

Figure 6 – Overlaid brain activity (INFUSION vs REST) in patients with lower urinary tract dysfunction/symptoms.

The activation pattern for the different patients are displayed in different colours; blue for the patient with non-neurogenic overactive bladder (NNOAB), pink for the patient with multiple sclerosis (MS) and yellow for the patient with spinal cord injury (SCI), respectively. For display reasons, results are shown at a threshold of $P < 0.001$ uncorrected. The coordinates correspond to the brain template of the Montreal Neurological Institute and Hospital. The colour coding at the image frame indicates the further subdivisions of activated brain areas within the prefrontal cortex (dorsolateral: Brodmann area [BA] 8, 9, 46; ventrolateral: BA 44, 45, 47; orbitofrontal/orbital: BA 47, 11; and frontopolar: BA 10).

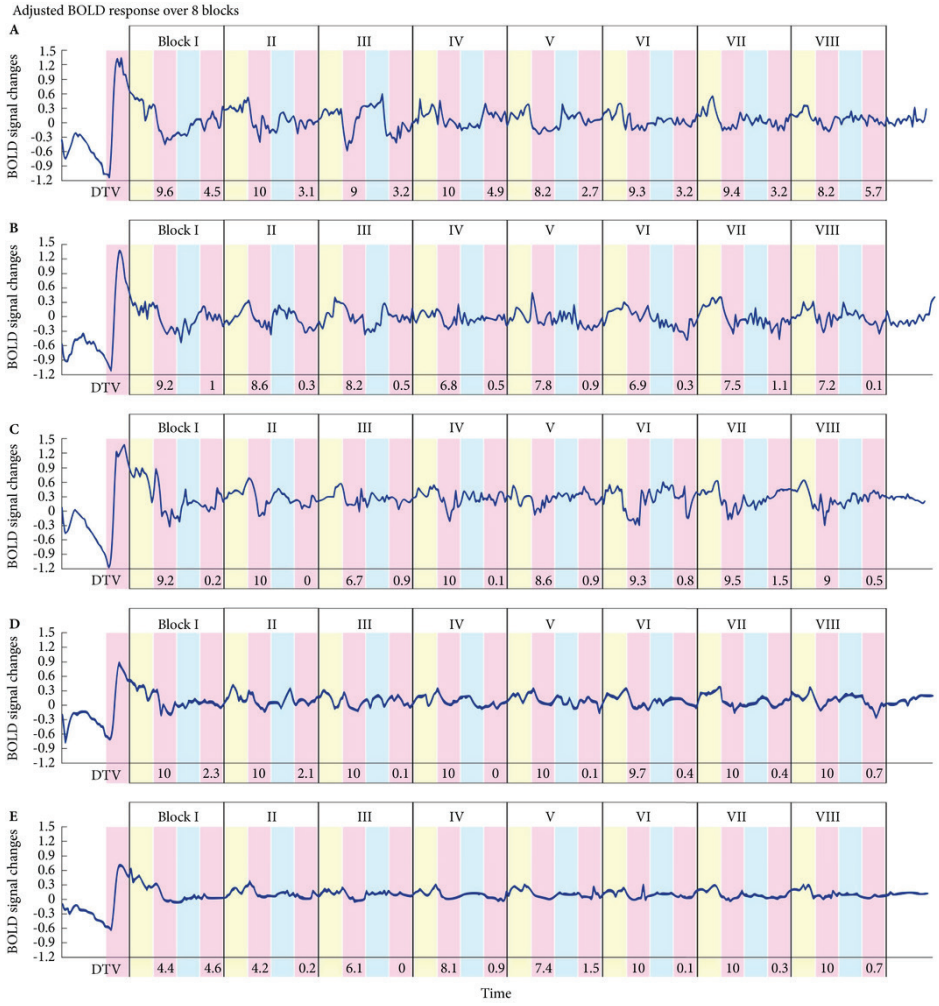

Figure 7 – Adjusted blood oxygen level□dependent (BOLD) response over eight blocks in two healthy subjects (male and female) and patients, in the voxel with the global maximum response during INFUSION vs REST.

The coordinates correspond to the brain template of the Montreal Neurological Institute and Hospital. (A) Healthy female subject (age 38 years, bladder pre-filling volume 300 mL): right middle frontal gyrus, x = 33, y = 53, z = -13. (B) Healthy male subject (age 37 years, bladder pre-filling volume 300 mL): right inferior frontal gyrus (triangle), x = 57, y = 32, z = 2. (C) Patients with nonneurogenic overactive bladder: right superior frontal medial gyrus, x = 12, y = 74, z = 1. (D) Patient with multiple sclerosis: right middle temporal gyrus, x = 54, y = - 49, z = 5. (E) Patient with spinal cord injury: left postcentral gyrus, x = -57, y = -19, z = 17. The colour backgrounds correspond to the different tasks, i.e. RATING/REST = pink, INFUSION/PLATEAU = yellow, WITHDRAWAL/PLATEAU = blue. Subjects rated desire to void (on a visual analogue scale from 0 to 10) after INFUSION and WITHDRAWAL are expressed in the RATING field. DTV, desire to void.

DISCUSSION

Main Findings

Our novel MR-compatible and MR-synchronised IDD, designed to stimulate the LUT during fMRI showed a consistent system performance with a high precision. Accuracy of the delivered volume was between 96.94 % and 99.39 %. All parts of the system within the MR scanner room are entirely non-ferromagnetic. Hence, MR compatibility could be proven by showing low decrease in SNR and tSNR. Feasibility test in humans showed robust supraspinal activation in areas related to LUT control. The BOLD signal increases in such areas were repeatedly related to the INFUSION and WITHDRAWL conditions. The real-time sensory feedback of healthy subjects and patients with LUT dysfunction correlated well with the IDD action, i.e. desire to void increased with infusion and decreased with fluid withdrawal. No adverse events occurred.

Findings in the Context of Existing Evidence

The decisive aspect in every fMRI experiment is the study design [8]. The important aspect of precise timing and stimulation intensity for better interpretation of task-related supraspinal activity has been shown already [12]. It has been proven that automated computerized systems are superior in temporal precision and accuracy to conventional methods [23]; however, because of the strong magnetic field and the high radio frequency energy, the MR scanner room is a challenging environment in which to implement an automated computerized stimulation system [24]. Whenever a computerized stimulation system is used, subjects' safety, MR compatibility and high performance accuracy have to be guaranteed [23]. Various study designs were used to evoke brain responses through LUT stimulation. Kuhtz-Buschbeck et al. [5] and our own group [6,25] applied periodic cycles of imagination of enhancing and suppressing the urge to void, contractions of the pelvic floor or manual filling of the bladder, to elicit supraspinal activity. Griffiths et al. [4] were the first to

implement an adapted urodynamic method to examine supraspinal responses attributable to bladder distension. The resulting supraspinal activation in response to urodynamic filling demonstrated increased brain activation in the interoceptive circuit [26,27]; however, BOLD signal responses and localization of BOLD signal changes in response to LUT stimulation tasks varied among studies, which might be, amongst other reasons, related to different scan protocols but also less accurate, non MR-synchronized task application. This latter aspect might be of specific relevance, considering that several studies on supraspinal LUT control could not provide significant results on a statistically corrected level, i.e. not using a correction for multiple comparisons [4,10,28]. An amelioration of significance levels using a standardized and tested device seems plausible and is supported by the significant multiple comparison corrected (FWE correction) findings in our group of 33 healthy subjects. Although urodynamic devices are used in daily routine they may have limitations in the setting of MR neuroimaging. They must be placed outside the scanner room and can only be connected to a transurethral catheter through extension hoses. According to the manufacturer specification for a standard urodynamic system, the range of the infusion rate of the pump module is limited (5–140 mL/min, with an accuracy of ±5%). The pump module is not designed for fluid withdrawal. No reports are available as to whether the extension hose additionally impairs the working accuracy; however, precise volume delivery is questionable and accuracy has not been evaluated during fMRI. Using an MR compatible syringe pump (e.g. HA2000WRMI; Harvard Apparatus, Massachusetts, USA; flow rate 0.0001 to 221 mL/min with an accuracy of ±0.5%) could overcome this problem. Nevertheless, the predetermined operator software does not allow any synchronization with the MR scanner. Each stimulation has to be edited manually, making exact timing more difficult to achieve. Furthermore, such systems do not offer a solution

by which additional data, e.g. behavioural data, could be implemented and recorded synchronously.

Based on our prototype [13], we developed a commercially available, MR-compatible and MR-synchronized IDD for routine use of bladder stimulation tasks in fMRI. The device provides a high accuracy in timing and filling volume with a continuously adaptable flow rate between 0.3 and 1200 mL/min. Using the IDD, we showed that supraspinal areas of the LUT control network can be significantly activated in a large sample of healthy subjects and also in an exemplary sample of patients. The temporal overlay of the adjusted BOLD signal increases demonstrated synchrony with the applied INFUSION and WITHDRAWAL conditions. In addition, the sensory responses of subjects and patients correlated well with the according IDD action, i.e. the desire to void was significantly higher and lower with INFUSION and WITHDRAWAL, respectively.

The current IDD version includes a fluid reservoir and a set of manual and check valves that enable a fast and complete evacuation of air (Figures 2C–F). In addition, the IDD is portable and easy to assemble, which enables convenient storage and use at multiple locations (Figures 2A and B). The whole system is available for ~$14,000, including a compact carrying case for safe transportation and a personal computer to run the system, which is reasonable compared with the costs for a conventional urodynamic system of $30,000–60,000. Furthermore, this device is expandable to allow additional measurements, e.g. implementation of pressure sensors to monitor intravesical pressure, suitable to be used in conjunction with other MR-synchronized devices, such as the HRS, and may be used for various applications including other organ systems such as the gastrointestinal tract, offering an all-in-one solution for MR synchronous measurements of visceral hollow organs.

Limitations

Currently, the IDD can only be used with a standardised 100 mL polypropylene syringe. However, it can be adjusted in order to operate with different volumes and syringes. This device is aimed to cause filling or distention and subsequent deflation or emptying of visceral organs such as the bladder. Hence, to investigate the central regulatory mechanisms during the initiation of micturition and actual micturition, such a device might not be useful.

CONCLUSIONS

The novel MR compatible and MR synchronised IDD, designed to stimulate the LUT during fMRI showed a high accuracy in timing and infusion volume during automated, repetitive bladder filling in the MR scanner, eliciting significant supraspinal activity in specific areas known to be involved in supraspinal LUT control. This novel IDD has the potential to improve accuracy and standardisation of neuroimaging protocols aiming to investigate supraspinal correlates of bladder filling and drainage. Using standardised protocols facilitates reproducibility of study outcomes and comparison between studies.

Acknowledgements
We would like to acknowledge the Swiss National Science Foundation, Wings for Life, Emily Dorothy Lagemann Stiftung and Swiss Continence Foundation (www.swisscontinencefoundation.ch) for financial support.

Competing interests
The authors declare that they have no competing interests.

Funding
We would like to acknowledge Wings for Life, Swiss National Science Foundation, Emily Dorothy Lagemann Stiftung, and Swiss Continence Foundation for financial support. The supporters had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions
Conceptualization: LL, MW, US, SCK, UM and TMK.
Methodology: LL, MW, UM and TMK.
Software: -.
Validation: LL, MW, US, UM and TMK.
Formal analysis: LL, MW, SCK and TMK.
Investigation: LL, MW, US and TMK.
Resources: -.
Data curation: LL and MW.
Writing (original draft preparation): LL, MW and TMK.
Writing (review and editing): LL, MW, US, SCK, UM and TMK.
Visualization: LL, MW, US, SCK, UM and TMK.
Supervision: UM and TMK.
Project administration: LL, MW and TMK.
Funding acquisition: UM and TMK.

Abbreviations
BA, Brodmann area; BOLD, blood oxygenation level-dependent; EPI, echo-planar imaging; fMRI, functional MRI; FWE, family-wise error; HRS, handheld response system; IDD, infusion-drainage device; LUT, lower urinary tract; MR, magnetic resonance; PAG, periaqueductal grey; SNR, signal-to-noise ratio; tSNR, time-variant SNR.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. a–e

Healthy subjects (n=33): supraspinal areas with significant (P < 0.05, FWE-corrected) task-related (INFUSION vs. REST) BOLD signal intensity increase.

Table S2.

Patients: Brain areas with significant (P < 0.05, FWE-corrected) task-related (INFUSION vs. REST) signal intensity increase, whole brain analysis.

REFERENCES

1. Fowler CJ, Griffiths DJ. A decade of functional brain imaging applied to bladder control. Neurourol Urodyn. 2010: 29:49-55
2. Fukuyama H, Matsuzaki S, Ouchi Y, et al. Neural control of micturition in man examined with single photon emission computed tomography using 99mTc-HMPAO. Neuroreport. 1996 Nov 25: 7:3009-12
3. Blok BF, Willemsen AT, Holstege G. A PET study on brain control of micturition in humans. Brain. 1997 Jan: 120 (Pt 1):111-21
4. Griffiths D, Derbyshire S, Stenger A, Resnick N. Brain control of normal and overactive bladder. J Urol. 2005 Nov: 174:1862-7
5. Kuhtz-Buschbeck JP, van der Horst C, Pott C, et al. Cortical representation of the urge to void: a functional magnetic resonance imaging study. J Urol. 2005 Oct: 174:1477-81
6. Zhang H, Reitz A, Kollias S, Summers P, Curt A, Schurch B. An fMRI study of the role of suprapontine brain structures in the voluntary voiding control induced by pelvic floor contraction. Neuroimage. 2005 Jan 1: 24:174-80
7. Ogawa S, Menon RS, Tank DW, et al. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. Biophys J. 1993 Mar: 64:803-12
8. Amaro E, Jr., Barker GJ. Study design in fMRI: basic principles. Brain Cogn. 2006 Apr: 60:220-32
9. Griffiths D, Tadic SD. Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. Neurourol Urodyn. 2008: 27:466-74
10. Krhut J, Holy P, Tintera J, Zachoval R, Zvara P. Brain activity during bladder filling and pelvic floor muscle contractions: a study using functional magnetic resonance imaging and synchronous urodynamics. Int J Urol. 2014 Feb: 21:169-74
11. Mehnert U, Boy S, Svensson J, et al. Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve--an fMRI study in healthy women. Neuroimage. 2008 Jul 1: 41:682-9
12. Buxton RB, Wong EC, Frank LR. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. Magn Reson Med. 1998 Jun: 39:855-64
13. Jarrahi B, Gassert R, Wanek J, Michels L, Mehnert U, Kollias S. Design and application of a new automated fluidic visceral stimulation device for human fMRI studies of interoception. IEEE Journal of Translational Engineering in Health and Medicine. 2016 April: 4:1-12
14. Jarrahi B, Wanek J, Mehnert U, Kollias S. An fMRI-compatible multi-configurable handheld response system using an intensity-modulated fiber-optic sensor. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society IEEE Engineering in Medicine and Biology Society Annual Conference. 2013: 2013:6349-52
15. Walter M, Michels L, Kollias S, van Kerrebroeck PE, Kessler TM, Mehnert U. Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms. BMJ Open. 2014: 4:e004357
16. Leitner L, Walter M, Freund P, et al. Protocol for a prospective magnetic resonance imaging study on supraspinal lower urinary tract control in healthy subjects and spinal cord injury patients undergoing intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity. BMC Urol. 2014: 14:68
17. Murphy K, Bodurka J, Bandettini PA. How long to scan? The relationship between fMRI temporal signal to noise ratio and necessary scan duration. Neuroimage. 2007 Jan 15: 34:565-74
18. Andersson JL, Hutton C, Ashburner J, Turner R, Friston K. Modeling geometric deformations in EPI time series. Neuroimage. 2001 May: 13:903-19
19. Friston KJ, Holmes AP, Poline JB, et al. Analysis of fMRI time-series revisited. Neuroimage. 1995 Mar: 2:45-53
20. Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med. 1990 Jul: 9:811-8

21. International conference on harmonisation. Guideline for good clinical practice E6(R1). Current Step 4 version dated 10 June 1996. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R1_Guideline.pdf. Accessed June 2016
22. Clinical investigation of medical devices for human subjects – Good clinical practice. ISO 14155:2011. Available at: http://www.iso.org/iso/catalogue_detail?csnumber=45557. Accessed June 2016
23. Gassert R, Burdet E, Chinzei K. MRI-Compatible Robotics. IEEE Eng Med Biol Mag. 2008 May-Jun: 27:12-4
24. Schaefers G. Testing MR safety and compatibility: an overview of the methods and current standards. IEEE Eng Med Biol Mag. 2008 May-Jun: 27:23-7
25. Mehnert U, Michels L, Zempleni MZ, Schurch B, Kollias S. The supraspinal neural correlate of bladder cold sensation–an fMRI study. Hum Brain Mapp. 2011 Jun: 32:835-45
26. Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nature reviews Neuroscience. 2002 Aug: 3:655-66
27. Jarrahi B, Mantini D, Balsters JH, et al. Differential functional brain network connectivity during visceral interoception as revealed by independent component analysis of fMRI TIME-series. Hum Brain Mapp. 2015 Nov: 36:4438-68
28. Griffiths DJ, Tadic SD, Schaefer W, Resnick NM. Cerebral control of the lower urinary tract: how age-related changes might predispose to urge incontinence. Neuroimage. 2009 Sep: 47:981-6

CHAPTER 8

General discussion

The primary aim of the thesis was to investigate the LUT function in health and disease, hence providing novel insight in long-term outcome of individuals with NLUTD and supraspinal LUT control. The multi-methodological approach, including surveillance urethro-cystoscopy, continuous cardiovascular monitoring during UDI, and neuro-imaging, facilitated the discovery of major findings.

Surveillance urethro-cystoscopy

This invasive diagnostic procedure has been used in clinical practice for decades to screen for and/or follow-up structural changes such as the presence of premalignant lesions or cancer (LE 1, GR A) [91] and functional changes (LE 3, GR B) [22]. The majority of studies previously published have investigated individuals with spinal cord injury (SCI), who frequently suffer from NLUTD. These individuals are thought to be at an increased risk for bladder cancer, that is up to 25 times compared to subject without underlying neurological disorder, when relying on indwelling catheters [92] due to chronic irritation of the bladder mucosa [93] and chronic urinary tract infections (UTI) [94]. With no consensus on how to manage (i.e. to detect and follow-up structural changes) individuals suffering from NLUTD and previous literature mainly focusing on bladder cancer in SCI population, we deemed it necessary to examine the value of urethro-cystoscopy as a suitable diagnostic method for surveillance in all individuals with NLUTD. In ***chapter 2,*** using surveillance urethro-cystoscopy and bladder washing cytology, relevant histological findings were discovered including a mucus-producing adenocarcinoma. Despite the study's limitations (i.e. suffering from NLUTD for at least 5 years), we feel that based on our findings urethro-cystoscopy (and bladder washing cytology) are of great value for surveillance of individuals with NLUTD. However, there

is still a need for prospective studies to investigate which sub-group of patients with NLUTD would profit most from surveillance urethro-cystoscopy and how (i.e. starting point and frequency).

Autonomic dysreflexia during urodynamic investigation

Individuals with NLUTD, are advised to attend regular evaluation of their LUT function [19]. Urodynamic investigation has been considered to be the gold standard to evaluate the presence and extent of NLUTD [23]. However, there is substantial evidence showing that only the repeatability of detrusor overactivity was excellent between the two UDIs [95]. For the remaining urodynamic parameters assessed (e.g. maximum cystometric capacity, compliance, and maximum detrusor pressure during storage phase) - there were wide 95% limits of agreement for differences in the parameters, indicating poor repeatability [95]. This is a significant limitation of this diagnostic procedure. As it is crucial for decision-making process (i.e. to commencing an appropriate treatment) to capture the entire extent of LUT dysfunction, which could be missed when only performing one UDI session, same session repeat UDI has been advocated [19, 95]. In individuals following SCI, caution should be raised when performing UDI as retrograde bladder filling can result in a sudden increase in SBP, known as AD [50]. Previous studies have reported incidence rates of AD during UDI up to 57% [96-100]. Cardiovascular monitoring allows one to detect changes in SBP, diastolic blood pressure (DBP) and heart rate (HR) – either episodically (e.g. every minute) or continuously (beat to beat). However, only episodic cardiovascular monitoring during UDI [96-98, 100] was applied prior to the start of our study. We know that the majority of UDI parameters show poor repeatability [95]. However, we do not know whether this fact has any implication of cardiovascular changes during same session repeat UDI. Lastly, there is a complete lack on women-specific data on AD during UDI. Taken all into account, we were the first to investigate

(*chapter 3*) the incidence of AD and the repeatability of cardiovascular changes during same session repeat UDI using continuous ardiovascular monitoring in women with SCI suffering from NLUTD. In contrast to the previous literature (37-57%) [96-98, 100], we found a significant higher incidence of AD of more than 70%. Furthermore, good repeatability of detecting AD in same session repeat UDIs might imply that one UDI would be sufficient. However, wide Bland and Altman 95 % limits of agreement indicate poor repeatability for continuous cardiovascular monitoring parameters, i.e., SBP, DBP and HR. Although these are novel findings, some limitations should be acknowledged. Same session repeat UDI may limit the outcome as physiological changes could be biased in the second UDI when performed immediately after the first UDI [101]. Considering that we only studied women with NLUTD, it is unclear whether the results could also be extrapolated to SCI patients without NLUTD. Future outlook - although reporting about the largest cohort of women with NLUTD suffering from suprasacral SCI, the influence of the duration of injury, completeness of SCI, lesion level and type of plegia on cardiovascular changes might be more distinctive in larger patient cohorts or in those with a more balanced distribution between patients with and without AD. Furthermore, it would be of great interest to continuously monitor cardiovascular parameters during long-term ambulatory urodynamics to further investigate the cardiovascular risk profile in patients with SCI and to compare these findings with same session repeat UDIs.

Urodynamic investigation in healthy subjects

What is considered to be the gold standard to assess NLUTD, can also be applied to further investigate LUT symptoms (LUTS) in non-neurogenic individuals (LE 3, GR B), e.g. refractory LUTS or specific indications. [22] However, individuals with LUTS do not necessarily present with pathological UDI findings [102]. Knowing what represents “normal organ function” is based on the

investigation of healthy subjects. Though, previous studies on UDIs in symptom-free healthy subjects reported a wide range of findings including pathological results [103-106]. Thus, the value of UDIs in asymptomatic healthy subjects (i.e. without any LUTS and underlying neurologic condition), is largely unknown. This void sparked our interest to assess whether UDI is appropriate to select healthy subjects as controls for comparative studies with individuals suffering from non-neurogenic LUTS as well as those with NLUTD. In ***chapter 4***, we investigated 42 healthy subjects (i.e. whose medical history, 3-day bladder diaries, questionnaires regarding LUTS, QoL, mental status and depression as well as neuro-urological assessment, free uroflowmetry, PVR, and urine samples were all without any pathological finding) using as same session repeat UDI. Our analysis revealed three interesting findings: 1) a high incidence rate of pathological UDI findings (>70%), 2) significant differences in UDI parameters between genders and 3) poor repeatability for UDI parameters (wide 95% limits of agreement for differences in the Bland and Altman method). Among our participants, DSD was the most frequently found pathological finding. One could argue that either detrusor sphincter dyscoordination or dysfunctional voiding might be a more precise term to describe our findings rather than DSD. However, detrusor sphincter dyscoordination, commonly used in German speaking countries for DSD in healthy individuals without any neurological condition, does not exist in the ICS terminology. Thus, according to ICS, DSD is defined as detrusor contraction concurrent with an involuntary contraction of the urethral and/or periurethral striated muscle during UDI that, typically revealed in patients with a suprasacral lesion [107]. Yet, an underlying neurological disorder is not a prerequisite. In fact, an incidence of undetected underlying neurological disorders in presumably healthy volunteers of more than 70% (30/42) is very unlikely. Taking into consideration the normal flow pattern and lack of PVR during free uroflowmetry, the high rate of DSD is most likely a phenomenon provoked by the examination

itself, i.e. stiff irrigating catheter and the unselective recording of the surface EMG. When applying generally agreed definitions [107], one will encounter a very high percentage of findings considered to be pathological. Therefore, we do not recommend using UDI to select healthy subjects as controls for comparative studies. We encourage to rather rely on bladder diaries, validated questionnaires and neuro-urological assessment [1]. For future studies, comparison between free uroflowmetry and invasive UDI both conducted with pelvic floor EMG could give further insights into catheter-induced changes. Thus, many important questions remain to be answered highly warranting further investigations in healthy subjects.

Investigation of Supraspinal Responses to Lower Urinary Tract Stimulation

From a variety of neuroimaging studies, our knowledge on what comprises the supraspinal LUT control has increased tremendously over the last two decades [17]. More recently, MRI has become one of the preferred neuroimaging technique allowing to investigate structural and functional properties of the brain and spinal cord non-invasively [108, 109]. However, when taking a closer look at the present literature, an inhomogeneous body of studies (i.e. very different subject populations, investigation methods, and scan protocols) is found. Therefore, one will find that the extent and intensity of the supraspinal activation can differ significantly between studies. Owing to the lack of a more standardized or sophisticated approach, we intended to draft study protocols combining a multi-modal neuro-imaging (i.e. structural and functional MRI) with a novel device that allow automatic, repetitive filling and draining of the bladder. The latter is intended to provide a recurrent stimulus to the LUT comprising the exact same features over and over again. In order to be able to create a stimulus to the LUT that is (almost) identical to the next one, we desire specific properties such as accurate timing (i.e. always the duration for filling and draining), equal force (i.e. always using the same

amount of thrust for filling and draining), and the same amount of fluid infused into and drained from the bladder. First (*chapter 5*), we outlined a study protocol to investigate supraspinal responses to LUT stimulation using neuroimaging in healthy subjects and individuals with non-neurogenic LUTS. Secondly (*chapter 6*), an adjusted protocol was established for individuals with SCI before and after treatment of NLUTD. According to the protocols (*chapter 5 and 6*), we evaluated the applicability of a novel IDD to investigate supraspinal responses to LUT stimulation using fMRI in healthy subjects and individuals with LUT dysfunction *(chapter 7)*. Our IDD showed excellent applicability both in a laboratory setting as well as in healthy subjects and individuals with LUT dysfunction, by providing LUT stimulation at high system accuracy resulting in significant supraspinal BOLD signal changes in interoceptive and LUT control areas in synchronicity to the applied stimuli. In light of this evidence, we deem our MR-compatible and MR-synchronized IDD, which is commercially available, portable and multi-configurable, capable of improving neuro-imaging studies on supraspinal LUT control. Potential future areas of application may include the investigation of supraspinal activity after onset of a specific condition (or multiple) affecting LUT function or short/long-term effect of novel LUT treatment options on supraspinal activity as well as multisite studies (i.e. comparability of LUT-related supraspinal activity across different locations) and repeatability analysis of LUT-related supraspinal activity. In line with this, we are currently concluding our investigation, which focussed on supraspinal changes in response to treatment of NLUTD (i.e. onabotulinumtoxinA) in patients with SCI.

References

1. Babjuk, M., et al. EAU Guidelines on non-muscle-invasive Bladder Cancer (TaT1 and CIS). 2017 2017/06/27]; Available from: http://www.uroweb.org/guidelines.
2. Gravas, S., et al. EAU Guidelines on Non-neurogenic male LUTS including benign prostatic obstruction. 2017 2017/04/14]; Available from: http://www.uroweb.org/guidelines.
3. Groah, S.L., et al., Excess risk of bladder cancer in spinal cord injury: evidence for an association between indwelling catheter use and bladder cancer. Arch Phys Med Rehabil, 2002. **83**(3): p. 346-51.
4. Hamid, R., et al., Screening cystoscopy and biopsy in patients with neuropathic bladder and chronic suprapubic indwelling catheters: is it valid? J Urol, 2003. **170**(2 Pt 1): p. 425-7.
5. Welk, B., et al., Bladder cancer in individuals with spinal cord injuries. Spinal Cord, 2013. **51**(7): p. 516-21.
6. Blok, B., et al. EAU Guidelines on Neuro-Urology. 2017 2017/04/14]; Available from: http://uroweb.org/guidelines.
7. Mangera, A. and C. Chapple, Modern evaluation of lower urinary tract symptoms in 2014. Curr Opin Urol, 2014. **24**(1): p. 15-20.
8. Bellucci, C.H., et al., Neurogenic lower urinary tract dysfunction - do we need same session repeat urodynamic investigations? J Urol, 2012. **187**(4): p. 1318-23.
9. Krassioukov, A., et al., International standards to document remaining autonomic function after spinal cord injury. J Spinal Cord Med, 2012. **35**(4): p. 201-10.
10. Giannantoni, A., et al., Autonomic dysreflexia during urodynamics. Spinal Cord, 1998. **36**(11): p. 756-60.
11. Huang, Y.H., et al., Autonomic dysreflexia during urodynamic examinations in patients with suprasacral spinal cord injury. Arch Phys Med Rehabil, 2011. **92**(9): p. 1450-4.
12. Huang, Y.H., et al., Blood pressure and age associated with silent autonomic dysreflexia during urodynamic examinations in patients with spinal cord injury. Spinal Cord, 2013. **51**(5): p. 401-5.
13. Liu, N., et al., Autonomic dysreflexia severity during urodynamics and cystoscopy in individuals with spinal cord injury. Spinal Cord, 2013. **51**(11): p. 863-7.
14. Sayilir, S., M. Ersoz, and S. Yalcin, Comparison of urodynamic findings in patients with upper and lower cervical spinal cord injury. Spinal Cord, 2013. **51**(10): p. 780-3.
15. Rahmanou, P., et al., Reliability testing of urodynamics, pressure flow studies and cough leak point pressure in women with urodynamic stress incontinence with and without detrusor overactivity. Int Urogynecol J Pelvic Floor Dysfunct, 2008. **19**(7): p. 933-8.
16. Katz, G.P. and J.G. Blaivas, A diagnostic dilemma: when urodynamic findings differ from the clinical impression. J Urol, 1983. **129**(6): p. 1170-4.
17. Mahfouz, W., et al., Normal urodynamic parameters in women. International Urogynecology Journal, 2012. **23**(3): p. 269-277.
18. Pfisterer, M.H., et al., Parameters of bladder function in pre-, peri-, and postmenopausal continent women without detrusor overactivity. Neurourol Urodyn, 2007. **26**(3): p. 356-61.
19. Wyndaele, J.J., Normality in urodynamics studied in healthy adults. J Urol, 1999. **161**(3): p. 899-902.
20. Wyndaele, J.J. and S. De Wachter, Cystometrical sensory data from a normal population: comparison of two groups of young healthy volunteers examined with 5 years interval. Eur Urol, 2002. **42**(1): p. 34-8.
21. Abrams, P., et al., The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn, 2002. **21**(2): p. 167-78.
22. Panicker, J.N., C.J. Fowler, and T.M. Kessler, Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. Lancet Neurol, 2015. **14**(7): p. 720-32.
23. Fowler, C.J. and D.J. Griffiths, A decade of functional brain imaging applied to bladder control. Neurourol Urodyn, 2010. **29**(1): p. 49-55.
24. Chow, M.S., et al., Functional magnetic resonance imaging and the brain: A brief review. World J Radiol, 2017. **9**(1): p. 5-9.
25. Martin, A.R., et al., Translating state-of-the-art spinal cord MRI techniques to clinical use: A systematic review of clinical studies utilizing DTI, MT, MWF, MRS, and fMRI. Neuroimage Clin, 2016. **10**: p. 192-238.

English Summary

Deutsche Zusammenfassung

Nederlandse Samenvatting

ENGLISH SUMMARY

This doctoral dissertation addresses multi-methodological approaches to investigate the LUT in health and disease, and starts with a general overview of LUT function and dysfunction.

In the first study *(Chapter 2)*, we examined the value of surveillance urethro-cystoscopy in patients with NLUTD. It has been reported that patients with NLUTD, mostly due to SCI, are at an increased risk for bladder cancer. In the framework of the largest prospective study to date, we used the data of 129 consecutive individuals suffering from NLUTD for at least 5 years. Using surveillance urethro-cystoscopy, we found relevant histological findings, including bladder cancer, in 5% of our patients suffering from NLUTD for at least 5 years. Contrasting most of the existing literature, our results suggest that surveillance urethro-cystoscopy is necessary. As a result of this study new guidelines advocating for the inclusion of every individual with NLUTD in the surveillance urethro-cystoscopy program were introduced.

In the next study *(Chapter 3)*, we investigated the overall incidence of AD and repeatability of cardiovascular changes in a series of 33 consecutive women with suprasacral SCI suffering from NLUTD. All patients underwent same session repeat UDI and synchronous continuous cardiovascular monitoring (SBP, DBP, and HR). We found a high overall AD incidence during UDI. Furthermore, patients with AD presented with significant increases in SBP and DBP as well as a significant decrease in HR compared to those without AD. The repeatability of AD between the two-same session UDIs was good. However, when applying the Bland and Altman method, wide 95% limits of agreement for differences in same session SBP, DBP and HR indicated poor repeatability. This study yielded important results into the field of neuro-urology, as there were no women-specific data available before. As a result of our study, the following recommendation has been imbedded into the European Association of Urology's Guidelines on Neuro-Urology: "Considering a high incidence of AD

with the relevant risks involved and poor repeatability of cardiovascular changes during UDI, continuous cardiovascular monitoring and reforming repeated measurements are strongly advised."

The aim of the next study *(Chapter 4)*, was to evaluate whether UDI, the gold standard to assess refractory LUTS, is appropriate to select healthy volunteers (i.e. without LUTS) as control subjects for comparative studies. Forty-two healthy subjects (22 women, and 20 men) were included into this prospective single-centre cohort study, recording a 3-day bladder diary, completed validated questionnaires regarding LUTS, and underwent neuro-urological assessment as well as free uroflowmetry. All 3-day bladder diaries, questionnaires, neuro-urological assessments and free uroflowmetries were within normal limits. In spite of this, more than 70% of our healthy subjects had pathological UDI findings. Given these results, it seems that UDI is not applicable in healthy subjects to define normal LUT function. Therefore, we do not recommend using UDI to select healthy control subjects.

The study presented in *chapter 5* outlines our intent 1) to identify brain networks of supraspinal LUT control in healthy subjects and 2) to detect abnormalities within these networks in patients with non-neurogenic LUTS. In order to facilitate these goals, we will utilize structural and fMRI techniques (in correlation with clinical measurements). This study aims to provide new insights into the supraspinal neuronal mechanisms and networks responsible for LUT control. The findings will help to verify, amend or adjust neuronal circuitry models established from findings in healthy controls, now in the context of patients with non-neurogenic LUTS. The use of newer imaging and evaluation techniques has the potential to serve as quantifiable outcome measures for therapy success and provide evidence for non-responders of LUTS treatment.

Using structural and fMRI, the following study outline *(Chapter 6)* focuses on the investigation of the supraspinal LUT control in healthy subjects and individuals with SCI undergoing intradetrusor onabotulinumtoxinA injections to

treat NLUTD. This study focuses at identifying structural and functional alterations in supraspinal networks of LUT control in SCI patients with NLUTD. Post-treatment MRI measurements in SCI patients will provide further insights into the intradetrusor onabotulinumtoxinA injections' effect on supraspinal LUT control.

The last study *(Chapter 7)* reports about the applicability and precision of a novel IDD for standardized filling paradigms in neuro-urology and fMRI studies of LUT function/dysfunction. The IDD is based on electrohydrostatic actuation which was previously proven feasible in a prototype setup. Our current design includes hydraulic cylinders and a motorized slider to provide force and motion. Methodological aspects have been assessed in a technical application laboratory as well as in healthy subjects and individuals with LUT dysfunction undergoing fMRI during LUT stimulation. According to our results, we were able to develop an MR-compatible and MR-synchronized IDD to routinely stimulate the LUT during fMRI in a standardized manner. The device provides LUT stimulation at high system accuracy resulting in significant supraspinal BOLD signal changes in interoceptive and LUT control areas in synchronicity to the applied stimuli. The IDD is commercially available, portable and multi-configurable. In our opinion, such a device may help to improve precision and standardization of LUT tasks in neuro-imaging studies on supraspinal LUT control, and may therefore facilitate multisite studies and comparability between different LUT investigations in the future.

DEUTSCHE ZUSAMMENFASSUNG

Die vorliegende Dissertation befasste sich mit verschiedenen methodologischen Ansätzen zur Untersuchung des unteren Harntrakts in gesunden Teilnehmern und Patienten.

In der ersten Studie (*Kapitel 2*) untersuchten wir die diagnostische Aussagekraft der kombinierten Harnröhren-/Harnblasenspiegelung, welche potentiell als eine regelmässige Kontrolluntersuchung dienen kann, für die Beurteilung von strukturellen Veränderungen im Bereich des unteren Harntrakts, wie zum Beispiel Krebs. Unser Kollektiv bestand aus Patienten mit nachgewiesener Funktionsstörung des unteren Harntrakts aufgrund einer neurologischen Erkrankung. Gemäss den bisherigen Angaben in der Fachliteratur ist diese Patientengruppe, vor allem aber jene mit einer Rückenmarkverletzung (RMV), im Vergleich zur gleichaltrigen Normalbevölkerung einem höheren Risiko für Blasenkrebs ausgesetzt. Im Rahmen dieser bis dato grössten prospektiven Studie untersuchten wir insgesamt 129 Patienten, davon 89 mit einer RMV, welche seit mindestens 5 Jahre lang an einer Funktionsstörung des unteren Harntrakts litten. Mittels der kombinierten Harnröhren-/Harnblasenspiegelung gelang es uns relevante histologische Befunde, inklusive Blasenkrebs, in 5% aller Patienten festzustellen. Im Gegensatz zu den bisherigen Berichten in der Fachliteratur, zeigten die Ergebnisse unserer Studie deutlich auf die Notwendigkeit der kombinierten Harnröhren-/Harnblasenspiegelung als regelmässige Kontrolluntersuchung hin. Als unmittelbare Folge unserer Studie, wird seit 2014 in der neuro-urologischen Abteilung der Universitätsklinik Balgrist die kombinierte Harnröhren-/Harnblasenspiegelung zur Kontrolle jedes Patienten, welcher an einer neurogenen Funktionsstörung des unteren Harntrakts leidet, regelmässig durchgeführt.

In der zweiten Studie (*Kapitel 3*) untersuchten wir die Inzidenz der autonomen Dysreflexie (AD) und die Wiederholgenauigkeit von kardiovaskulären

Veränderungen während urodynamischer Messungen. Die Messung beinhaltete die Einlage eines Messkatheters durch die Harnröhre in die Harnblase. Anschliessend wurde die Harnblase mit steriler Kochsalzlösung gefüllt, wobei gleichzeitig sowohl der Druck als auch das Volumen in der Harnblase bestimmt wurden. Die AD ist durch einen Anstieg des systolischen Blutdrucks (SBD) von mehr als 20mmHg vom Ausganswert in Patienten/Patientinnen mit einer RMV definiert. Der SBD kann extrem hohe Werte (250mmHg und mehr) erreichen, so dass es zu einer lebensbedrohlichen Situation kommt, z.B. durch eine Hirnblutung oder einem Herzinfarkt, welche tödlich enden kann. Als Auslöser einer AD gelten Reize, sowohl schmerzhaft als auch nicht schmerzhaft, welchen Ihren Ursprung unterhalb der RMV haben und letztlich zu einer unausgeglichenen Reflexantwort des sympathischen Nervensystems führen. Insgesamt wurden 33 Patientinnen, welche an einer Funktionsstörung des unteren Harntrakts aufgrund einer RMV litten, untersucht. Alle Frauen nahmen an zwei direkt nacheinander folgenden urodynamischen Messungen teil. Synchron zu diesen Untersuchungen wurde der SBD und diastolische Blutdruck (DBD) als auch die Herzfrequenz (HF) kontinuierlich aufgezeichnet. Im Vergleich zur bisherigen Fachliteratur, konnte eine deutlich höhere Inzidenz an AD nachgewiesen werden. Des Weiteren stellten wir fest, dass Patienten mit nachgewiesener AD im Vergleich zu jenen ohne AD einen signifikant grösseren Anstieg des SBD und DBD, aber auch eine signifikant grössere Abnahme der HF hatten. Die Übereinstimmung des Auftretens der AD in beiden aufeinander folgenden urodynamischen Messungen war beachtlich. Im Gegensatz dazu zeigte sich jedoch bei der Verwendung der Bland und Altman Methode eine ungenügende Übereinstimmung zwischen den Veränderungen des SBD, DBD und HF während der direkt nacheinander durchgeführten urodynamischen Messungen. Zusammengefasst besteht somit eine ungenügende Wiederholgenauigkeit von kardiovaskulären Veränderungen. Diese Studie ist nicht nur deshalb so wichtig für das Gebiert der Neuro-

Urologie, da bisher keine frauenspezifischen Ergebnisse vorlagen, sondern das aufgrund der Erkenntnisse aus dieser Studie die folgende Empfehlung in den neuro-urologischen Richtlinien der Europäischen Gesellschaft für Urologie ausgesprochen wurde: “Angesichts der hohen Inzidenz an AD, dem damit verbundenen Risiko und der ungenügende Wiederholgenauigkeit von kardiovaskulären Veränderungen während urodynamischer Messungen, ist sowohl die kontinuierliche Überwachung kardiovaskulärer Parameter als auch das wiederholden der urodynamischer Messungen strengstens empfohlen.”
In der dritten Studie (*Kapitel 4*) befassten wir uns mit der Frage, ob die urodynamische Messung, welche als Mass aller Dinge in der Untersuchung von therapieresistenten Beschwerden des unteren Harntrakts gilt, als Auswahlkriterium für gesunde Probanden (d.h. ohne Beschwerden des unteren Harntrakts) benutzt werden kann, um diese als Teilnehmer für Vergleichsstudien, zum Beispiel gegenüber Patienten, rekrutieren zu können. Vierundvierzig gesunde Teilnehmer (22 Frauen und 20 Männer) wurden in dieser prospektiven Studie an unserem Zentrum eingeschlossen. Alle Teilnehmer führten ein Blasentagebuch über 3 Tage, beantworteten validierte Fragebögen in Bezug auf das Vorliegen von Beschwerden des unteren Harntrakts, der Lebensqualität und kognitive Funktionen, erhielten eine neuro-urologische Untersuchung und nahmen an einer Harnstrahlmessung teil. Alle zuvor genannten Untersuchungen fielen unauffällig aus, d.h. es wurden keine pathologischen Befunde nachgewiesen. Im Gegensatz dazu, konnten in mehr als 70% unserer gesunden Teilnehmer pathologische (d.h. nicht den Referenzwerten entsprechende) Aufzeichnungen während den urodynamischen Messungen dokumentiert werden. Anhand dieser Tatsache erscheint die urodynamische Messung als ungeeignet, um eine normale Funktion des unteren Harntrakts in gesunden Teilnehmer definieren zu können. Somit können wir die urodynamische Messung als Auswahlkriterium für gesunde Teilnehmer nicht empfehlen.

In der vierten Studie lag unser Interesse daran einerseits Studienprotokolle zu entwerfen, welche die Untersuchung der supraspinalen Kontrolle des unteren Harntrakts mittels multi-modaler neuronaler Bildgebung standardisiert (*Kapitel 5 und 6*) und andererseits die Anwendbarkeit und Präzision einer neuartigen Apparatur mit welcher die Füllung beziehungsweise Entleerung der Harnblase zeit- und volumengenau durchgeführt werden kann (*Kapitel 7*). Zuerst (*Kapitel 5*) erstellten wir ein Studienprotokoll, welches im Vergleich zu den bisherigen Studien auf diesem Gebiet als methodische Verbesserung angesehen werden kann. Dieses Protokoll, beinhaltet einerseits die Identifizierung jener Hirnareale, welche dem Netzwerk der supraspinalen Kontrolle des unteren Harntrakts zugeordnet sind, in gesunden Teilnehmern und andererseits die Entdeckung von strukturellen und funktionellen Veränderungen in diesem Netzwerk in Patienten, welche an einer nicht-neurologischen Störung des unteren Harntrakts leiden. Um dieses Ziel zu verwirklichen, integrierten wir die Korrelation von struktureller und funktioneller Magnetresonanztomographie (MRT) mit klinischer Parametern. Auf diese Weise könnten neue Einblicke in die supraspinale Verarbeitung innerhalb des Netzwerkes, welches für die Steuerung des unteren Harntrakts verantwortlich, gewonnen werden. Die daraus resultierenden Erkenntnisse könnten dazu dienen, um die neuronalen Schaltkreise in Patienten, welche an einer nicht-neurologischen Störung des unteren Harntrakts leiden, mit einer gezielten Therapie positiv zu beeinflussen, so dass bestenfalls eine Normalisierung der supraspinalen Kontrolle des unteren Harntrakts erreicht werden kann. Des Weiteren könnte diese Kombination von verschiedenen neuronalen Bildgebungstechniken dazu benutzt werden, die supraspinalen Veränderungen zu quantifizieren, um zwischen Therapieerfolg und –versagen zu unterscheiden.
Als zweiten Schritt (*Kapitel 6*) erstellten wir ein weiteres Studienprotokoll. Ziel war es therapie-assoziierte Veränderungen in Hirnarealen, die zum supraspi-

nalen Netzwerkes zur Kontrolle des unteren Harntrakts gehören, zu erkennen. Die Zielgruppe besteht aus Patienten mit einer Funktionsstörung des unteren Harntrakts aufgrund einer chronischen RMV. Durch die Verwendung struktureller und funktioneller MRT kann das supraspinale Netzwerk der Patienten einerseits im direkten Vergleich mit sich selbst, d.h. vor und während der Therapie, aber auch andererseits gegenüber gesunden Teilnehmern, welche ebenfalls zweimal gemessen werden, vergleicht werden. Die Therapie besteht in der standardisierten Applikation von OnabotulinumtoxinA, besser bekannt auch als BOTOX, in die glatte Muskulatur der Harnblase (Detrusor). Schlussendlich können somit Unterschiede zwischen der Anatomie (strukturell) und der Funktionsweise der supraspinalen Netzwerke zwischen Patienten mit einer RMV-bedingten Funktionsstörung des unteren Harntrakts und gesunden Teilnehmern einerseits und therapie-assoziierte Veränderungen innerhalb des supraspinalen Netzwerkes von Patienten aufgezeigt werden.

Als dritten und letzten Schritt in dieser Studie (*Kapitel 7*) untersuchten wir die Anwendbarkeit und Präzision einer durch uns entwickelten Apparatur mit welcher die Harnblase zeit- und volumengenau gefüllt und entleert werden kann. Diese spezielle MRT-taugliche Konstruktion ermöglicht eine präzise, sich wiederholende Stimulation des unteren Harntrakts, sodass Stimulus-spezifische supraspinale Verarbeitungsprozesse mittels funktioneller MRT aufgezeichnet werden können. Die aktuellste Version unserer Konstruktion, basierend auf einem erfolgreichen getesteten Prototyp, verbindet hydraulische und elektrische Elemente zu einem in sich geschlossenen System, dem elektro-hydrostatische Aktuatoren. Dieser Aktuator, auch Antriebselement genannt, wandelt ein elektrisches Steuersignal in eine Bewegung um. Die Anwendbarkeit und Präzision wurde sowohl unter Laborbedingungen als auch an Studienobjekten, d.h. in gesunden Teilnehmern sowie Patienten mit einer Funktionsstörung des unteren Harntrakts, im MRT untersucht. Entsprechend unserem Er-

gebnissen konnten wir aufzeigen, dass unsere MRT-taugliche und MRT-synchrone „Füll- und Entleerungs-Einheit“ unter beiden Messbedingungen eine sehr hohe Funktionsgenauigkeit aufweist, sodass zeit- und volumengenaue Füllungen bzw. Entleerung wiederholt durchgeführt werden können. Somit konnten signifikante, Stimulus-abhängige Signalveränderungen im supraspinalen Netzwerk, welches für die Steuerung des unteren Harntrakts verantwortlich ist, mit dem MRT entdeckt und dokumentiert werden. Unsere Konstruktion ist kommerziell erhältlich, leicht zu transportieren und kann bedarfsgerecht auf verschiedenste Weise angepasst werden. Unserer Meinung nach kann diese Apparatur die Präzision und Standardisierung von Studien, welche neuronale Bildgebungstechniken zur Untersuchung der supraspinalen Kontrolle des unteren Harntrakts verwendet, deutlich verbessern. Des Weiteren kann mit Hilfe dieser Apparatur die Durchführung zukünftiger Multizentrumsstudien zur Untersuchung des unteren Harntrakts standardisiert und als direkt vergleichbar konzipiert und realisiert werden.

NEDERLANDSE SAMENVATTING

Dit proefschrift bespreekt een aantal multimodale technieken die gebruikt kunnen worden bij het bestuderen van de lage urinewegen bij gezonde vrijwilligers en bij patiënten.

Het eerste hoofdstuk (*Hoofdstuk 1*) geeft een algemeen overzicht betreffende functie en dysfunctie van de lage urinewegen. In een eerste studie (*Hoofdstuk 2*), werd de diagnostische waarde van een urethrocystoscopie (blaasspiegeling) bestudeerd bij patiënten met neurogeen blaaslijden. Het is bekend dat patiënten met neurogeen blaaslijden (in het bijzonder ten gevolge van een dwarslaesie) een verhoogd risico vertonen op het ontstaan van blaaskanker. In deze prospectieve studie, de grootste tot op heden, werden de gegevens beschreven van 129 opeenvolgende patiënten die minstens 5 jaar neurogeen blaaslijden hadden. Tijdens follow-up urethrocystoscopie, werden bij 5% van deze patiënten klinisch relevante afwijkingen gevonden, waaronder blaaskanker. In tegenstelling met de beschikbare literatuur suggereren deze resultaten dat een regelmatige controle met urethrocystoscopie aangewezen is bij deze patiëntengroep.

In de tweede studie (*Hoofdstuk 3*), werd het optreden van autonome dysreflexie (AD) en andere cardiovasculaire veranderingen beschreven bij 33 opeenvolgende vrouwen met neurogeen blaaslijden ten gevolge van een supra sacrale dwarslaesie. Een dergelijke studie werd nog niet eerder bij een vergelijkbare patiëntenpopulatie uitgevoerd. Alle patiënten ondergingen gelijktijdig een urodynamisch onderzoek met continu cardiovasculaire monitoring (systolische en diastolische bloeddruk, hartslagfrequentie). Tijdens het urodynamisch onderzoek werd een hoge incidentie van AD gevonden en patiënten met AD vertoonden een significante verhoging van zowel de systolische en diastolische bloeddruk als van de hartslagfrequentie in vergelijking met patiënten zonder AD. Het optreden van AD tijdens

opeenvolgende urodynamische onderzoeken was voorspelbaar, maar de andere cardiovasculaire factoren bleken moeilijk voorspelbaar. Als gevolg van deze resultaten werden de richtlijn van de Europese Vereniging voor Urologie (EAU) aangepast en deze raadt nu systematische cardiovasculaire monitoring aan tijdens een urodynamisch onderzoek bij patiënten met neurogeen blaaslijden ten gevolge van een dwarslaesie.

In de volgende studie (*Hoofdstuk 4*), werd een urodynamisch onderzoek uitgevoerd bij gezonde vrijwilligers omdat dit waardevol zouden kunnen zijn bij de interpretatie van gegevens van studies met patiënten met een dysfunctie van de lage urinewegen. Daartoe werd bij 42 gezonde vrijwilligers (22 vrouwen en 20 mannen) een prospectieve cohort studie uitgevoerd in één onderzoekscentrum. Deze vrijwilligers hielden gedurende 3 dagen een mictiedagboek bij en vulden een aantal gevalideerde vragenlijsten in. Verder ondergingen zij een klinisch neuro-urologisch onderzoek en een urineflowmeting werd uitgevoerd. Ondanks het feit dat deze vrijwilligers geen klachten hadden en de andere gegevens binnen de limieten van het normale waren, werd bij meer dan 70% van deze 'gezonde' vrijwilligers minimaal één afwijking gevonden bij het urodynamisch onderzoek. Op basis van dit resultaat moet geconcludeerd worden dat een urodynamisch onderzoek niet geschikt is om de normale functie van de lage urinewegen te bestuderen en dus ook niet aangeraden kan worden bij toekomstige vergelijkende studies.

Het volgende hoofdstuk (*Hoofdstuk 5*) beschrijft een protocol om de netwerken ter hoogte van de hersenen te visualiseren tijdens fMRI studies, die mogelijk verantwoordelijk zijn voor de supra spinale controle van de lage urinewegen bij gezonde vrijwilligers. Tevens worden de resultaten gepresenteerd van een studie die tracht de afwijkingen te detecteren in de werking van deze netwerken bij patiënten met niet-neurogeen bepaalde dysfunctie van de lage urinewegen. Voor deze studie werden de gegevens van de fMRI beeldvorming gecorreleerd met de klinische gegevens. De

bedoeling van deze studie was het verder bevestigen dan wel verbeteren en eventueel aanpassen van de beschikbare modellen die de neuronale circuits beschrijven die verantwoordelijk zijn voor de neurologische controle van de lage urinewegen. Deze modellen worden verwacht behulpzaam te zijn als aanvullende diagnostiek bij patiënten met neurogeen blaaslijden.

In een volgend hoofdstuk (*Hoofdstuk 6*) wordt een studieprotocol beschreven waarin fMRI beeldvorming gebruikt wordt om de supra-spinale controle van de lage urinewegen te bekijken bij gezonde vrijwilligers en patiënten met een dwarslaesie die een behandeling ondergingen met onabotuline toxine A injecties in de detrusor. Deze fMRI beeldvorming moet toelaten inzicht te verkrijgen in de centrale effecten van onabotuline injecties in de detrusor bij patiënten met een dwarslaesie.

In het laatste hoofdstuk (*Hoofdstuk 7*) wordt verslag gedaan van de toepasbaarheid en de validiteit van een nieuw ontwikkeld apparaat waarmede de urineblaas op een gestandaardiseerde manier gevuld en gemeten kan worden tijdens een urodynamisch onderzoek. Het bestudeerde ontwerp omvat hydraulische en elektrische elementen die de kracht en de snelheid van de vulling bepalen. Deze nieuwe techniek werd bestudeerd zowel onder laboratorium omstandigheden als bij gezonde vrijwilligers en patiënten met lage urineweg dysfuncties die een fMRI onderzoek ondergingen. Deze apparatuur laat toe ook tijdens een MRI onderzoek om op een gestandaardiseerde manier een urodynamisch onderzoek uit te voeren en hierdoor accurate en significante signalen op te wekken in gebieden van de hersenen die verantwoordelijk zijn voor de controle van de lage urinewegen. Dit apparaat laat toe om op een betrouwbare en gestandaardiseerde manier de blaasvulling te veranderen tijdens beeldvorming door middel van fMRI. Het laatste hoofdstuk (Hoofdstuk 8) omvat de globale conclusies van dit proefschrift.

CHAPTER 9

Valorisation

INTRODUCTION

Lower urinary tract (LUT) dysfunctions are highly prevalent [1], significantly reduce the quality of life of affected individuals [2], and cause an enormous burden on healthcare system and society [3]. In individuals with an underlying neurological disorder, such as spinal cord injury (SCI) or MS, LUT dysfunction are labelled as neurogenic, i.e. NLUTD and pose a long-term threat to the upper urinary tract (UUT) [4]. When NLUTD is improperly managed, affected individuals may establish renal insufficiency or even failure long-term [5, 6]. Furthermore, NLUTD is associated with the development of LUT malignancies, such as bladder cancer [7]. Individuals suffering from SCI are prone to autonomic dysfunctions [8]. As an example, cardiovascular disease as a result of autonomic dysfunction is the leading cause of death in individuals with SCI [8]. In particular, individuals with an injury at or above the spinal segment T6 are at risk to experience autonomic dysreflexia (AD) [9]. The latter is elicited through noxious or innocuous stimuli originating from below the lesion level [9]. The urinary bladder is one of the most frequent causes for AD [10]. This potentially life-threatening situation, characterized by a sudden increase in systolic blood pressure (SPB) >20 mmHg from baseline [9], which can be accompanied with clinical symptoms such as headache, flushing, piloerection, or profound sweating, can result in brain hemorrhage, stroke, or even death [11]. In order to reduce the detrimental long-term effect of LUT dysfunctions in individuals with and without underlying neurological disorder, appropriate assessments, treatment and follow-up strategies must be established. Furthermore, to increase the beneficial effect of currently available treatment options or to develop novel therapeutics, we need to expand our knowledge about supraspinal LUT control.

ASSESSMENTS

To find out whether the LUT is functioning properly or not, several assessments are available (and should be performed). Obtaining past and present medical history including bowel, sexual, and neurological history are highly recommended [4]. Furthermore, bladder diaries, specific questionnaires on patient's quality of life (QoL) as well as urinary, bowel, sexual, and neurological symptoms provide excellent information [4]. A thorough physical examination should include the palpation of genital and reproductive organs as well as a neuro-urologic status. The latter comprises the assessment of sensations and reflexes of the spinal segments Th10 to S5 [4]. Diagnostic procedures, such as urinalysis, blood chemistry, free uroflowmetry, urinary tract imaging (e.g. ultrasound) are well-established in daily practise of urologists [4]. Invasive diagnostic tools such as urethro-cystoscopy and urodynamic investigation (UDI), are used to identify LUT dysfunctions worldwide. The combination of both will allow one to detect anatomical and functional changes of LUT.

OUTCOME

The results of our studies provided new insights on healthy subjects and patients with NLUTD alike, as we were able to answer specific research questions. For the clinical research studies 1 to 3 (Chapters 2 to 4), we utilized recommended assessment strategies. As such, urethro-cystoscopy (Chapter 2) revealed the presence of muscle-invasive bladder cancer and other (partially premalignant) changes of the bladder mucosa in patients with NLUTD. Furthermore, we detected more than 70% of our female SCI patients are exposed to autonomic dysreflexia during UDI (Chapter 3) and provided evidence that UDI is not a valid instrument to identify "healthy" subjects (as 70% of the "normal controls" had pathological UDI findings).

With regards to our experimental studies 4 to 6 (Chapters 5 to 7), we successfully implemented the assessment of structural and functional magnetic resonance imaging according to our study protocols (Chapter 5 and 6) while utilizing our novel infusion-drainage devise (Chapter 7) at a high system accuracy to elicit supraspinal activity in interoceptive and LUT control areas.

IMPACT ON ONGOING WORK

Given our results from Chapter 3 and the well-known potential health risk involved with AD, we continued to investigate individuals with SCI in order to find predictors for AD during UDI. We were successful and revealed two significant independent predictors, i.e. level of SCI and presence of neurogenic detrusor overactivity. This study, which is currently under consideration for publication, will help urologist to take better care of individuals with SCI, but most importantly to protect patient who are at the highest risk for AD when assessing LUT function during UDI. With the positive experience from our experimental study designs (Chapter 5 and 7), we were able to successfully investigate task-specific supraspinal activity related to LUT stimulations. First, we could provide evidence for the repeatability of supraspinal activity in healthy subjects [12]. In addition, we revealed differences in supraspinal activity related to LUT stimulations between healthy subject and individuals with 1) non-neurogenic overactive bladder [13] and 2) spinal cord injury [14]. Furthermore, we found differences in supraspinal activity related to LUT stimulation in healthy subjected when using body warm and cold saline [15].

OUTLOOK

In light of the promising results from our experimental studies, presented in this thesis and the previous section related to ongoing research, we feel con-

fident to find significant changes in supraspinal activity related as an indicator for efficacy of treatment, which has been applied to improve LUT function in individuals with either LUT dysfunction or NLUTD. Furthermore, we are confident to find a significant beneficial effect of NLUTD treatment, such as onabotulinumtoxinA or fesoterodine, to reduce frequency and severity of AD, and to improve LUT and cardiovascular function as well as quality of life in individuals with SCI.

REFERENCES

1. 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;108(7):1132-8.
2. Ganz ML, Smalarz AM, Krupski TL, Anger JT, Hu JC, Wittrup-Jensen KU, et al. *Economic costs of overactive bladder in the United States*. Urology 2010;75(3):526-32, 32 e1-18.
3. 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 pro-ductivity, sexuality and emotional well-being in men and women: results from the EPIC study*. BJU Int 2008;101(11):1388-95.
4. Blok, B., et al. EAU Guidelines on Neuro-Urology. 2017 2017/04/14]; Available from: http://uroweb.org/guideline/neuro-urology/.
5. Lawrenson, R., et al., *Renal failure in patients with neurogenic lower urinary tract dysfunction.* Neuroepidemiology, 2001. 20(2): p. 138-43.
6. Hackler R.H., *A 25-year prospective mortality study in the spinal cord injured patient: comparison with the long-term living paraplegic.* J Urol. 1977. 117(4): p. 486-8.
7. Zhou, J., et al., *Lower Urinary Tract Symptoms and Risk of Bladder Cancer in Men: Results From the Health Professionals Follow-up Study.* Urology, 2015. 85(6): p. 1312-8.
8. Cragg, J.J., et al., *Cardiovascular disease and spinal cord injury: results from a national population health survey.* Neurology, 2013. 81(8): p. 723-8.
9. Krassioukov, A., et al., *International standards to document remaining autonomic function after spinal cord injury.* J Spinal Cord Med, 2012. 35(4): p. 201-10.
10. Faaborg, P.M., et al., *Autonomic dysreflexia during bowel evacuation procedures and bladder filling in subjects with spinal cord injury.* Spinal Cord, 2014. 52(6): p. 494-8.
11. Wan, D. and A.V. Krassioukov, *Life-threatening outcomes associated with autonomic dysreflexia: a clinical review.* J Spinal Cord Med, 2014. 37(1): p. 2-10.
12. Walter M., et al. *Repeatability of supraspinal responses to automated, repetitive bladder filling - an fmri study*; Available from: https://www.ics.org/Abstracts/Publish/326/000346.pdf.
13. Walter M., et al. *Different supraspinal responses to automated, repetitive bladder filling in oab patients compared to healthy subjects - an fmri study*; Available from: http://www.jurology.com/article/S0022-5347(16)02203-5/pdf.
14. Leitner L., et al. Supraspinal control of lower urinary tract function in patients with spinal cord injury: an fmri study; Available from: http://www.jurology.com/article/S0022-5347(15)00516-9/pdf.
15. Walter M., et al. Supraspinal activity to bladder cold sensation in healthy subjects - an fmri study; Available from: http://www.jurology.com/article/S0022-5347(15)01106-4/pdf.

Acknowledgment

First, I would very much like to thank professor Philip van Kerrebroeck (University of Maastricht), who agreed to act as the first promoter for this thesis, for this academic guidance, intellectual support, and beyond. I also would like to thank professor Gommert van Koeveringe (University of Maastricht), who agreed to act as the second promotor for this thesis.

I am very grateful to professor Armin Curt (University of Zürich), who agreed to act as a copromotor for this thesis at Zürich, not only for his commitment toward with thesis but also for his advice in research and life. I am very grateful to Thomas Kessler (University of Zürich), not only for introducing me to the world of Neuro-Urology, his intellectual and financial support of this thesis but also for his advice in life. I am very grateful to Ulrich Mehnert for his intellectual and financial support of this thesis.

Furthermore, I would very much like to thank Lorenz Leitner with whom I have spent countless hours working together on almost every research project and Stephanie Knüpfer for her companionship and intellectual support – but foremost both for their friendship. I would like to thank Johann Wanek for his technical and intellectual support on our device used in chapter 5, 6, and 7. I would like to thank Roger Lüchinger for this technical support on MRI for the chapter 5, 6, and 7. Research is always a collaborative effort. Therefore, I would like to thank my remaining co-authors for their excellent work: Beata Bode-Lesniewska (chapter 2), Jörg Diefenbacher (chapter 7), Patrick Freund (chapter 6), Behnaz Jarrahi (chapter 7), Spyros S. Kollias (chapter 5, 6, and 7), Martina D. Liechti (chapter 7), Lars Michels (chapter 5, 6, and 7), Ulla Sammer (chapter 2 and 4), and Martin Schubert (chapter 3).

Furthermore, I would like to thank Catherine, my parents Doris and Rudolf, my sister Chris, my niece Kim, my nephew Jan, and all my other friends for their endless support and understanding the sacrifices I have made in pursuing a PhD. To my brother Rudolf, we miss you - each and every day.

Curriculum vitae

Matthias Walter was born in Eilenburg, Germany, on December 12th, 1977. After obtaining his high school diploma from Albert-Schweitzer-Gymnasium in Bad Düben in 1996, he graduated in 2006 with a degree in medicine from the Friedrich-Schiller-University Jena, Germany. In 2014, he completed his MD thesis about the outcome and complications of intrathecal baclofen therapy in children at the University of Zurich. His residency included clinical training in General Surgery, Pediatric Surgery and Urology. Between December 2011 and 2014 he worked as a full-time PhD candidate at the Spinal Cord Injury Centre and Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland. He completed his residency in Urology and passed the board certification examination of the Swiss Association of Urology at the Cantonal Hospital Aarau in September 2016. He has been board certified as a Urologist by the Swiss Medical Association (FMH)y and is a fellow of the European Board of Urology (FEBU). Since November 2016, he is a postdoctoral research fellow at the International Collaboration on Repair Discoveries (ICORD), Blusson Spinal Cord Centre, University of British Columbia, Vancouver, Canada.

List of publications

(last update February 8, 2018)

Original studies (peer reviewed)

Christison K*, **Walter M***, Wyndaele JJM, Kennelly M, Kessler TM, Noonan VK, Fallah N, Krassioukov AV.
Intermittent catheterization: The devil is in the details. J Neurotrauma. 2018 Feb 1. doi: 10.1089/neu.2017.5413. [Epub ahead of print] (*Equal contribution)

Leitner L*, **Walter M***, Jarrahi B, Wanek J, Diefenbacher J, Michels L, Liechti MD, Kollias SS, Kessler T.M., Mehnert U
A novel infusion-drainage device to assess lower urinary tract function in neuroimaging. BJU Int. 2017 Feb;119(2):305-316. (*Equal contribution)

Leitner L*, **Walter M***, Sammer U, Knüpfer SC, Mehnert U, Kessler T.M.
Urodynamic investigation: A sensible tool to define normal lower urinary tract function? PLoS One. 2016 Oct 13;11(10):e0163847. (*Equal contribution)

Leitner L, Sammer U, **Walter M**, Knüpfer SC, Schneider MP, Seifert B, Tornic J, Mehnert U, Kessler TM.
Antibiotic prophylaxis may not be necessary in patients with asymptomatic bacteriuria undergoing intradetrusor onabotulinumtoxinA injections for neurogenic detrusor overactivity. Sci Rep. 2016 Sep 12;6:33197.

Leitner L, Guggenbühl-Roy S, Knüpfer SC, **Walter M**, Schneider MP, Tornic J, Sammer U, Mehnert U, Kessler TM.
More Than 15 Years of Experience with Intradetrusor OnabotulinumtoxinA Injections for Treating Refractory Neurogenic Detrusor Overactivity: Lessons to Be Learned. Eur Urol. 2016 Sep;70(3):522-8.

Walter M, Wetterauer C, Bruder E, Obermann EC, Subotic S, Wyler S:
Protocol for a Renal cell carcinoma in a young adult - Do we need further investigations? Urol Case Rep. 2016 Mar 12;6:27-9

Walter M*, Knüpfer SC*, Leitner L, Mehnert U, Schubert M, Curt A, Kessler TM:
Autonomic dysreflexia and repeatability of cardiovascular changes during same session repeat urodynamic investigation in women with spinal cord injury.
World J Urol. 2016 Mar;34(3):391-7. (*Equal contribution)

Sammer U*, **Walter M***, Knüpfer S, Mehnert U, Bode-Lesniewska B, Kessler TM:
Do we need surveillance cystoscopy in patients with neurogenic lower urinary tract dysfunction? PLoS One. 2015 Oct 29;10(10):e0140970. (*Equal contribution)

Walter M, Altermatt S, Furrer C, Meyer-Heim A:
Intrathecal baclofen therapy in children with acquired brain injuries after drowning: a case series. Brain Inj. 2015;29(1):98-103.

Walter M, Altermatt S, Furrer C, Meyer-Heim A:
Intrathecal baclofen therapy in children with severe spasticity: Outcome and complications. Dev Neurorehabil. 2014 Dec;17(6):368-74.

Leitner L*, **Walter M***, Freund P, Mehnert U, Michels L, Kollias S, Kessler TM:
Protocol for a prospective magnetic resonance imaging study on supraspinal lower urinary tract control in healthy subjects and spinal cord injury patients undergoing intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity. BMC Urol. 2014 Aug 18;14(1):68. (*Equal contribution)

Walter M, Michels L, Kollias S, van Kerrebroeck PE, Kessler TM, Mehnert U:
Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms. BMJ Open. 2014 May 21;4(5):e004357.

Reviews

Walter M, Sammer U, Kessler TM:
Chronisches Schmerzsyndrom des Beckens [Chronic pelvic pain syndrome: neurostimulation, neuromodulation and acupuncture]. Urologe A. 2012 Dec;51(12):1683-91